Pharmacological control of transient lower oesophageal sphincter relaxations

Ilmars Lidums
MBBS FRACP

Gastrointestinal Medicine Unit
Department of Medicine
Royal Adelaide Hospital

Submitted for the degree of Doctor of Philosophy
The University of Adelaide
November 1999

To Zinta,

for her love, support and devotion

Contents

Thesis Summary

The work contained in this thesis investigates pharmacological control of transient lower oesophageal sphincter relaxations as a treatment of gastro-oesophageal reflux. Two major classes of pharmaceutical agents were explored; anticholinergic agents and the $GABA_B$ agonist, baclofen.

Transient lower oesophageal sphincter relaxation is the principal mechanism of reflux in normal subjects and in the majority of patients with gastro-oesophageal reflux disease. The anticholinergic agent, atropine, has previously been shown to inhibit gastro-oesophageal reflux in normal subjects by inhibition of transient lower oesophageal sphincter relaxations. Because reflux occurs during absent basal lower oesophageal sphincter pressure in a significant minority of patients with gastro-oesophageal reflux disease, the effects of atropine may not be the same as in normal subjects. The effect of atropine in reflux patients was therefore examined. This study showed that atropine inhibits reflux in patients with reflux disease largely by inhibition of transient lower oesophageal sphincter relaxations.

The site at which atropine exerts its effect on transient lower oesophageal sphincter relaxations is unknown. Transient lower oesophageal sphincter relaxation is believed to be neurally mediated through vagal pathways, stimulated by gastric distension and integrated in the brainstem. Atropine could potentially act centrally, in the brainstem, or peripherally, by altering the mechanical properties of the proximal stomach. Therefore, the site of action of atropine was investigated by examining the effect of atropine on proximal gastric function and the effect of a peripherally acting anticholinergic agent on the triggering of transient lower oesophageal sphincter relaxations. These studies support the notion that atropine inhibits transient lower oesophageal sphincter relaxations by acting centrally in the brainstem.

As gamma-amino-butyric acid (GABA) is a major inhibitory neurotransmitter within the central nervous system and inhibitory $GABA_B$ receptors are abundant in the brainstem, the effects of the $GABA_B$ receptor agonist baclofen on transient lower oesophageal sphincter relaxations and gastro-oesophageal reflux were investigated in normal subjects. This study showed that baclofen significantly inhibited the rate of transient lower oesophageal sphincter relaxations and thereby reduced the rate of reflux episodes, suggesting that $GABA_B$ receptor agonists may have a potential therapeutic role in the treatment of reflux disease.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief this thesis contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying.

Ilmars Lidums

November 1999

Acknowledgments

After arriving at Royal Adelaide Hospital in 1995, I was under the mistaken impression that I would be "dabbling" in a short research project for six months. I was quickly overcome by Prof John Dent's infectious enthusiasm for research in gastrointestinal motility. Before I knew it, I was (again) enrolled as a university student. Thank you, John, for giving me the initial interest in research and ongoing guidance in my career.

Dr Richard Holloway was my principal supervisor. Richard, you always had the patience to discuss any questions or ideas that I had. When experimental results seemed confusing, you were able to assist me in clear and logical interpretation of data and formation of concepts. I am sincerely grateful for your dedication and support.

Dr Geoff Hebbard wrote the software program for the barostat and gave me guidance in using the barostat. Helen Checklin gave much valuable assistance in recruiting volunteers and performing the studies. Marcus Tippett was always available for technical advice. Ashley Blackshaw answered any queries I had about oesophageal physiology. I wish to thank David Hetzel, Hugh Harley and Russell Fitch for allowing me to study their patients.

There were various overseas visitors who gave me much valuable advice in the design of study protocols and interpretation of data, including Ravi Mittal, Roberto Penagini, Melvin Sampson, Daniel Sifrim and Murray Barclay. I am grateful to Anders Lehmann from Astra Hässle, Sweden, for his support in the baclofen project and to Maria Flärdh for performing the baclofen assays.

Special thanks to the Research Review Committee of the Royal Adelaide Hospital. This work was supported by a Dawes Scholarship which made it possible for me to undertake this research doctorate.

Chapter 1

Anatomy and innervation of the oesophageal body

1.1 Gross Anatomy

The oesophagus is a muscular hollow tube extending from the pharynx to the stomach. In the adult, its length ranges from 25 to 35 cm. Its lateral diameter is about 30 mm and anteroposterior diameter 19 mm. The origin of the oesophagus is at the lower border of the cricoid cartilage, at the level of C6, and it ends at the level of T10 or T11. (1)

The oesophagus can be anatomically divided into the cervical, thoracic and abdominal segments. The cervical oesophagus is 5 to 6 cm in length. It extends from the cricoid cartilage and cricopharyngeal muscle at the level of C6, to the thoracic inlet at the level of T1. Anteriorly, its relations are the larynx and trachea. Anterolaterally on each side are the carotid sheath, inferior thyroid artery, lobe of the thyroid gland and recurrent laryngeal nerve. In addition, on the left side is the thoracic duct which reaches its highest point at the level of C7. Posteriorly lie the alar fascia, the prevertebral fascia, the longissimus cervicis muscle and the vertebrae.

The thoracic oesophagus extends from the level of T1 to T10 or T11. Anteriorly, its relations are: trachea, aortic arch, right pulmonary artery, left main bronchus, left atrium, anterior vagal trunk and oesophageal hiatus. Posteriorly, its relations are: vertebral column, longus colli muscle, left thoracic duct from T7 to T4, azygos and hemiazygos veins and aorta. The right lateral aspect contains the azygos vein, right main bronchus, right lung and right vagus nerve. The left lateral aspect contains the aortic arch and descending thoracic aorta, left subclavian artery, left recurrent laryngeal nerve, left vagus nerve and thoracic duct (T4 to C7).

The abdominal oesophagus is 0.5 to 2.5 cm in length and lies at the level of T11 or T12. Anteriorly is the left lobe of the liver. Posteriorly are the crura of the diaphragm, left inferior phrenic artery and the aorta. To the right side is the caudate lobe of the liver; to the left is the fundus of the stomach.

The arterial supply of the oesophagus arises from three principal arteries: the inferior thyroid artery, bronchial arteries and the left gastric artery. In addition, the oesophagus receives blood from the descending thoracic aorta, right intercostal arteries and the left inferior phrenic artery. These arteries penetrate the muscularis mucosa to supply the muscular layers, before joining a plexus of vessels in the submucosa.

The venous drainage of the oesophagus consists of three sections. The upper one third of the oesophagus drains into the superior vena cava, the middle third into the azygos system and lower third into the gastric veins and then portal vein. Therefore, the upper two thirds of the oesophagus drain into the systemic system, the lower one third into the portal system. Numerous anastomoses exist between these two systems.

The lymphatics of the oesophagus are highly interconnected and widely distributed. Lymph vessels run longitudinally and are continuous with the lymphatics of the pharynx above and the stomach below. The upper one third of the oesophagus drains to the cervical lymph nodes, middle third to mediastinal nodes and lower third to the coeliac and gastric nodes.

1.2 Microscopic Anatomy

The oesophagus is lined with non-keratinising, stratified squamous epithelium. It consists of a basal layer of two or three cells with dark nuclei and a series of layers of squamous cells. The squamous cells are shed at the surface and continuously replaced by cell proliferation in the basal layers. Histochemical staining reveals that the squamous cells are filled with glycogen as well as small lipid droplets and phospholipids, although this is less abundant in the basal layers. (2) Extensions of the lamina propria, dermal pegs, protrude into the epithelium. These dermal pegs extend almost half way to the free luminal border and contain capillaries. (3) The lamina propria consists of loose connective tissue that contains mononuclear cells, lymphocytes and occasional plasma cells. In addition, there are mucus-

producing tubular glands. The deepest layer of the mucosa is the muscularis mucosa, a thin band of smooth muscle separating the lamina propria from the underlying submucosa.

The submucosa is the strongest layer of the oesophageal wall. It consists of loose connective tissue with fibrous and elastic elements and an arteriolar plexus. Meissner's nerve plexus lies in the submucosa.

Deep to the submucosa are the major muscles of the oesophagus; the internal circular and external longitudinal muscle layer. The upper quarter of the oesophagus is striated muscle. The second quarter is composed of a mixture of striated and smooth muscle fibres. The lower half contains only smooth muscle fibres. Between the circular and longitudinal muscle layers lies Auerbach's myenteric plexus.

The oesophagus does not have a serosa, but has an adventitia, consisting of loose fibro-elastic connective tissue.

1.3 Innervation of the oesophageal body

Oesophageal body function is modulated by both extrinsic and intrinsic pathways. (4) The extrinsic innervation is provided by vagal (parasympathetic) and spinal (sympathetic) nerves, and intrinsic innervation is provided by neurons of the myenteric plexus and submucous plexus. The proximal striated muscle oesophagus has sparse intrinsic innervation and is regulated predominantly by extrinsic control. The smooth muscle oesophagus is controlled by the intrinsic plexi which may be influenced by the extrinsic innervation.

1.3.1 Extrinsic innervation

1.3.1.1 Vagal (parasympathetic) innervation

The vagus contains both afferent and efferent nerves and conveys information between the oesophagus and the brainstem.

Vagal afferent pathways

Vagal afferent neurons are unmyelinated and their cell bodies are found in the nodose ganglia, lying just below the jugular foramen. Using retrograde labelling, Collman et al showed that in cats, the vagal afferent cell bodies from the striated muscle oesophagus are concentrated in the rostral portion of the nodose ganglion. (5) In contrast, cell bodies from the smooth muscle oesophagus are found diffusely throughout the nodose ganglion with a paucity at the rostral end. Vagal afferent fibres from the nodose ganglia then pass to the brainstem, where they terminate in a very discrete portion of the medial division of the nucleus of the solitary tract a region termed the central subnucleus. (6, 7) Vagal afferents are believed to mediate physiological sensations, although recent evidence suggests that they may influence spinal nociceptive transmission. (8)

Vagal motor pathways

Vagal motor neurons that innervate the oesophagus occupy the cell bodies of the nucleus ambiguus and dorsal motor nucleus. The nucleus ambiguus is found in the ventrolateral medulla and is the major source of motor neurons that supply the striated muscle fibres of the upper oesophagus. In cats, striated muscle efferents are found in the rostral portion of the nucleus ambiguus although a small proportion arise from the dorsal motor nucleus of the vagus. (9) These motor nerves contact striated muscle fibres of the oesophagus via motor endplates.

The vagal motor pathways to the smooth muscle oesophagus differ from that to the striated muscle segment. The dorsal motor nucleus of the vagus is the major source of motor neurons that supply the smooth muscle fibres of the oesophagus. In cats, most efferent fibres to the smooth muscle are present in two groups in the dorsal motor nucleus of the vagus, one rostral and one caudal to the obex. (9) Preganglionic fibres then synapse on the cell bodies of motor neurons that are located within the ganglia of the myenteric plexus.

Higher central nervous system control of vagal nuclei

The brainstem vagal nuclei, which provide the circuitry for basic reflex control of gastrointestinal function are modulated by higher brain centres, comprising the cerebral cortex and various subcortical structures including the internal capsule, subthalamus, amygdala, hypothalamus, substantia nigra and pontomesencephalic reticular formation. (4) The solitary tract nucleus relays the information received from the abdominal viscera, including the oesophagus, and then sends information to higher centres, as well as receiving information from these centres. These connections with the higher centres are important in influencing sensory and motor oesophageal signals and integrating oesophageal and other gastrointestinal activities with cardiovascular and respiratory activities that occur in autonomic reflexes such as vomiting. These central connections also provide a pathway by which various emotions may modulate gastrointestinal function.

1.3.1.2 Spinal pathways

Afferent and efferent spinal innervation were previously referred to collectively as "sympathetic" innervation. However, now it is more accepted to refer to the spinal afferents in the spinal cord as "spinal visceral afferents" and restrict the term "sympathetic innervation" to spinal efferents. (10)

Spinal visceral afferent pathways

Spinal visceral afferents pass from the oesophageal muscle via prevertebral and paravertebral ganglia to the spinal cord. Spinal afferents have cell bodies in the dorsal root ganglia. In cats, sensory neurons from oesophageal striated muscle project to spinal cord segments C1 to T8, and from oesophageal smooth muscle project to spinal cord segments C5 to L2. (5) Spinal visceral afferent nerves transmit nociceptive information to the central nervous system.

Spinal (sympathetic) efferent pathways

The oesophagus receives efferent sympathetic innervation. (4) Preganglionic neurons have cell bodies in the intermediate grey region of the spinal cord at the level of T5 and T6 and terminate in the spinal ganglia. Retrograde tracing techniques have shown that the proximal oesophagus derives its sympathetic innervation from the cervical and upper thoracic ganglia whereas the distal oesophagus, in contrast, derives its sympathetic innervation from the coeliac ganglion. (7) Postganglionic sympathetic neurons project to the ganglia of the myenteric and submucous plexi of both the smooth and striated muscle segments of the oesophagus as well as to blood vessels. The precise role of the sympathetic efferent pathway to the oesophagus remains to be defined. However, in other areas of the gastrointestinal tract, they play a role in inhibiting gastrointestinal function.

1.3.1.3 Neural pathways involved in swallowing

Swallowing is a complex event requiring the coordinated contraction of muscles in the pharynx as well as the oesophagus. The neural control of swallowing is governed by a central pattern generator, which organises the excitation of motor neurons controlling the muscles of swallowing. The swallowing centre is located in the medulla and pons, and has three functional components: an afferent system, an efferent motor system and a complex system of interneurons forming the central pattern generator. (11) The afferent system consists of the trigeminal nerve, glossopharyngeal nerve, and superior laryngeal branch of the vagus nerve. The efferent system consists mainly of the cranial motor nuclei of the

trigeminal, facial, vagal and hypoglossal nerves. The central pattern generator contains interneurons that are found in two main regions. The dorsal region, located in the solitary tract nucleus and adjacent reticular formation, is involved in initiation and organisation of the entire swallow sequence. The ventral region, located in the lateral reticular formation above the nucleus ambiguus, is involved as a connecting pathway to the various motor neurons involved in the swallowing sequence as well as coordination of swallowing with the respiratory centre.

Sensory afferents from the oesophagus terminate in a specific region of the medial portion of the nucleus of the solitary tract a region termed the central subnucleus. (6) Cell bodies in the central subnucleus project to motor neurons in the nucleus ambiguus which then supply the striated muscles involved in swallowing. Therefore, there is a simple relay of information between the nucleus of the solitary tract, receiving sensory information from the oesophagus, and the motor neurons of the nucleus ambiguus, which innervate the muscles of deglutition.

1.3.2 Intrinsic innervation

The intrinsic nerve supply of the oesophagus consists of groups of small ganglia that are connected by bundles of nerve processes forming two major plexuses, Auerbach's myenteric plexus and Meissner's submucous plexus. (12) The submucous plexus of the oesophagus is very sparse. The myenteric plexus lies between the longitudinal and circular muscle layers of the oesophagus and serves as a relay between the vagal fibres and smooth muscle cells.

A myenteric plexus also exists in the striated muscle oesophagus. It is believed that in the striated muscle oesophagus, efferent vagal motor fibres do not synapse on cells of the myenteric plexus but pass directly to innervate striated muscle cells, similar to those of skeletal muscle fibres elsewhere. (4) Therefore, the function of the myenteric plexus in striated muscle oesophagus remains obscure.

Oesophageal smooth muscle is innervated by two types of postganglionic motor neurons that arise from the myenteric plexus: one mediates cholinergic excitation of both longitudinal and circular layers of smooth muscle through muscarinic receptors and the other mediates nonadrenergic noncholinergic (NANC) inhibition mainly of the circular layer. (13-16) The major neurotransmitter released by the latter neuron is most likely to be nitric oxide, (17, 18) although other candidates including purine nucleotides and peptide hormones such as vasoactive intestinal polypeptide have been implicated. (4, 19) Both types of postganglionic motor neurons are excited by cholinergic input from preganglionic vagal fibres and intramural interneurons. Cholinergic excitation of the excitatory neuron is nicotinic, whereas that of the NANC neuron can be nicotinic or muscarinic (M1 receptors). (11)

A population of cells that are neither nerve or muscle are found in the circular muscle layer of the oesophagus. These cells, called interstitial cells of Cajal, are found inserted between nerves and smooth muscle, and form intimate gap junctions with smooth muscle cells. (20-22) Interstitial cells of Cajal and nerves form networks that run along the long axis of the oesophagus and seem ideally suited to facilitate communication in this direction. Morphological and functional studies in other areas of the gut, have suggested that interstitial cells of Cajal have three major functions: they serve as pacemakers, they may facilitate active propagation of electrical events and they may mediate neurotransmission. (23) Their precise role in the smooth muscle oesophagus remains undefined. The interstitial cells of Cajal seem to be structurally organised in such a manner that they may play a role in neural control of oesophageal motility.

Chapter 2

Normal function of the oesophageal body

2.1 Introduction

In the oesophagus, material is transported aborally by a propagated contraction, known as peristalsis. Oesophageal peristalsis is divided into primary and secondary types. Primary peristalsis is initiated by swallowing and secondary peristalsis is triggered by oesophageal distension, independent of swallowing.

2.2 Primary peristalsis

2.2.1 Function

Primary peristalsis is the classic coordinated motor pattern of the oesophagus and is initiated by the act of swallowing. (11) During the oropharyngeal phase of swallowing, a bolus is voluntarily moved into the pharynx and pharyngeal contractions are initiated. Then peristalsis becomes entirely involuntary. A pharyngeal contraction transfers the bolus through an open upper oesophageal sphincter into the oesophagus. This is followed by rigorous closure of the upper oesophageal sphincter and laryngeal descent, the combination of which has been called the "grabbing effect", and which functions to transfer luminal contents distal to the laryngeal inlet at the end of the pharyngeal swallow. (24) A caudally progressing wave of circular muscle contraction then propels the food bolus along the length of the oesophagus through a relaxed lower oesophageal sphincter into the stomach.

2.2.2 Motor patterns

Tone

Using conventional perfused manometry, the musculature of the oesophageal body appears to have no rhythmic or tonic contraction at all at rest. The only movements that are seen at rest are motions associated with the heart or aorta or with respiration. (25) During inspiration, intraluminal pressure ranges from -5 to -15 mmHg and during expiration from -2

to +5 mmHg. Using a computerised isobaric recording system, however, Mayrand and Diamant have demonstrated that active tone is present in the smooth muscle oesophagus and can be inhibited by a smooth muscle relaxant. (26)

Peristalsis

Primary peristalsis involves both the longitudinal and circular muscles. The exact role of the longitudinal muscle is unknown, but it may assist in bolus transport by making the oesophagus more rigid. In studies in cats, Dodds et al showed that wire markers affixed to the oesophageal wall move proximally during peristalsis, presumably as the result of longitudinal muscle contraction. (27) Using miniature strain gauges implanted in the opossum oesophagus, Sugarbaker et al showed that during swallowing, oesophageal circular and longitudinal smooth muscle contracts in a sequential fashion. (28) Peristaltic oesophageal shortening has been demonstrated in the human oesophagus and occurs most prominently in the distal oesophagus. (29, 30) Longitudinal muscle shortening during peristalsis causes transient elevation of the squamocolumnar junction above the diaphragm which may be important in the development of hiatus hernia. (31) This will be discussed in detail in chapter 7 of this thesis.

The mechanical function of the oesophageal circular muscle is more clearly understood. A narrow zone of circular muscle contraction progresses caudally along the oesophageal body, propelling the food bolus to the stomach. In the human oesophagus, this wave of circular muscle contraction is preceded by an initial brief period of inhibition. This has been demonstrated by Sifrim et al, using an intra-oesophageal balloon to create an artificial high-pressure zone. (32) They found that swallowing induces a relaxation beginning simultaneously at various levels of the oesophagus and lasting longer in progressively more distal oesophageal segments. Following this wave of oesophageal inhibition, a small rise in pressure may occur which appears to be related to entry of the swallowed bolus into the oesophagus. (25) Subsequently, the peristaltic wave occurs and proceeds throughout the entire length of the oesophageal body.

The peristaltic pressure wave is generated by smooth muscle contraction causing active apposition of the oesophageal wall. Peristaltic wave amplitude is an indication of the maximal force of this apposition. Using topographic plotting techniques, to analyse the spatial relationship of primary peristalsis, Clouse et al showed that a high amplitude, proximal oesophageal contraction segment is separated from the distal region by a trough in contraction amplitudes at the transition zone of skeletal and smooth muscle. (33, 34) They also demonstrated a second trough in the contraction sequence separating the distal oesophagus into two contraction segments. Similarly, using mathematical modelling and computer simulations combined with concurrent manometric and videofluoroscopic data to analyse oesophageal function, Brasseur et al demonstrated two separate contraction waves, one above and one below the transition zone of the oesophageal body. (35, 36) The actual peristaltic wave amplitude in the oesophageal body is quite variable depending on the measurement method. Peristaltic amplitudes of at least 30 mmHg are required for complete clearance of liquids in the distal oesophagus. (37, 38)

The average velocity of the peristaltic wave is 3 or 4 cm/sec. However, it varies from 3 to 3.5 cm/sec in the upper oesophageal body, 5 cm/sec in the middle of the oesophageal body, to 2 cm/sec in the very distal few centimetres of the oesophageal body. (39) Overall, it takes 6 to 8 seconds for the peristaltic wave to traverse the oesophageal body. Using simultaneous manometry and fluoroscopy, Hewson et al found that normal oesophageal clearance of barium occurs over a wide range of peristaltic velocities, (1.4 to 5.6 cm/s) but is impaired if the peristaltic velocity is greater than 6.25 cm/s. (40) The duration of oesophageal contraction is 2 to 4 seconds, increases distally and rarely exceeds 7 seconds. (39) Oesophageal clearance of liquid is not affected by large variations in the duration of oesophageal contractions as long as the peristaltic amplitude is at least 30 mmHg. (37)

Oesophageal manometric parameters display considerable intersubject (41) and intrasubject variability. (42) Wave amplitude shows the least intrasubject variability and wave duration

the greatest degree of variability. Water swallows are associated with greater peristaltic amplitude, slower propagation velocity and a longer wave duration than a dry swallow. (43) Other factors which may influence oesophageal manometric parameters include, posture, (44) the age of the subject, bolus temperature and intra-abdominal pressure. (25, 45)

Following a swallow, there is a refractory time period during which a second swallow will not generate a peristaltic wave. (46, 47) If the swallow interval is less than 5 seconds, the peristaltic response is ablated. If repeated swallows are performed with less than 15 seconds between them, a reduction in amplitude of the peristaltic is seen. (48) In the clinical evaluation of primary peristalsis, it is important that swallows be spaced at least 15 seconds apart from the preceding swallow because of the refractory period after peristalsis.

2.2.3 Control of primary peristalsis

The control of primary peristalsis is complex and different mechanisms are involved in the striated and smooth muscle segments of the oesophagus.

Striated muscle component

The striated muscle segment of the oesophageal body like other skeletal muscle, receives only an excitatory somatic innervation. Axons from nerve cell bodies of the nucleus ambiguus project via branches of the vagus nerve and eventually terminate in motor end plates on striated muscle cells. At rest, inactivity of the striated muscle of the oesophageal body is due to inactivity of these nerves. The striated muscle of the oesophagus contracts only when the motor neurons supplying it are activated. A peristaltic contraction represents a vigorous discharge of these somatic nerves. The contraction is peristaltic because the motor units are activated in a craniocaudal neural sequence along the oesophageal body. Therefore, the central nervous system is responsible for this peristaltic sequence through sequential activation of the lower motor neuron. (4)

Smooth muscle component

The control mechanisms that modulate primary peristalsis in the smooth muscle oesophagus are far more complex than that of the striated muscle oesophagus. There are different levels of control: the central nervous system, and peripheral neural and myogenic control.

The central nervous system is responsible for the initiation of swallow-induced peristaltic contractions of the smooth muscle portion of the oesophagus. This conclusion is based on the observation that bilateral cervical vagotomy abolishes swallow-induced peristalsis in the smooth muscle segment. (25, 49, 50) However, unlike peristalsis in the striated muscle segment of the oesophagus, the progressive nature of the peristaltic contraction in the smooth muscle segment is not programmed by the central nervous system, but instead by the neuromuscular mechanisms within the wall of the oesophagus. This is demonstrated in studies by Dodds et al, in which mass stimulation of all the fibres in the transected vagus nerves of the opossum causes a progressive, peristaltic contraction of the smooth muscle segment of the oesophagus. (51, 52) In the Rhesus monkey, Janssens et al demonstrated that after transection and reanastomosis of the smooth muscle segment of the oesophagus, primary peristalsis deteriorates due to interruption of intramural fibres, suggesting that neuromuscular mechanisms within the wall of the oesophagus are important in the control of progression of primary peristalsis. (53)

The peripheral neuromuscular mechanisms that produce peristalsis in the smooth muscle oesophagus are not completely understood. The propagating peristaltic contraction is associated with a biphasic electrophysiological response of the circular smooth muscle. (54) Almost immediately after swallowing, the circular muscle becomes hyperpolarised nearly simultaneously along the entire length of the smooth muscle segment. This hyperpolarisation corresponds to the wave of inhibition which precedes a primary peristaltic contraction (32) and is due to the release of nitric oxide from inhibitory nerves. (18) The smooth muscle

membrane then depolarises and spike potentials are generated, which are correlated with smooth muscle contraction. The spike potentials are produced by the opening of calcium channels and the inward movement of calcium ions, which then activates a contraction. (55)

The entire sequence of motor and electrophysiological events described above can be duplicated in vitro in strips of oesophageal smooth muscle by stimulating intrinsic oesophageal nerves to produce a stereotyped mechanical response. (18) During stimulation, there is hyperpolarisation of the circular muscle, but no change in muscle tone. Cessation of the electrical stimulus is followed by a short period of mechanical quiescence, called the "latency", followed by a circular muscle contraction, called the "off response". (56) The latency period is shortest in the proximal part of the smooth muscle oesophagus and longest in the distal part. This latency gradient appears to be important in the control of the timing of peristalsis in the smooth muscle oesophagus.

In anaesthetised opossums, tetrodotoxin does not block the propagation of contractions from direct muscle stimulation, suggesting that the myogenic system is capable of generating and propagating contractions independently of any neural input. (57)

Nitric oxide produced in response to intrinsic nerve stimulation plays an important role in the neuromuscular control of oesophageal peristalsis. Inhibition of nitric oxide production abolishes nerve stimulation induced hyperpolarisation, the depolarisation, the "off response" circular muscle contraction and the latency gradient. (18, 58)

Swallow-induced peristalsis is influenced by two different nerves: muscarinic, cholinergic, excitatory nerves and nitrergic inhibitory nerves. (56) Inhibition of cholinergic innervation decreases the amplitude of peristaltic contractions. (51, 52, 59) Inhibition of nitric oxide synthesis shortens the time interval between swallowing and the onset of peristaltic contractions in the smooth muscle oesophagus and renders the peristaltic contractions almost simultaneous. (60, 61) Therefore, cholinergic innervation plays a role in determining

peristaltic amplitude and nitrergic inhibitory innervation plays a role in timing of the peristaltic sequence.

2.3 Secondary peristalsis

2.3.1 Function

Secondary peristalsis is a propulsive oesophageal contraction that is triggered by oesophageal distension. (62) and is the major mechanism that maintains the oesophageal body empty (45) Oesophageal distension may be caused by a bolus such as oesophageal content which remains after a primary peristalsis, or by refluxed gastric contents. Secondary peristalsis usually starts at or above the level of the stimulus and closely resembles swallow-induced peristalsis.

2.3.2 Motor patterns

In contrast to primary peristalsis, the oesophageal response to oesophageal distension can be quite variable, depending on the nature and site of the distension stimulus. Injection of liquid or gas into the oesophageal body lumen usually produces a peristaltic contraction that traverses the entire oesophageal body. Schoeman and Holloway showed that regardless of the level of injection, the secondary peristaltic response starts in the proximal oesophagus and traverses the entire length of the oesophageal body. (63) The response rates increases significantly as the injected volume increases and is similar for equal bolus volumes of air and water. Secondary peristaltic amplitude is less than that of primary peristalsis.

In contrast, focal oesophageal distension with a transiently inflated balloon produces a pattern of peristaltic contraction which is different from that seen after air or water boluses. During balloon distension, a high amplitude synchronous contraction is seen proximal to the balloon while there is no oesophageal body motor response distal to the balloon. When the

balloon is deflated, the synchronous contraction ceases and a peristaltic contraction wave progresses distally from the level of the balloon distension. (63, 64) Using high-frequency intraluminal ultrasonography, Yamamoto et al demonstrated that during balloon distension, the high amplitude synchronous contraction seen proximal to the balloon is associated with an increase in the thickness of both longitudinal and circular muscle layers of the oesophagus and the lack of pressure response distal to the balloon is associated with a decrease in the thickness of the two muscles. (65)

Secondary peristaltic amplitude elicited by balloon distension is less than that of primary peristalsis while propagation velocity is similar. (63) Balloon distension of the mid oesophagus is more likely to elicit a peristaltic contraction than distension of the upper or lower oesophagus. The reason for this is unclear but may be related to the transition zone of striated and smooth muscle seen at this level. In addition, distension with a 3 cm diameter balloon is more effective than a 1 cm diameter balloon.

With prolonged balloon inflation, a high amplitude synchronous contraction is seen above the balloon and motor quiescence below the balloon. This forceful contraction is sustained if the balloon is allowed to slowly move down the oesophagus. (66) Prolonged balloon distension is analogous to acute oesophageal obstruction and the oesophageal response is an important mechanism for the clearance of an oesophageal bolus.

These studies demonstrate clearly that the site, volume, nature and timing of the distension stimulus is important in determining the secondary peristaltic response. The peristaltic response is an important mechanism for the clearance of an oesophageal bolus which has either not been cleared by primary peristalsis or is due to refluxed gastric contents.

2.3.3 Control of secondary peristalsis

There are little data available about the control of secondary peristalsis. Substantial disparity in the available data may reflect species differences as well as differences in the nature of the oesophageal distension stimulus. Balloon inflation of opossum oesophagus for ten seconds, evokes simultaneous phasic contractions at several sites proximal to the distension site that are abolished by bilateral cervical vagotomy or atropine. (67) However, bilateral vagal cooling does not affect the oesophageal smooth muscle response to balloon distension distal to the distension site. (68) In addition, the opossum oesophagus can be made to elicit a secondary contraction after it has been removed from the animal and placed in an organ bath. (69) These studies suggest that proximal contractions depend on centrally mediated vagal pathways and responses below the distension stimulus are mediated by intramural neuromuscular mechanisms. (70)

Oesophageal balloon distension causes a prompt membrane hyperpolarisation of oesophageal smooth muscle distal to the balloon. Upon balloon deflation, rapid depolarisation occurs that is associated with spike bursts and muscle contraction. This response has a progressively longer latency in the distal oesophagus, that results in a peristaltic contraction. (71) Membrane hyperpolarisation is due to nonadrenergic noncholinergic inhibitory neurotransmitter, presumably nitric oxide. (18)

There are few studies that have directly compared primary and secondary peristalsis in humans. Paterson et al (72) found that the oesophageal contractions distal to balloon distension are of lower amplitude, less atropine sensitive and more often nonperistaltic compared to swallow induced oesophageal contractions, suggesting that there are two different neural pathways. In contrast, Schoeman and Holloway (63) found that the characteristics of secondary peristalsis induced by injection of air or water into the oesophageal lumen, are similar to those of primary peristalsis and concluded that there is a common neural mechanism. The reason for these different conclusions may be explained by

the differences in the methodology. Balloon distension is probably not the best stimulus for testing secondary peristalsis and produces a completely different response to air or water injection into the oesophagus, which produces a peristaltic contraction of the oesophageal body, more closely mimicking a primary peristaltic contraction.

Chapter 3

Anatomy and innervation of the lower oesophageal sphincter

3.1 Gross Anatomy

The presence of a physiological sphincter at the lower end of the oesophagus has been known for years. (73) However, studies on the anatomical structure of the lower oesophageal sphincter are technically difficult. This difficulty may account for the previously confusing and contradictory information seen in the literature. (74)

The lower oesophageal sphincter is 2.5 to 4 cm in length. The proximal part of the lower oesophageal sphincter lies in the hiatal canal formed by the right crus of the diaphragm and the distal part is intra-abdominal. In fresh human cadavers, Liebermann-Meffert et al (75) identified a ring of thickened muscle tissue representing the lower oesophageal sphincter. This muscle thickening is asymmetrical and the greatest thickness is at the greater curvature. The ring of thickened muscle tissue is split into two segments; muscle bundles on the greater curvature side form long oblique loops to the stomach, and muscle bundles on the lesser curvature side form short transverse muscle clasps. Using high-frequency catheter-based ultrasound transducers, Liu et al (76) demonstrated in healthy human subjects that the inner circular and outer longitudinal muscle layers at the level of the lower oesophageal sphincter are thicker than the corresponding muscle layers in the body of the oesophagus.

3.2 Microscopic Anatomy

The circular muscle of the lower oesophageal sphincter is connected with some of the smooth muscle fibres of the stomach. The muscle is thicker than in the adjacent oesophagus. This increase in thickness is due to an increase in the muscle mass of the inner muscle coat. (75)

Electron microscopy reveals that the circular smooth muscle fibres of the opossum lower oesophageal sphincter has irregular surfaces with numerous protrusions, in contrast to the smooth surfaces of the rest of the circular muscle fibres of the oesophagus. (77) This may

be a reflection of the tonically contracted state of the sphincter. Also, there is an increased amount of connective tissue between the sphincter muscle bundles. The mitochondria of the sphincter differ from the rest of the oesophagus. Although the number of mitochondria per muscle cell does not differ, mitochondria in the sphincter are larger and more centrally located in the muscle cell. In addition, the endoplasmic reticulum is more abundant in the sphincter muscle cells than in the circular muscle cells of the rest of the opossum oesophagus. (78)

Interstitial cells of Cajal (ICC) are abundant in the lower oesophageal sphincter and may play a role in control of motility although their precise role remains undefined. (20) The myenteric plexus of the sphincter is interspersed within several muscle planes of the circular and longitudinal muscle. This is in contrast to the rest of the oesophagus where the myenteric plexus lies between the circular and longitudinal muscle layers. The ganglion cell density of the myenteric plexus was previously thought to be decreased in the sphincter region compared to the rest of the oesophagus. (79) However, this observation may have been due to technical artefact, related to dissection, to the staining procedure, and to methods of quantification. A more recent study in opossums has shown that the ganglion cell density is actually increased in the sphincter region. (80)

3.3 Innervation

Lower oesophageal sphincter function is modulated by both an extrinsic pathway, provided by vagal (parasympathetic) and spinal (sympathetic) nerves, and an intrinsic pathway, provided by neurons of the myenteric plexus and submucous plexus.

3.3.1 Extrinsic innervation

3.3.1.1 Vagal (parasympathetic) innervation

The vagus contains both afferent and efferent nerves and conveys information between the lower oesophageal sphincter and brainstem.

Vagal afferent pathways

Vagal afferent neurons of the lower oesophageal sphincter are found in the nodose ganglion and in cats, have a similar distribution to that of oesophageal smooth muscle vagal afferents, occurring predominantly in the caudal portion. (5)

Vagal motor pathways

The dorsal motor nucleus of the vagus is the major source of motor neurons that supply the smooth muscle fibres of the lower oesophageal sphincter. The vagal motor pathways to the lower oesophageal sphincter are similar to that of the smooth muscle of the oesophageal body although in cats, the cell bodies in the dorsal motor nucleus of the vagus are located more caudally. (9)

Preganglionic parasympathetic motor fibres synapse with postganglionic nerve cells in the myenteric plexus, where they release acetylcholine that activates muscarinic and nicotinic receptors of myenteric nerve cells.

3.3.1.2 Spinal pathways

Spinal visceral afferent pathways

Spinal sensory neurons from the lower oesophageal sphincter project to more caudal segments of the spinal cord than those from the oesophageal smooth muscle, and are found in spinal cord segments T1 to L3. (5)

Spinal (sympathetic) efferent pathways

Preganglionic neurons have cell bodies in spinal segments T6 to T10 and terminate in the cervical ganglia, coeliac ganglion and ganglia of the paravertebral chains. Postganglionic sympathetic neurons project to the ganglia of the myenteric and submucous plexi.

3.3.2 Intrinsic innervation

As in the smooth muscle oesophageal body, there are two types of postganglionic motor neuron: one mediates cholinergic excitation of smooth muscle through muscarinic receptors and the other mediates nonadrenergic noncholinergic inhibition. (81) Cholinergic excitatory innervation of the feline lower oesophageal sphincter is mediated by M3 muscarinic receptors (82) The inhibitory innervation is most likely to be mediated by nitric oxide, (17, 18, 83) although other candidates including purine nucleotides and peptide hormones such as vasoactive intestinal polypeptide have been implicated. (4, 19) Using retrograde labelling in the guinea pig, Brookes et al showed that inhibitory motor neurons have longer projections and are located exclusively oral to the lower oesophageal sphincter muscle, in contrast to excitatory motor neurons which have shorter projections and are located more closely to the sphincter muscle. (84) In addition, gastric motor neurons innervate the gastric oblique fibres, which merge with the lower oesophageal sphincter and play a role in maintaining the antireflux barrier. (84)

Chapter 4

Normal function of the lower oesophageal sphincter

4.1 Function

The lower oesophageal sphincter is a physiological structure which is readily identified grossly in living tissue as a ring of contracted muscle at the oesophagogastric junction. Tonic contraction of the lower oesophageal sphincter is the major antireflux mechanism. Relaxation of the lower oesophageal sphincter allows trans-sphincteric flow in both directions.

4.2 Motor patterns

4.2.1 Basal lower oesophageal sphincter tone

The lower oesophageal sphincter is tonically contracted at rest. This zone of raised intraluminal pressure at the oesophagogastric junction shows radial asymmetry, as detected by withdrawal of a radially perfused catheter. (85) The highest pressures are recorded in the left orientation. This radial asymmetry has been explained on the basis of thickness of the lower oesophageal sphincter muscle on the left side, (75) extrinsic compression by the crural diaphragm, as well as due to folds created in the mucosa by the contraction. Liu et al have recently re-examined this issue using intra-oesophageal ultrasound and oesophageal manometry. (86) Ultrasound images showed that the circular muscle is radially asymmetrical and is thicker on the left side and posteriorly. They concluded that the radial asymmetry is related to the non-circular shape of the lower oesophageal sphincter. In a more recent study, using concurrent manometry, fluoroscopy and endoscopically placed clips at the squamocolumnar junction, Kahrilas et al demonstrated that the normal radial asymmetry of the oesophagogastric junction is due to extrinsic compression by the crural diaphragm, as the lower oesophageal sphincter was radially symmetric in patients with hiatus hernia. (87) Radial asymmetry may be an important reason for the wide variation in normal values for lower oesophageal sphincter pressure. Depending on the recording conditions, normal basal pressure can vary between 5 and 65 mmHg.

The anatomical position of the lower oesophageal sphincter relative to the abdominal and thoracic cavities varies with respiration, and is of importance in the determination of sphincter pressure. Elevations of intra-abdominal pressure, such as during abdominal straining, transmit equal pressures to the gastric contents and the intra-abdominal component of the lower oesophageal sphincter. Therefore, the sphincter muscle does not require any additional contraction force, to prevent reflux of gastric contents into the oesophagus.

The crural diaphragm is an important factor in the genesis of inspiration related pressure increases of the lower oesophageal sphincter and in humans has been reported to increase pressure by 10 to 20 mmHg. (88) In cats, during central apnoea or diaphragmatic paralysis with neuromuscular blockade, the crural diaphragm is inactive and disappearance of the inspiratory-induced increase in sphincter pressure occurs. (89) In contrast, increasing the amount of diaphragmatic contraction causes a progressive increase in the amplitude of inspiratory-induced pressure increases in the lower oesophageal sphincter. Therefore, active crural diaphragmatic contraction is responsible for increases in sphincter pressure and the magnitude of diaphragmatic contraction influences the magnitude of the pressure increase.

Basal lower oesophageal sphincter pressure shows considerable minute-to-minute variation. External influences such as meals, the interdigestive motor cycle, exercise and sleep status are important contributors to this variability. These will be discussed in detail at the end of this chapter.

4.2.2 Lower oesophageal sphincter relaxation

The lower oesophageal sphincter relaxes to allow trans-sphincteric flow in both directions. There are three patterns of lower oesophageal sphincter relaxation: swallow-induced and oesophageal distension-induced sphincter relaxation, which are important in aborad oesophageal transport, and transient lower oesophageal sphincter relaxation, which allows for retrograde flow of gas or liquid.

4.2.2.1 Swallow-induced lower oesophageal sphincter relaxation

On swallowing, the lower oesophageal sphincter relaxes and allows normal oesophageal emptying of liquids and solids. Relaxation minimises resistance to flow across this segment. The relaxation usually starts at the time of swallowing. Lower oesophageal sphincter relaxation lasts 3 to 10 seconds, as the peristaltic contraction traverses the oesophageal body, and is terminated by arrival of the peristaltic wave as it sweeps across the sphincter. The relaxation is then followed by a brief forceful contraction that soon subsides back to the tonic contraction of the resting state. This hypercontraction has not been studied in detail but seems to be an extension of the peristaltic wave that sweeps the oesophageal body. (25) The lower oesophageal sphincter relaxes with all swallows, even if there is failure of the oesophageal peristaltic wave. Inhibition of the crural diaphragm has been demonstrated during swallow-induced lower oesophageal sphincter relaxation in anaesthetised cats, (90) but has not been demonstrated in humans (91).

4.2.2.2 Oesophageal distension-induced lower oesophageal sphincter relaxation

Oesophageal distension is able to induce relaxation of the lower oesophageal sphincter. The larger the distension volume, the greater the magnitude of sphincter relaxation. (92) The lower oesophageal sphincter starts to relax at the onset of oesophageal distension and recovers during small volume but persists during large volume prolonged distension. (93) If the distending bolus progresses aborad, then the sphincter relaxation persists until the secondary peristaltic wave reaches the lower oesophageal sphincter. The relaxation is then followed by a return to the basal level of contraction.

4.2.2.3 Transient lower oesophageal sphincter relaxation

This term describes a distinctive type of lower oesophageal sphincter relaxation which is not triggered by swallowing or oesophageal distension. Transient lower oesophageal sphincter relaxation is the major mechanism of belching (94) and is the predominant mechanism of gastro-oesophageal reflux. (95) Transient lower oesophageal sphincter relaxations are of longer duration than swallow-induced lower oesophageal sphincter relaxation, lasting from 10 to 45 seconds (96) and are usually complete, to within 2 mmHg of intragastric pressure. A detailed review of the triggering and control of transient lower oesophageal sphincter relaxation is covered in detail in chapter 8 of this thesis.

4.3 Control

4.3.1 Control of basal lower oesophageal sphincter pressure

Control of resting lower oesophageal sphincter tone is a complex process, dependent on a combination of myogenic, neural and hormonal factors. Biochemical and electrophysiological studies of the circular muscle of the lower oesophageal sphincter support the notion that the lower oesophageal sphincter smooth muscle is different from that of the oesophageal body.

4.3.1.1 Myogenic control

Early in vitro studies investigating the length-tension characteristics of the lower oesophageal sphincter muscle showed that strips of muscle from the sphincter region generated much greater force than did those from adjacent oesophageal body. This force was not affected by tetrodotoxin suggesting that it was myogenic in nature. Therefore, the sphincter was thought of as a muscle that maintains a tonic contraction at rest as a result of some unique property of the sphincter muscle itself. (97)

The process of maintaining lower oesophageal sphincter tone requires energy and energy metabolism differs between the circular muscle of the lower oesophageal sphincter and that of the oesophageal body. In vitro, lower oesophageal sphincter tone is supported entirely by aerobic mechanisms, as oxygen availability is reduced, tone is reversibly reduced. In contrast, in the smooth muscle of the oesophageal body, phasic contractions are supported under anaerobic conditions. (98, 99) When the sphincter muscle is stretched, oxygen consumption rises steeply, much more so than does that of smooth muscle of the oesophageal body. In the smooth muscle of the sphincter, mitochondria are larger, endoplasmic reticulum is more developed and the concentration of mitochondrial enzyme cytochrome c oxidase is lower than in the oesophageal body (78, 100), giving further evidence that myogenic tone of the sphincter muscle requires energy and that energy metabolism is different in the sphincteric muscle.

Prolonged lower oesophageal sphincter muscle tonic contraction depends on the interaction of actin and myosin. Contraction is related to calcium-dependent myosin phosphorylation, as levels of myosin phosphorylation decrease during maintenance of basal sphincter tone or sphincter relaxation and increase during phasic contraction. (101) Maintenance of lower oesophageal sphincter tone depends on intracellular and intracellular calcium. Depletion of either source of calcium reduces basal sphincter tone. (55, 102, 103) The sphincter muscle may be more capable of utilising intracellular calcium than the oesophageal body, as evidenced by the more developed endoplasmic reticulum, which is important in the regulation of cytoplasmic calcium.

There are special electrophysiological properties of the plasma membrane which are important in the lower oesophageal sphincter tone. Electrophysiological studies in the opossum and cat have shown that continuous spike activity is generated in the smooth muscle of the lower oesophageal sphincter and this is independent of neural activity. (104, 105) This spike activity is may play a role in the maintenance of sphincter tone. However, if

this spike activity is abolished, sphincter pressure is reduced but not totally abolished suggesting that spike activity is not the sole factor in the maintenance of basal sphincter tone. Other studies have suggested that the sphincter muscle maintains a basal tone partly because it has a more positive resting membrane potential than adjacent muscle tissues. (106) However, recent studies have failed to show any differences in resting membrane potential between sphincteric and oesophageal smooth muscle. (103, 107)

Spontaneous lower oesophageal sphincter tone is antagonised by protein kinase C (PKC) inhibitors, suggesting that PKC activation is important for this function. Sohn et al (108) showed that the PKC-dependent contraction of the circular smooth muscle is mediated by the calcium-dependent PKC-beta isoenzyme. In contrast, contraction of the oesophageal body is mediated by the calcium-independent PKC-epsilon isoenzyme.

These biochemical and electrophysiological studies outlined above support the notion that the lower oesophageal sphincter smooth muscle is different from that of the oesophageal body and that myogenic properties of the sphincter muscle itself are important in the maintenance of basal lower oesophageal sphincter tone.

4.3.1.2 Neural control

It is clear that control of basal lower oesophageal sphincter tone is not entirely myogenic but can be altered by the actions of nerves and hormones. Neural control of the lower oesophageal sphincter shows marked species variation. In humans, dogs, cats and guinea pigs the resting lower oesophageal sphincter tone is influenced by excitatory as well as inhibitory vagal fibres, (9, 59, 81, 109, 110) whereas in the opossum inhibitory vagal fibres predominate. (68, 111-113) In dogs, cholinergic blockade has been shown to reduce lower oesophageal sphincter pressure. (59, 114, 115) Similarly, vagal blockade by vagal cooling or vagotomy also causes a reduction in lower oesophageal sphincter pressure. (109, 110, 116) These observations support the concept that in dogs, resting tone is influenced by

excitatory muscarinic vagal fibres. In the opossum, there is no significant change in basal sphincter pressure after vagal cooling, vagotomy, cholinergic blockade or tetrodotoxin, suggesting that sphincter tone in the opossum is predominantly myogenic and not vagally driven. (68, 111-113) In humans, atropine reduces lower oesophageal sphincter pressure. (59, 117-119) The effect of bilateral vagal cooling in humans is unknown. The effect of vagotomy on lower oesophageal sphincter pressure in humans is difficult to study because the efferent vagal fibres controlling the lower oesophageal sphincter enter the oesophagus at some point above the usual site of truncal vagotomy. (120) However, it is likely that in humans, basal lower oesophageal sphincter tone is maintained by cholinergic neural input. (59)

Sympathetic nerve fibres are excitatory in the lower oesophageal sphincter of the cat. (121, 122) The sympathetic response of the lower oesophageal sphincter is mediated by alpha-adrenergic receptors located on cholinergic parasympathetic motor neurons. (123) Stimulation of sympathetic fibres in the cat causes facilitation of the vagal excitatory fibres and inhibition of vagal inhibitory fibres. Alpha-adrenergic antagonists cause a small and transient reduction in the force of sphincter closure in the opossum, suggesting that the role of the sympathetic nerves in the maintenance of basal tone is of minor importance. (124) Furthermore, Behar showed that in cats, basal lower oesophageal sphincter contraction occurs with stimulation of splanchnic nerves but is not influenced by section of the splanchnic nerves or coeliac ganglion. (125) The role of the sympathetic nervous system on maintenance of lower oesophageal sphincter tone in humans is unclear. Isoproterenol, a selective beta receptor agonist inhibits (126) and propranolol, a beta receptor antagonist transiently increases (127) the lower oesophageal sphincter resting tone in humans. However, there is no evidence that in humans, the sympathetic nervous system plays a major role in the maintenance of lower oesophageal sphincter basal tone.

4.3.1.3 Hormonal control

There are a number of circulating hormones and other biologically active substances that can affect lower oesophageal sphincter tone. However, the existence of these effects does not necessarily indicate that these substances have a physiological role in the control of lower oesophageal sphincter tone. No one hormone has been demonstrated to be the major determinant of sphincter tone. There has previously been much controversy regarding the role of endogenous gastrin in the control of the lower oesophageal sphincter. Whilst exogenous gastrin increases sphincter pressure, (128-130) other more critical experiments show that endogenous gastrin has very little influence, if any, on basal sphincter pressure. (131-133)

Numerous other hormones and biological agents have been shown to influence basal tone of the lower oesophageal sphincter. These agents include motilin, substance P, galanin, bombesin, histamine, prostaglandin, secretin, cholecystokinin, vasoactive intestinal polypeptide and dopamine. The physiological role of these agents in the generation of basal sphincter tone is unclear. As discussed later in this chapter, some of the agents may but may be important in influencing sphincter tone after a meal or during a migrating motor complex or influencing sphincter relaxation.

4.3.2 Control of lower oesophageal sphincter relaxation

Control of lower oesophageal sphincter relaxation is a complex process. Lower oesophageal sphincter relaxation can be induced by swallowing or oesophageal distension or may occur spontaneously, independent of swallowing such as occurs during transient lower oesophageal sphincter relaxation. Relaxation of the lower oesophageal sphincter is neurogenic in nature, although different pathways are utilised, depending on the mechanism of sphincter relaxation.

Swallow-induced relaxation of the lower oesophageal sphincter is vagally mediated, and abolished by bilateral cervical vagotomy or vagal cooling. (68, 134) Swallow-induced lower oesophageal sphincter relaxation is mediated by vagal preganglionic efferent fibres which synapse with inhibitory postganglionic neurons in the myenteric ganglia. (111) The synaptic transmission is cholinergic and combines with nicotinic and muscarinic receptors on the postganglionic neuron. In the opossum, this response is antagonised by a combination of atropine and hexamethonium. (111) Postganglionic neurons terminate in the circular muscle of the lower oesophageal sphincter and release an inhibitory neurotransmitter which is neither adrenergic, nor cholinergic and has previously been referred to as the nonadrenergic noncholinergic inhibitory neurotransmitter. (111) The nature of this neurotransmitter is discussed later in this section. Relaxation of the lower oesophageal sphincter is a combination of cessation of tonic cholinergic excitation and active inhibition of the sphincter muscle through nonadrenergic noncholinergic pathways.

Oesophageal distension can mediate relaxation of the lower oesophageal sphincter and is usually associated with secondary peristalsis. The neural pathways involved in oesophageal distension mediated lower oesophageal sphincter relaxation are different for the smooth and striated portions of the oesophagus. In the opossum, vagal cooling or vagal blockade does not affect lower oesophageal sphincter relaxation associated with distension of the smooth muscle portion of the oesophagus. (68) Therefore, local neuromuscular factors within the oesophagus appear to be sufficient to produce distension mediated lower oesophageal sphincter relaxation independently of central nervous system pathways. In contrast, in the dog, the lower oesophageal sphincter response to distension of the striated muscle oesophagus is inhibited by bilateral vagal nerve blockade, indicating a dependence on central nervous system connections in this species. (110)

Transient lower oesophageal sphincter relaxation appears to be neurally mediated (135) It is thought that gastric distension triggers transient lower oesophageal sphincter relaxations and the sensory signals then project via afferent fibres to the vagal nuclei in the brainstem. In

dogs, transient lower oesophageal sphincter relaxations are inhibited by vagal cooling (116) or vagotomy. (136) It is thought that the neural efferent pathway is the same as that involved in swallowing. The control of transient lower oesophageal sphincter relaxation is discussed in detail in chapter 8.

Electrophysiological studies in the opossum (54, 107) and cat (106) reveal that relaxation of the lower oesophageal sphincter is accompanied by hyperpolarisation of the sphincter circular muscle. However, in contrast to the circular muscle of the oesophageal body, the hyperpolarisation lasts for a much longer time (7.0 ± 0.4 seconds versus 1.5 ± 0.1 seconds, in the opossum) and is not followed by membrane depolarisation or spike potentials. (107) This prolonged hyperpolarisation may serve as a mechanism inhibiting the sphincter muscle as peristaltic contractions sweep the oesophagus.

There is convincing evidence to suggest that nitric oxide is the neurotransmitter involved in relaxation of the lower oesophageal sphincter. (17, 18, 58, 137-139) Inhibitors of nitric oxide synthesis can completely block nerve-induced relaxation of the lower oesophageal sphincter and the membrane hyperpolarisation that accompanies this relaxation. (18, 107) In addition, exogenous nitric oxide can mimic some events produced by nerve stimulation; it relaxes the lower oesophageal sphincter and hyperpolarises circular smooth muscle from the sphincter. (18, 58, 107) Nitric oxide is produced by the metabolism of l-arginine through the action of nitric oxide synthase, an enzyme present in myenteric neurons, (140) and nitric oxide synthase enzymatic activity can be measured in smooth muscle tissue from the lower oesophageal sphincter and in the nucleus tractus solitarius and nucleus ambiguus. (141, 142) Nerve-mediated relaxation of the lower oesophageal sphincter is preceded by a rise in cGMP. (143) Similarly, exogenous nitric oxide stimulates the production of cGMP in the smooth muscle of the lower oesophageal sphincter. Patients with achalasia have been reported to have absence of nitric oxide synthase in the myenteric plexus of the oesophagogastric junction. (144) The lack of this key enzyme, associated with impaired local production of nitric oxide may be responsible for the defective lower oesophageal

sphincter function in this condition. Inhibitors of nitric oxide increase the resting tone of the lower oesophageal sphincter (61) and inhibit lower oesophageal sphincter relaxation in humans (145) further supporting the hypothesis that control of the lower oesophageal sphincter is a nitric oxide-dependent process.

Vasoactive intestinal polypeptide (VIP) was until recently, believed to be the nonadrenergic, noncholinergic inhibitory neurotransmitter in the lower oesophageal sphincter. VIP immunoreactivity is present in myenteric neurons of the human lower oesophageal sphincter (146) and is released during nerve-mediated relaxation. (147) VIP causes lower oesophageal sphincter relaxation. This inhibition is not a neurally mediated response, but a direct effect on the smooth muscle cells, as the neural blocker, tetrodotoxin, does not block this effect. (148) Specific VIP antisera (19, 147, 149) as well as putative VIP antagonists (150) have been reported to inhibit the neurally mediated relaxation of the lower oesophageal sphincter both in vivo and in vitro. Furthermore, patients with achalasia, a condition characterised by failure of lower oesophageal sphincter relaxation have been reported to have a marked paucity of VIP immunoreactive neurons in the region of the lower oesophageal sphincter. (146) However, the observation that VIP appears to induce lower oesophageal sphincter relaxation via activation of adenylate cyclase whereas nerve-mediated lower oesophageal sphincter relaxation causes activation of guanylate cyclase (143) casts doubt on the role of VIP as a nonadrenergic, noncholinergic inhibitory neurotransmitter in the lower oesophageal sphincter.

Calcitonin gene-related peptide (CGRP) may be a potential inhibitory neurotransmitter in the lower oesophageal sphincter. CGRP immunoreactivity localises in neurons of the myenteric plexus and causes a dose-dependent relaxation of the lower oesophageal sphincter which was previously thought to be mediated at two levels: at the sphincteric smooth muscle and at the nonadrenergic, noncholinergic inhibitory neurons. (151) However, in a recent study in the opossum, CGRP-induced sphincter relaxation was not blocked by tetrodotoxin, arguing against the hypothesis that CGRP causes lower oesophageal sphincter relaxation by

activating myenteric neurons supplying the sphincter. (152) Therefore, CGRP may be an inhibitory modulator of lower oesophageal sphincter tone, rather than a nonadrenergic, noncholinergic inhibitory neurotransmitter although further studies are required.

Other biological agents are capable of relaxing the lower oesophageal sphincter, such as cholecystokinin, somatostatin, dopamine, prostaglandins and ingestion of certain food elements. Some of these agents will be discussed in the following section.

4.4 Other factors influencing sphincter pressure

4.4.1 Migrating Motor Complex

Basal lower oesophageal sphincter pressure shows considerable minute to minute variability. Some of this variability may be related to the cycle of the gastric interdigestive migrating motor complex (MMC). During phase 1 of the MMC, lower oesophageal sphincter pressures are lowest, during late phase 2, sphincter pressure has a "roller coaster" pattern, and during phase 3, sphincter pressures are maximal. (153) This variability is also seen in the opossum, is abolished by anaesthesia, feeding and atropine (154) and is mediated by motilin. (155)

4.4.2 Meals

Basal lower oesophageal sphincter pressure is reduced after a meal. (95, 156) A fatty meal, which is a potent stimulus for cholecystokinin (CCK) release, has been shown to reduce lower oesophageal sphincter pressure. (157, 158) Whether this postprandial reduction in lower oesophageal sphincter pressure is mediated by CCK is controversial. A reduction in sphincter pressure is only seen after high doses of exogenous CCK (159, 160) and after a fatty meal, postprandial changes in lower oesophageal sphincter pressure do not correlate with plasma CCK levels. (161) Following a fatty meal, the CCK-A receptor antagonist,

loxiglumide attenuates the fall in basal lower oesophageal sphincter pressure in obese subjects (162) and in patients with gastro-oesophageal reflux disease. (163) However, in normal healthy subjects, loxiglumide significantly reduces the fall in lower oesophageal sphincter pressure after an intraduodenal meal but has no effect after oral ingestion of a meal. Therefore, other factors, such as neural mechanisms may be involved in the reduction in basal lower oesophageal sphincter pressure after a fatty meal.

4.4.3 Sleep

During sleep, there is an increase in basal lower oesophageal sphincter pressure. This effect is seen in normal healthy volunteers and in patients with gastro-oesophageal reflux disease. (95, 164) Transient lower oesophageal sphincter relaxations do not occur during stable sleep (95) but may be seen during short arousal periods. This will be discussed in detail in chapter 8 of this thesis.

4.4.4 Exercise

Exercise is associated with a reduction in basal lower oesophageal sphincter pressure. (156) However, the magnitude and type of physical exercise has no effect on lower oesophageal sphincter pressure.

Chapter 5

Anatomy and innervation of the stomach

5.1 Gross Anatomy

The stomach is a sac-like, roughly J-shaped, hollow organ located in the upper abdomen. Superiorly, the stomach is connected to the oesophagus and inferiorly, is joined to the duodenum. The stomach is fixed in position only at the oesophagogastric junction and to the duodenum and is quite mobile between these two attachments. Anteriorly, its relations are the anterior abdominal wall, the left costal margin, the left lung, the left lobe of the liver and the diaphragm. Posteriorly, its relation are the spleen, the left adrenal gland, the upper part of the left kidney, the pancreas, the transverse colon and the diaphragm. The location of the stomach, as well as its size and shape can vary substantially with age, body habitus and degree of distension. (165)

The lesser curve of the stomach forms the right margin of the stomach and extends from the cardia to the pylorus. The lesser omentum extends from the lesser curve to the liver. The greater curve of the stomach is much longer than the lesser curve and extends from the left of the cardia, over the dome-shaped fundus and then sweeps around to the inferior part of the pylorus. The greater omentum extends from the lower part of the greater curve to the transverse colon. In the lower part of the lesser curve is a sharp, constant notch-like indentation called the incisura angularis or angulus.

The stomach is divided into various anatomical regions. The cardia lies immediately adjacent to the oesophagogastric junction. The fundus is dome shaped and lies above a horizontal line through the cardia. The gastric body, or corpus, extends from the cardia and fundus to the level of the angulus. The antrum lies between the angulus and the pylorus. The pylorus is the most distal and tubular part of the stomach and has a thick muscular wall which forms the pyloric sphincter. The pylorus terminates in a septum of connective tissue which marks the gastroduodenal junction.

Rather than dividing the stomach into anatomical regions, it may be functionally more relevant to divide the stomach into two distinct regions. (166) The proximal stomach, consisting of the cardia, fundus and proximal corpus largely serves as a reservoir. The distal stomach consists of the distal corpus and antrum and plays an important role in grinding of food and delivering chyme to the small intestine. The function of these two distinct regions will be further discussed in chapter 6 of this thesis.

The muscle coat of the gastric wall is composed of three layers. (167) The outer longitudinal layer is most prominent along the greater and lesser curves. The middle circular layer is more uniformly distributed throughout the stomach and is thickened at the pylorus to form the pyloric sphincter. The muscle fibres of the innermost oblique layer loop over the fundus and pass down along the anterior and posterior walls, running parallel with the lesser curve. Muscle bundles from the greater curvature side of the lower oesophageal sphincter form long oblique loops to the stomach, thereby forming close connections between the stomach and lower oesophageal sphincter. (75)

The arterial supply of the stomach arises from branches of the coeliac artery, which arises from the aorta immediately inferior to the aortic hiatus of the diaphragm at the level of L1. Venous drainage of the stomach is into the portal circulation. The lymphatic drainage of the stomach follows the arteries and ultimately drains into the coeliac lymph nodes.

5.2 Microscopic Anatomy

The gastric mucosa has a glandular structure and is lined by simple columnar epithelium interspersed by numerous pits. Epithelial cells are responsible for the production of mucus, acid and pepsin. (168) Gastric glands lie in the lamina propria and open into the bottom of these pits. The lamina propria also consists of loose connective tissue interspersed with smooth muscle and lymphoid cells. The muscularis mucosa is a thin band of smooth muscle separating the lamina propria from the underlying submucosa.

The submucosa contains dense connective tissue, blood vessels and nerves. Beneath the submucosa are bundles of smooth muscle cells, which comprise the three layers of the muscle coat of the gastric wall.

The serosa is a thin layer which lies external to the muscle layer. It consists of loose connective tissue, rich in blood and lymph vessels and is covered by a mesothelium.

5.3 Innervation of the stomach

The stomach is richly innervated by extrinsic and intrinsic nerves. The extrinsic innervation is provided by vagal (parasympathetic) and spinal (sympathetic) nerves, and intrinsic innervation is provided by neurons of the myenteric and submucous plexus and is an important component of the enteric nervous system.

5.3.1 Extrinsic innervation

5.3.1.1 Vagal (parasympathetic) innervation

Vagal afferent pathways

Approximately 95% of abdominal vagal fibres are afferent. (169) Cell bodies of the vagus nerve are found in the nodose ganglia. Vagal afferent neurons project to nucleus of the solitary tract and the area postrema. (170, 171) Neurons in the solitary tract and area postrema project to the dorsal motor nucleus of the vagus and intermediolateral column of the spinal cord, forming the basis for vago-vagal and vago-spinal reflexes involved in the control of gastric motility. Connections from the nucleus of the solitary tract also project to higher centres. (172) The vagal afferent fibres are activated by mechanoreceptors and chemoreceptors. Mechanoreceptors respond to gastric wall tension, generated by both passive distension and active contraction. (173-175)

Vagal motor pathways

Cell bodies of the vagal preganglionic neurons are located in the medulla within the dorsal motor nucleus of the vagus. (171) In addition, a few cell bodies arise from the nucleus of the solitary tract and nucleus ambiguus. (176) Preganglionic vagal efferent fibres synapse with neurons of the gastric myenteric plexus.

5.3.1.2 Spinal pathways

Spinal visceral afferent pathways

Spinal sensory neurons have their cell bodies in spinal dorsal root ganglia between T7 and T11 and are activated by mechanoreceptors located in the gastric wall. Spinal afferents mediate nociceptive information to the central nervous system. In addition, spinal afferents interact with vagal afferents in the reflex control of gastric function.

Spinal (sympathetic) efferent innervation

The sympathetic nerve supply to the stomach is derived from spinal cord segments T6 to T9 which contain the cell bodies of the preganglionic neurons. Fibres then pass alongside the splanchnic nerves to reach the coeliac ganglion. Postganglionic fibres arising from the coeliac ganglia then pass to the stomach supplying intramural ganglia and blood vessels with minimal innervation of muscle. (4)

5.3.2 Intrinsic innervation

The intrinsic nerve supply of the stomach is provided mainly by neurons located in the ganglia of the myenteric plexus. The myenteric plexus lies between the longitudinal and circular muscle layers and the ganglia are more densely distributed in the antrum. The neurons receive sensory fibres from receptors in the muscle and mucosa and the axons synapse with secretory and smooth muscle cells.

There are two types of vagal efferent fibres: the large diameter, low-threshold excitatory fibres and the smaller diameter, high-threshold inhibitory fibres. (4) The excitatory fibres are cholinergic and are blocked by atropine. The inhibitory fibres are not blocked by atropine or adrenergic blockers and are designated nonadrenergic noncholinergic nerves. The neurotransmitter released by inhibitory fibres is most likely to be nitric oxide (177, 178) although other candidates including purine nucleotides and peptide hormones such as vasoactive intestinal polypeptide have been implicated. (4, 19) Whether the three muscular layers of the gastric wall differ in their innervation remains to be determined.

A population of cells that are neither nerve or muscle, the interstitial cells of Cajal, are distributed within the intrinsic nerve network of the myenteric plexus. They may play a role in mediating nerve to muscle communication and are believed to possess pacemaking activity. (23, 179)

Chapter 6

Normal function of the stomach

6.1 Motor patterns

As Cannon pointed out years ago, (180) the stomach can be separated into two distinct motor regions, each with a unique physiological function. Cannon gave mixtures of bismuth and food to cats and observed their gastric movements fluoroscopically. He identified the proximal stomach as the gastric reservoir and showed that peristaltic contractions occurred in the distal stomach. More recent experimental evidence has expanded Cannon's original concept, and proposed the two-component model of gastric emptying. (166) The proximal stomach is important in accommodation of a meal and gastric emptying of liquids. The distal stomach has a role in the grinding of solid particles and controlling the emptying of food, but is also important in the gastric emptying of liquids. (181)

During fasting, the stomach exhibits a distinct cyclical motor pattern, the migrating motor complex, which migrates from the stomach down the entire small intestine. The migrating motor complex occurs every 90-120 minutes and consists of three phases. (182) Phase 1, the longest part of the cycle, is a period of motor quiescence. Phase 2 is characterised by irregular muscle contractions. Phase 3 consists of forceful rhythmic contractions at a frequency of three per minute. During phase 3, large indigestible residue may leave the stomach. The migrating motor complex is important for the emptying of large indigestible solids from the stomach and therefore prevent the formation of bezoars.

Immediately after ingestion of food, the "fasting" pattern is replaced by a "fed" pattern, which consists of irregular but persistent phasic contractile activity. This non-cyclical continuous pattern of activity persists as long as food remains in the stomach. It is not clear whether the maintenance of a "fed" pattern is directly due to the presence of nutrients in the stomach or indirectly through maintenance of gastric distension. When the stomach is nearly empty and intestinal chyme is absorbed, the cyclical pattern returns.

The conversion to a "fed" pattern and duration of this postprandial pattern is dependent on the physical state and nutrient content of the meal. Water does not significantly disturb fasting activity. However, fats are potent in their ability to maintain a "fed" pattern. The vagus also plays a role in conversion to the postprandial pattern as vagal cooling changes the usual "fed" pattern in dogs to a phase 3 like activity. (183)

Mechanical stimulation of the pharynx or distension of the oesophagus induces a short-lived relaxation of the proximal stomach. (184, 185) This reflex is called receptive relaxation and was first described by Cannon and Lieb to describe the relaxation of the proximal stomach during sham feeding. (186) Receptive relaxation is dependent on an intact vagus nerve. (187)

Gastric distension induces a prolonged relaxation of the proximal stomach and is called gastric accommodation. During feeding, the proximal stomach expands its volume without changing intragastric pressure. Most of this expansion is seen along the greater curve of the proximal stomach. (188, 189) The gastric accommodation reflex maintains intragastric pressure within a narrow range, even though intragastric volume may fluctuate widely. (190)

The proximal stomach plays a major role in gastric emptying of liquids. (166) In dogs, fundectomy or proximal gastric vagotomy causes larger increases in pressure during gastric distension leading to more rapid gastric emptying of liquids. (191, 192) In humans, increased intragastric pressure using a barostat is associated with an acceleration of gastric emptying of liquids. (193, 194) These findings have been used as evidence to support the hypothesis that the primary control of gastric emptying of liquids lies in the proximal stomach. Furthermore, the proximal stomach probably regulates transfer of solid food to the distal stomach, thus participating in the control of solid emptying. (181)

The main function of the distal stomach is regulation of gastric emptying of solids. Food is transferred from the proximal stomach to the antrum. The antrum plays a role in grinding and

mixing of solids. This is achieved by coordinated activity of the distal stomach and pylorus. Distal gastric peristaltic contractions propel solid food towards the pylorus. However, because of the large size of the food particles and rapid closure of the pylorus, the solids are trapped in the terminal antrum. The advancing terminal antral contraction wave, generating pressures of more than 100 mmHg, grinds together the food particles, triturating them. (182) The solid particles are not able to pass through the closed pylorus and are then retropelled. This sequence of propulsion, grinding and retropulsion gradually breaks down the solid particles into a size that is allowed to pass through the pylorus. (166)

In dogs, resection or denervation of the antrum impairs antral trituration and alters the rate of gastric emptying of solids. (195, 196) These findings illustrate the importance of the antrum in the regulation of gastric emptying of solids.

In addition to its major role in the gastric emptying of solids, the distal stomach may also have a role in the emptying of liquids. (197) A positive correlation between antral motor activity and rate of gastric emptying of a liquid meal has been observed. (198-200) In pigs, surgical resection and re-anastomosis of the antrum has been shown to impair gastric emptying of liquid, by modulating the function of the pylorus. (201)

6.2 Control

In the stomach, there is a hierarchy of control mechanisms that act on the gastric smooth muscle. Myogenic control is not sufficient for physiological functioning of the stomach and requires modulatory control by the enteric nervous system. Both the myogenic components and the enteric nervous system are modulated by two extrinsic control systems: extrinsic innervation, comprising vagal and spinal pathways, and circulating hormones.

6.2.1 Intrinsic control

The smooth muscle cells in the wall of the proximal stomach have unique electrophysiological characteristics. Pacesetter or action potentials are not observed. (202) Instead, cells from the proximal stomach have low resting negative membrane potentials which are steady and show little fluctuation. (182) Resting tone of the proximal stomach is determined by small and sustained changes in membrane potential. This region of the stomach exhibits predominantly tonic muscular activity and not peristalsis.

Smooth muscle cells in the wall of the distal stomach exhibit periodic episodes of spontaneous depolarisation called pacesetter potentials. (182) Each pacesetter potential consists of an initial rapid depolarisation followed by a rapid partial repolarisation. The subsequent time course varies with the site of origin of the cell. In the body of the stomach and proximal antrum, a sustained monophasic plateau action potential occurs, causing a muscle contraction. In the distal antrum, muscle contraction is determined by spike wave action potentials. Therefore, gastric contraction is a motor manifestation of the pacesetter potential. The greater the amplitude and duration of the action potential, the greater the amplitude and duration of contraction.

The frequency of pacesetter potential varies with position along the stomach. The frequency is highest in an area in the mid-body, along the greater curve and this zone is considered to be the gastric pacemaker. It is thought that the cells which are involved in the generation and propagation of pacesetter potentials are the interstitial cells of Cajal. (23, 179) Pacemaker potentials initiated at the site of the gastric pacemaker propagate aborally along the longitudinal axis of the circular muscle towards the duodenum. Conduction velocity of the pacesetter potential is faster along the greater than along the lesser curve, so that the propagated wave reaches the pylorus at each curve simultaneously. (202) The frequency of muscle contraction is limited by the frequency of pacesetter potentials which in the human antrum is three per minute.

There are many factors which determine the intensity and duration of pacesetter potentials and whether they trigger an action potential and muscle contraction. In vitro muscle stretch increases conduction velocity of pacesetter potentials. (203) In vivo, this effect may be important in the postprandial period to enhance propulsion. Acetylcholine, gastrin and cholecystokinin (CCK) increase the duration and amplitude of the plateau potential and contraction. Noradrenaline and neurotensin produce opposite effects. (202)

6.2.2 Extrinsic control

Vagal stimulation produces both excitatory and inhibitory effects on proximal gastric tone. (204) The excitatory effects, predominantly an increase in proximal gastric tone, are blocked by atropine, suggesting that they are muscarinic in nature. (205) The inhibitory effects, corresponding to relaxation of the wall of the proximal stomach, are blocked by nitric oxide synthase inhibitors, suggesting that nitric oxide may play an important role. (177, 206, 207)

In the dog, reversible vagal blockade by cooling, produces a reversible reduction in gastric tone. (208) The excitatory vagal input is mediated by a cholinergic muscarinic mechanism because pharmacological blockade by atropine mimics the effect of vagal blockade by cooling, and bethanechol, a cholinergic agonist increases gastric tone regardless of vagal blockade. Therefore, under fasting basal conditions, proximal gastric tone is maintained by a dominant vagal cholinergic excitatory input.

In the ferret, vagal blockade by vagotomy produces a reduction in gastric tone. (209) Following pretreatment with atropine, acute vagotomy in the ferret increases vagal tone. This effect is reversed by stimulating the vagus nerve below the point of sectioning, suggesting a tonic vagal inhibitory influence, in addition to the excitatory input.

In humans, cholinergic blockade with atropine has not demonstrated any effect on fasting or postprandial proximal gastric tone. (210, 211) However, relatively low doses of atropine were used in these studies. Based on the findings described above, it is possible that proximal gastric relaxation could be mediated by a decrease in the excitatory vagal input, or an increase in the inhibitory vagal input, or a combination of both.

The distal stomach is also influenced by excitatory as well as inhibitory vagal pathways. Vagotomy causes delayed emptying of solids, attributed to a decreased force of antral peristalsis. (191) Therefore, antral motor activity is maintained by a vagal excitatory input. Vagal stimulation increases the force of antral contractions.

Inhibitory vagal pathways to the distal stomach are nonadrenergic, noncholinergic in nature. Nitric oxide inhibitors increase (212) and nitric oxide donors decrease (213) the force of antral contractions, suggesting that nitric oxide may be the nonadrenergic noncholinergic inhibitory mediator in the distal stomach.

Sympathetic neural input appears to inhibit gastric motility. Electrical stimulation of feline spinal sympathetic efferent fibres produces an inhibitory effect on proximal gastric tone. (214) Noradrenaline released from the spinal sympathetic nerves inhibits the release of acetylcholine from cholinergic nerves, thereby inhibiting gastric smooth muscle activity. (188) If the vagus is intact, acute sympathectomy alone has no significant effect on the pressure response to volume-controlled gastric distension. (215) However, following vagotomy, sympathectomy reduces gastric compliance. (215) Furthermore, splanchnic nerve stimulation relaxes the proximal stomach by a mechanism independent of cholinergic tone. (216) These experiments suggest that the vagus may influence the expression of splanchnic nerve activity, although the site and mechanism of this interaction is unknown.

Stimulation of spinal sympathetic efferent fibres produces an adrenergic inhibitory effect on antral motor activity. The site of inhibition is either in the myenteric plexus, where most

spinal sympathetic efferents end, or at muscle coats, where some sympathetic fibres ramify. Noradrenaline reduces the force and duration of antral contractions.

There are a number of circulating hormones and other biological substances that affect gastric motor activity. Cholecystokinin (CCK) reduces proximal gastric tone at supra-physiological doses but has no effect at doses that produce plasma levels within the physiological range. (217) After a fatty meal, changes in postprandial proximal gastric tone do not correlate with plasma CCK levels. (161) The CCK-A antagonist, loxiglumide has been reported to have no effect on postprandial gastric relaxation, (218, 219) although one study reported a reduction in the postprandial gastric relaxation after duodenal infusion of a liquid meal. (220) Therefore, based on these observations, CCK does not seem to play a significant physiological role in the hormonal control of proximal gastric function.

Motilin may modify the control of the migrating motor complex. There is a peak of serum concentration of motilin at the time of phase 3 of the cycle. If motilin levels are reduced, the phase 3 contractions become less intense. (221, 222) In dogs, intravenous doses of gastrin, CCK, vasoactive intestinal polypeptide (VIP), somatostatin, glucagon and dopamine all reduce intragastric pressure as measured by a distending gastric balloon. (223-225) Gastrin and CCK increase the amplitude and duration of the plateau potential and the force and duration of rhythmic contractions, whereas noradrenaline and VIP have opposite effects. (226-228) These observation suggest that hormones play a role in gastric motility. However, it is difficult to determine the precise role of individual endogenous hormones in the gastric regulation because there are so many hormones acting together and simultaneously with a number of reflexes to control gastric motility. It seems likely that hormones operate in collaboration with extrinsic nerves, to control the enteric nervous system and gastric smooth muscle cells.

Chapter 7

The antireflux barrier and mechanisms of gastro-oesophageal reflux

7.1 The antireflux barrier

7.1.1 Introduction

Before discussing the mechanisms of gastro-oesophageal reflux, it is important to first understand the mechanisms that normally prevent reflux of gastric contents into the oesophagus. In humans, there is a positive pressure gradient between the abdominal and thoracic cavity. This pressure gradient increases during events associated with abdominal straining, such as exercise, coughing and sneezing, favouring movement of gastric contents into the oesophagus. Under normal physiological circumstances, gastro-oesophageal reflux is prevented by an antireflux barrier located at the oesophagogastric junction. The functional integrity of the antireflux barrier has been attributed to intrinsic lower oesophageal sphincter pressure, the intra-abdominal segment of the lower oesophageal sphincter, extrinsic compression of the lower oesophageal sphincter by the crural diaphragm, integrity of the phreno-oesophageal ligament, and maintenance of an acute angle at the junction of the oesophagus and stomach (angle of His).

7.1.2 Role of the lower oesophageal sphincter in the antireflux barrier

The lower oesophageal sphincter is the major antireflux barrier at the oesophagogastric junction. The intrinsic tone of the lower oesophageal sphincter and the resultant high pressure zone provides an important barrier for the prevention of reflux from the intra-abdominal stomach into the lower pressure intrathoracic oesophagus.

A basal lower oesophageal sphincter pressure of 5 to 10 mmHg above intragastric pressure is generally adequate to prevent gastro-oesophageal reflux even during episodes of increased intra-abdominal pressure (164) suggesting that basal lower oesophageal sphincter pressure in the range of 15 to 25 mmHg is a more than adequate reserve needed to prevent reflux. Studies using concurrent oesophageal manometry and pH monitoring in normal healthy

volunteers and in patients with gastro-oesophageal reflux disease, have shown that lower oesophageal sphincter pressure must fall to virtually zero, compared to intragastric pressure, for acid reflux to occur, although transient increases in intra-abdominal pressure, may intermittently overcome a hypotensive sphincter. (95, 164)

The surgical literature suggests that the length of the intra-abdominal portion of the lower oesophageal sphincter is another important barrier to reflux. (229-231) The abdominal segment may play a role in preventing reflux during periods associated with increased intra-abdominal pressure. Zaninotto et al suggested that maintenance of a competent antireflux barrier requires an adequate sphincter pressure as well as a minimal length of sphincter exposed to the positive pressure of the abdomen. (229) O'Sullivan et al demonstrated that after Hill, Belsey and Nissen antireflux procedures, sphincter pressure and intra-abdominal length are increased. (230) However, the importance of the intra-abdominal location of the lower oesophageal sphincter as an independent factor in the maintenance of an antireflux barrier, has not been adequately studied.

During respiration and activities that increase intra-abdominal pressure, such as abdominal compression and straight-leg raising, there is an increase in lower oesophageal sphincter pressure. (88, 232) This increase in pressure is associated with an increase in crural diaphragm activity, and is discussed in detail in the next section.

7.1.3 Crural diaphragm

7.1.3.1 Anatomy

The diaphragm is composed of two embryologically distinct components. The crural diaphragm originates from the oesophageal dorsal mesentery and the costal diaphragm from the pleuroperitoneal membrane. (233) These two components are regarded as functionally separate muscles, with the crural diaphragm having a role relating to the oesophagus. The

crural diaphragm arises from the first three or four lumbar vertebrae on the right, or the first two or three lumbar vertebrae on the left, as well as from the intervertebral discs and the anterior longitudinal ligament. (1) The crural fibres pass upwards and forwards and insert into the transverse ligament of the central tendon of the diaphragm. The crural diaphragm forms the diaphragmatic hiatus which is a canal through which the oesophagus passes to reach the abdomen. This canal is about 2.5 cm in length and its major component is the right crus of the diaphragm. (234) Anatomically, the fibres of the crural diaphragm are orientated in such a way as to be able to "pinch" the lower oesophageal sphincter during diaphragmatic contraction, thereby forming part of the antireflux barrier.

7.1.3.2 Innervation

The crural diaphragm is innervated by the phrenic nerves. Although the diaphragmatic hiatus is primarily composed of muscle fibres from the right crus, it is innervated by both the right and left phrenic nerves. A concentration of inspiratory neurons in the ventrolateral portion of the nucleus of the tractus solitarius, called the dorsal respiratory group, serve as the principal rhythmic respiratory drive to phrenic motorneurons. (235)

For respiration, the two parts of the diaphragm act as a unit and contract together during inspiration and relax during expiration. There is experimental evidence that the costal and crural diaphragm are innervated independently. During inspiration, initiation of contraction of the crural diaphragm actually occurs a fraction of a second earlier than the costal diaphragm. (236) Electromyographic studies in cats (90) and dogs (237) have shown that during distension of the lower oesophagus, there is inhibition of crural diaphragm electrical activity throughout the distension period whilst the outer hiatal fibres and dome fibres are generally unaffected. During vomiting and eructation, the inner hiatal fibres remain electrically inactive, while the outer hiatal fibres and dome fibres show a strong burst of spike potentials.

Transient lower oesophageal sphincter relaxation, the principal mechanism of gastro-oesophageal reflux, is associated with inhibition of crural diaphragm electrical activity. (91, 238) This will be discussed in detail in chapter 8 of this thesis.

The relationship between swallowing and inhibition of the crural diaphragm is variable. In anaesthetised cats, pharyngeal stimulation induces swallows that initiates oesophageal peristalsis, causes simultaneous relaxation of the lower oesophageal sphincter and inhibition of crural electrical activity. (90) In dogs, swallowing produces only partial inhibition of crural diaphragm electrical activity. (238) In humans, swallow-induced lower oesophageal sphincter relaxation does not appear to be associated with crural diaphragm inhibition. (91) The differences between these studies may be attributed to species and methodological differences.

While the central control of the respiratory function of the diaphragm has been extensively investigated, little is known about the role of the central neurons that modulate the gastrointestinal function of the diaphragm. The nucleus of the tractus solitarius contains not only the dorsal respiratory group of neurons responsible for rhythmic respiration, but also receives afferent input from the vagus and glossopharyngeal nerves. By recording the activity of medullary respiratory neurons in decerebrate, spontaneously breathing cats during oesophageal distension and swallowing, Altschuler et al found that activity of inspiratory neurons were unaltered during mechanical distension of the oesophagus, and concluded that additional, as yet unidentified central pathways must exist for the control of the gastrointestinal function of the crural diaphragm. (239)

7.1.3.3 Role of the crural diaphragm in the antireflux barrier

Both the lower oesophageal sphincter and crural diaphragm contribute to the intraluminal pressure at the oesophagogastric junction. Klein et al showed that in patients who have previously had an oesophagogastrectomy, the crural diaphragm alone can maintain a high

pressure zone at the abdominothoracic junction. (240) Respiration-induced changes in oesophagogastric junction pressure have been attributed to contractions of the crural diaphragm during inspiration. This was first demonstrated by Boyle et al by anchoring the manometric catheter to the feline lower oesophageal sphincter, to prevent movement artefact between the catheter and lower oesophageal sphincter during respiration. (89) In this study, the amplitude of the pressure oscillation was directly proportional to the depth of inspiration and these oscillations were abolished by paralysis of the skeletal muscle. In a more recent study in humans, using crural diaphragm electromyographic recordings, Mittal et al showed that there is an increase in lower oesophageal sphincter pressure with inspiration and this increase in pressure is associated with an increase in crural diaphragm activity. (88) The crural diaphragm is also able to maintain pressure at the oesophagogastric junction during activities other than respiration, such as abdominal compression, coughing, straight-leg raising and any physical activity that increases intra-abdominal pressure. (232) In a study to determine the importance of the crural diaphragm in the prevention of gastro-oesophageal reflux, Mittal et al demonstrated that in cats, crural myotomy increases the frequency of acid reflux. (241)

These observations outlined above have advanced the "two-sphincter hypothesis" of oesophagogastric junction competence, suggesting that both the lower oesophageal sphincter and the crural diaphragm serve a sphincteric function. During expiration, the lower oesophageal sphincter pressure is considered to be adequate to maintain oesophagogastric junction competence and prevent gastro-oesophageal reflux. However, during deep inspiration, the pressure difference between the stomach and oesophagus increases, in favour of gastro-oesophageal reflux. (242) This tendency towards reflux is counteracted by an increase in oesophagogastric junction pressure caused by contraction of the crural diaphragm. Crural diaphragm function is even more important if the basal lower oesophageal sphincter pressure is low or absent.

As discussed in section 7.2.3, a hiatus hernia compromises the sphincteric function of the crural diaphragm, impairing oesophagogastric junction competence and thereby exacerbating gastro-oesophageal reflux.

As discussed further in chapter 8 of this thesis, during a transient lower oesophageal sphincter relaxation, there is simultaneous relaxation of the lower oesophageal sphincter and crural diaphragm. (91, 238) In normal subjects, if lower oesophageal sphincter pressure is abolished by atropine, reflux only occurs during periods of transient inhibition of the crural diaphragm, indicating that absent lower oesophageal sphincter pressure does not induce reflux if crural diaphragm contraction is preserved. (243)

7.1.4 Other anatomical factors

7.1.4.1 Phreno-oesophageal ligament

The phreno-oesophageal ligament, the third component of the antireflux barrier, is a continuation of the transversalis fascia from the undersurface of the diaphragm. The major component of this ligament extends cranially, and attaches to the muscular layers of the oesophagus, above the squamocolumnar junction. The other, less defined portion of this ligament descends caudally to insert into the lower portion of the abdominal oesophagus and the cardia of the stomach. (74) The phreno-oesophageal ligament forms a sheath around the oesophagus and acts as an anchor to fix the lower oesophagus to the diaphragm. As the anterior and lateral parts of the phreno-oesophageal ligament are thin and mobile, the posterior part represents the prime attachment of the oesophagogastric junction.

7.1.4.2 Angle of His

The oblique angle at which the oesophagus enters the stomach, the cardio-oesophageal angle of His creates an anatomical valve mechanism, thought to be important in maintaining the

antireflux barrier. The valve created by the angle of His has been the subject of much research and controversy. In the 1950's and 1960's, it was postulated that an acute angle of His prevented reflux and an obtuse angle predisposed to reflux. (1) More recently, a retrospective review reported that 3.3% of patients have some irregularity in the angle of His, apparently explaining their reflux. (244)

7.1.4.3 Flap valve

The sling muscle fibres of the gastric cardia may also contribute to the antireflux barrier. The orientation of these muscle fibres results in a "flap valve" mechanism, whereby pressure in the fundus creates a flap of tissue that closes against the distal oesophagus as demonstrated in cadavers. (245)

7.2 Mechanisms of reflux

7.2.1 Introduction

The lower oesophageal sphincter and crural diaphragm form the antireflux barrier which prevents the reflux of stomach contents into the oesophagus. Gastro-oesophageal reflux arises because of defective lower oesophageal sphincter function. However, other factors, such as the presence of a hiatus hernia and delayed gastric emptying may contribute.

The understanding of the pathogenesis of gastro-oesophageal reflux has undergone several changes over the years. In the 1940's and 1950's, the most commonly held theory was that reflux was attributed to the presence of a sliding hiatus hernia. In the 1970's, the importance of hiatus hernia in reflux was disputed, as most patients with hiatus hernia had no evidence of gastro-oesophageal reflux disease, and a proportion of patients had gastro-oesophageal reflux disease in the absence of a hiatus hernia. Reflux was thought to occur as a result of a chronically weak lower oesophageal sphincter. (246) However, this theory failed to explain

why some patients with decreased basal lower oesophageal sphincter pressure maintain an adequate antireflux barrier and why a proportion of patients with gastro-oesophageal reflux disease have normal basal lower oesophageal sphincter pressure. More recent studies, using concurrent pH and oesophageal motility measurements to analyse individual reflux events, have shown that under resting conditions, lower oesophageal sphincter pressure is absent during reflux episodes. However, absent lower oesophageal sphincter pressure during most reflux episodes is not due to a chronically weak or absent lower oesophageal sphincter pressure but due to transient lower oesophageal sphincter relaxation. (95) In normal healthy volunteers reflux occurs almost exclusively during transient lower oesophageal sphincter relaxation. (95) In patients with gastro-oesophageal reflux disease, most reflux episodes also occur during transient lower oesophageal sphincter relaxations, although low basal lower oesophageal sphincter pressure is the predominant mechanism in some patients. (164, 247-249)

7.2.2 Lower oesophageal sphincter

7.2.2.1 Defective basal lower oesophageal sphincter pressure

At rest, reflux only occurs if the pressure of the lower oesophageal sphincter falls below 2 mmHg. (95, 164, 248) In a study investigating reflux mechanisms in ambulant patients with gastro-oesophageal reflux disease, Penagini et al found that in 4 of 11 patients (36%) persistently absent basal lower oesophageal sphincter pressure was the most prevalent mechanism of reflux. (249) With increasing severity of oesophagitis, absent basal lower oesophageal sphincter pressure becomes an increasingly more prevalent mechanism of reflux. (248)

In humans, lower oesophageal sphincter pressure appears to be dependent on tonic cholinergic vagal input as atropine reduces lower oesophageal sphincter pressure. (59, 118, 119, 243) Some patients with gastro-oesophageal reflux disease have vagal neuropathy as

evidenced by impaired gastric secretory response to insulin-induced hypoglycaemia and abnormal cardiovascular reflexes. (250-252) Although unproven, these indirect observations suggest that impaired cholinergic vagal input to the lower oesophageal sphincter may be responsible for the defective sphincter pressure present in some patients with reflux disease.

There is evidence that sphincter muscle dysfunction is a cause of defective basal sphincter pressure in some patients with reflux disease. Patients with smooth muscle myopathies such as scleroderma or mixed connective tissue disease develop reflux oesophagitis as a result of impairment of basal lower oesophageal sphincter pressure and have atrophy of the smooth muscle of the lower oesophageal sphincter and replacement by fibrous tissue. (76) In patients with gastro-oesophageal reflux disease, lower oesophageal sphincter responses to direct muscle stimulants are abnormally low, suggesting a decreased contractile function of the smooth muscle of the lower oesophageal sphincter. (253)

It is unclear as to whether reflux oesophagitis causes, or is a result of hypotonia of the lower oesophageal sphincter. Acid induced injury to the feline oesophagus reduces basal lower oesophageal sphincter pressure which then returns to normal with healing of the oesophagitis. (254) However, these findings have not been reproduced in humans. In patients with apparently successfully treated reflux oesophagitis as evidenced by healing of all macroscopic mucosal breaks, lower oesophageal sphincter pressure fails to improve after healing, suggesting that hypotonia of the lower oesophageal sphincter is irreversible. (255-257) Furthermore, after cessation of antireflux therapy, there is rapid relapse of oesophagitis. (258) Whether oesophagitis contributes significantly to hypotonia of the lower oesophageal sphincter is unproven although the possibility exists that oesophagitis irreversibly causes hypotonia of the lower oesophageal sphincter which does not improve with healing.

7.2.1.2 Lower oesophageal sphincter relaxation

Lower oesophageal sphincter relaxation is an important mechanism of reflux in normal subjects and in patients with gastro-oesophageal reflux disease. There are two distinct patterns of lower oesophageal sphincter relaxation; swallow-induced and those independent of swallowing, referred to as transient lower oesophageal sphincter relaxation.

Swallow-induced lower oesophageal sphincter relaxation

The lower oesophageal sphincter relaxes in response to swallowing. Despite swallowing being a frequent event, swallow-induced lower oesophageal sphincter relaxation uncommonly causes reflux and accounts for only 5 to 10% of reflux episodes. (95, 164, 247, 248) One of the reasons why reflux does not occur commonly during swallow-induced lower oesophageal sphincter relaxation is that the duration of relaxation is brief, usually lasting for less than 5 seconds. Secondly, even if reflux occurs, the refluxate is limited to the distal oesophagus by the oncoming peristaltic wave which sweeps down the oesophagus quickly clearing the refluxate back into the stomach. Swallow-induced reflux is more common in patients with hiatus hernia, as discussed later in this chapter.

Most reflux episodes attributed to swallow-induced lower oesophageal sphincter relaxation, are associated with impaired oesophageal peristalsis (95, 248) whereby the oesophageal peristaltic wave is absent or fails to traverse the entire oesophageal body. The lower oesophageal sphincter relaxation is of longer duration than those associated with normal peristalsis and subsequent clearance of the acid refluxate is impaired.

Transient lower oesophageal sphincter relaxation

Transient lower oesophageal sphincter relaxations are sphincter relaxations not triggered by swallowing. They are the single most common mechanism of gastro-oesophageal reflux in

normal healthy subjects, accounting for 70 to 100% of reflux episodes. (95, 156, 164, 247, 248, 259) In patients with gastro-oesophageal reflux disease, most reflux episodes also occur during transient lower oesophageal sphincter relaxations, (247-249, 259) although low basal lower oesophageal sphincter pressure is an increasingly predominant mechanism as the severity of oesophagitis increases. (248, 260) In a study investigating reflux mechanisms in ambulant patients with gastro-oesophageal reflux disease, Penagini et al found that in 7 of 11 patients (64%) transient lower oesophageal sphincter relaxation was the most prevalent mechanism of reflux. (249)

Not all transient lower oesophageal sphincter relaxations are accompanied by reflux. In normal healthy volunteers, 40 to 50% of transient lower oesophageal sphincter relaxations are accompanied by acid reflux whereas in patients with gastro-oesophageal reflux disease, the proportion rises to 60 to 70%. (156, 164, 247, 249, 261) The factors that determine the likelihood of reflux during a transient lower oesophageal sphincter relaxation are incompletely understood. Maximal sphincter relaxation is of longer duration than that seen during a swallow-induced sphincter relaxation. (96) The peristaltic wave which sweeps down the oesophagus after swallowing and prevents reflux, is absent during a transient lower oesophageal sphincter relaxation. Therefore, transient lower oesophageal sphincter relaxation represents a truly unguarded moment during which reflux can occur. Abdominal straining is seen in 15 to 20% of transient lower oesophageal sphincter relaxations and increases the likelihood of reflux from 30 to 60%. (156, 249) However, other factors may be important in determining whether reflux occurs during a transient lower oesophageal sphincter relaxation. These include the presence of a hiatus hernia, degree of oesophageal shortening, body position and intragastric volume and pressure. (135, 262) Triggering and control of transient lower oesophageal sphincter relaxations will be covered in detail in chapter 8 of this thesis.

7.2.3 Effect of hiatus hernia

Hiatus hernia was once regarded synonymous with gastro-oesophageal reflux disease. In the 1970's, reflux was thought to occur as a result of a chronically weak lower oesophageal sphincter and it became clear that the prevalence of a sliding hiatus hernia is age-related. As a result, the importance of hiatus hernia in reflux disease was disputed. However, recent studies have re-examined the role of hiatus hernia in the pathogenesis of gastro-oesophageal reflux disease.

Although only 50 to 60% of patients with hiatus hernias have endoscopic evidence of oesophagitis, up to 94% of patients with oesophagitis have a hiatus hernia. (263-265) The epidemiological association between oesophagitis and hiatus hernia makes it difficult to dispute a potential pathophysiological role for hiatus hernia in reflux disease.

A hiatus hernia might impair oesophagogastric junction competence by several potential mechanisms. Studies of the antireflux mechanism during stress manoeuvres such as leg raising and abdominal compression suggest a buttressing effect of the crural diaphragm on the lower oesophageal sphincter. (89, 232, 266) Therefore, both the crural diaphragm and the lower oesophageal sphincter contribute to oesophagogastric junction competence. Prevention of reflux during abrupt increases of intra-abdominal pressure, such as occur during bending or coughing, depends on both the lower oesophageal sphincter pressure and augmentation of the crural diaphragm sphincter pressure. A recent study, combining manometry and fluoroscopy suggested that patients with a hiatus hernia, regardless of whether or not they had a hypotensive lower oesophageal sphincter, are more susceptible to gastro-oesophageal reflux induced by abrupt increases in intra-abdominal pressure. (267) Although neither hiatus hernia nor hypotensive lower oesophageal sphincter pressure alone results in severe gastro-oesophageal incompetence, the two conditions interact with each other to increase susceptibility to reflux. Statistical modelling of the determinants of oesophagogastric junction competence, suggests that the susceptibility to reflux in the

presence of a hypotensive lower oesophageal sphincter is greatly compounded by hiatus hernia. (267) Therefore, a patient with a hypotensive lower oesophageal sphincter and a large hiatus hernia is much more likely to reflux during abdominal straining than is a patient with a hypotensive lower oesophageal sphincter but without a hiatus hernia. These data suggest that in patients with hiatus hernia, sphincter function of the crural diaphragm is impaired, and there is loss of diaphragmatic support of the lower oesophageal sphincter, and together, these defects predispose to reflux during episodes of increased intra-abdominal pressure, events normally associated with augmentation of crural diaphragm contraction.

In patients with hiatus hernia, displacement of the intra-abdominal portion of the lower oesophageal sphincter into the negative intrathoracic pressure environment, may be an important factor contributing to reflux. (234) Using oesophageal metal mucosal clip markers, Kahrilas et al showed that in subjects with hiatus hernia, if the squamocolumnar junction is more than 2 cm proximal to the diaphragmatic hiatus, there is loss of the intra-abdominal aspect of the high pressure zone. (31) As a result, transient increases in intra-abdominal pressure, which normally transmit pressure to the intra-abdominal segment of the lower oesophageal sphincter and help to maintain sphincter closure, splay open the lower oesophageal sphincter and result in reflux. In a more recent study, using concurrent manometry, fluoroscopy and endoscopically placed clips at the squamocolumnar junction, Kahrilas et al demonstrated that hiatus hernia reduces lower oesophageal sphincter pressure and impairs its response to abdominal compression by spatially separating the pressure components derived from the intrinsic lower oesophageal sphincter and the extrinsic compression of the oesophagus within the hiatal canal. (87)

Contraction of the crural diaphragm during inspiration can lead to compartmentalisation of the stomach between the lower oesophageal sphincter and crural diaphragm, and formation of a hernial sac, thereby predisposing patients with hiatus hernia to reflux. (242, 268) Negative intrapleural pressure may force open the lower oesophageal sphincter, with subsequent reflux of the hernial sac contents into the oesophagus.

Another mechanism by which a hiatus hernia contributes to reflux is by impairment of oesophageal acid clearance. A number of studies have demonstrated that the presence of a hiatus hernia compromises fluid emptying from the distal oesophagus. Using concurrent pH recording and scintiscanning, Mittal et al showed that regardless of the presence of oesophagitis, patients with hiatus hernia have impaired oesophageal acid clearance because there is re-reflux from the hernial sac during swallowing. (269) Swallowing brings about lower oesophageal sphincter relaxation causing the trapped gastric acid in the hernial sac to flow backward into the oesophagus. Using concurrent videofluoroscopy and manometry, Sloan et al also demonstrated that patients with non-reducing hiatus hernias have impaired oesophageal acid clearance time because of the constant backwash of gastric contents that occurs with swallowing. (267) Almost half of these patients exhibited early retrograde flow from the ampulla occurring coincident with lower oesophageal sphincter relaxation, analogous to re-reflux in Mittal's study.

7.2.4 Role of the stomach

Although gastro-oesophageal reflux is due to a defective sphincter mechanism at the oesophagogastric junction, the stomach is an important component of the reflux process. The stomach secretes acid and pepsin and is an important reservoir for the refluxate. Distension of the proximal stomach is the major stimulus for the triggering of transient lower oesophageal sphincter relaxations and will be discussed in chapter 8 of this thesis.

Delayed gastric emptying could, theoretically, increase the volume of gastric contents and contribute to the pathogenesis of gastro-oesophageal reflux. However, conflicting results have been obtained when studying gastric emptying of both solids and liquids in patients with gastro-oesophageal reflux disease. (252, 270-281) These discrepancies could be attributed to different techniques for the measurement of gastric emptying and variations in the patient population studied. The larger studies have generally shown that gastric emptying

is delayed in about 40% of patients with reflux oesophagitis. (252, 272, 273) The delay in gastric emptying is most marked for solids or semisolids but gastric emptying of liquids is also delayed in about 20% of patients with reflux disease. Gastric emptying has been shown to be significantly delayed in patients with oesophagitis compared to those without oesophagitis who were similar to normal subjects. (271) Whether the delayed gastric emptying is a cause, or consequence, of oesophagitis is unknown. Gastric emptying does not improve after medical healing of the oesophagitis. (282) Scarpignato and Franze demonstrated a correlation between the half-emptying time for solids and the degree of oesophageal acid exposure. (278) Similarly, Micali et al found a significant correlation between the gastric emptying half-time of solids and the degree of oesophageal lesions in patients with gastro-oesophageal reflux disease and speculated that the impaired gastric emptying is responsible for the appearance of oesophagitis. (275) Taken together, these studies demonstrate that gastric emptying is an important component of the reflux process.

Little is known about the patterns of gastric motility in reflux disease. Antral motility has been shown to be reduced in patients with reflux oesophagitis. (270) However, in this study, antral function analysis was limited to measuring the rate and duration of antral contractions and did not include any analysis of the patterns of antral pressure waves or their relationship to pyloric activity.

Motor function of the gastric fundus has received little attention in relation to the pathophysiology of gastro-oesophageal reflux. This region is involved in accommodation of a meal and the control of intragastric pressure. In theory, therefore, a disturbance in the control of intragastric pressure may be important in the pathogenesis of reflux. Patients with gastro-oesophageal reflux disease have been shown to exhibit significantly lower gastric pressure responses to distension by an intragastric bag. (283) In contrast, Penagini et al found no difference in fasting compliance in patients with reflux disease compared to normal control subjects. (280) This disparity may be due to methodological differences as well differences in the control population studied. However, Penagini et al did demonstrate that

reflux disease is associated with delayed recovery of proximal gastric tone after a meal, which may explain, at least in part, the delayed gastric emptying with reflux disease. Penagini et al also showed that in reflux disease, maximal postprandial relaxation volume of the proximal stomach is unchanged, although Zerbib et al demonstrated that it is increased. (284) This disparity may be due to the use of control subjects who were younger and had a lower body mass index than their reflux patients in the latter study.

In severe reflux oesophagitis, duodenal juice may contribute to the aggressiveness of the refluxate that enters the oesophagus. (285-287) Antropyloroduodenal incoordination may allow bile reflux from the duodenum to the stomach. (288) Excessive duodenogastric reflux may be contribute significantly to the development of gastro-oesophageal reflux disease complications, such as oesophageal strictures and Barrett's oesophagus. (285, 287, 289) The mechanism underlying the increased duodenogastric reflux in gastro-oesophageal reflux disease is not well understood. Contrary to what one might anticipate, measurements of transpyloric flow using ultrasound imaging suggest that the pressure gradient from stomach to duodenum is usually positive in reflux disease compared with that in normal subjects, a circumstance that would prevent duodenogastric reflux. (290) Clearly, this area is in need of more research.

7.2.5 Role of oesophageal body: oesophageal acid clearance

Once an episode of gastro-oesophageal reflux has occurred, the efficiency with which the refluxate is cleared from the oesophagus is the major determinant of the time that the oesophageal mucosa is exposed to the damaging effects of gastric contents. It is important that the refluxate be evacuated from the oesophagus as quickly as possible.

7.2.5.1 Normal clearance mechanisms

Oesophageal acid clearance after gastro-oesophageal reflux is a two-step process. First the bulk of the refluxate volume is cleared by oesophageal peristalsis, and second, residual acid is neutralised by alkaline saliva.

Oesophageal peristalsis

In normal subjects, volume clearance occurs largely through primary peristalsis. (95) After a reflux event, virtually all acid volume is emptied from the oesophagus by one or two peristaltic sequences. (291) Ambulatory oesophageal manometry and pH studies in normal subjects have shown that in the upright posture, primary peristalsis is the most common oesophageal clearance event. However, if the subjects are supine (and presumably asleep), the initial clearance event in most reflux episodes is secondary peristalsis. (156)

Role of saliva

Although oesophageal peristalsis is able to return nearly all the reflux material to the stomach, that in itself does not alter the pH of the small amount which still remains in contact with oesophageal mucosa. Rather the restoration of oesophageal pH is achieved with subsequent swallows. The need for repeat swallows suggests that alkaline saliva is an important component of the neutralisation process of refluxed acid. Using radiolabelled acidified water, Helm et al investigated clearance of acid from the oesophagus (291) and demonstrated that if saliva is aspirated from the mouth, oesophageal acid clearance is prolonged, verifying that the saliva rather than the clearing action of subsequent peristalsis is essential to the restoration of oesophageal pH. Increasing salivary flow with oral lozenges or administration of bethanecol improves oesophageal clearance in normal subjects with normal oesophageal peristaltic function. Conversely, inhibition of salivary flow with atropine

prolongs oesophageal acid clearance. (292, 293) The ability of saliva to neutralise refluxed acid is due primarily to its bicarbonate concentration. (293)

7.2.5.2 Acid clearance in gastro-oesophageal reflux disease

In an early study, Booth et al demonstrated that oesophageal acid clearance is invariably prolonged in patients with gastro-oesophageal reflux disease. (294) Similarly, using 24 hour oesophageal pH monitoring, DeMeester et al demonstrated that patients with predominantly recumbent reflux have prolonged mean oesophageal acid clearance times compared with normal controls. (295) Subsequent studies have demonstrated substantial overlap in acid clearance times between reflux patients and normal controls. There is heterogeneity in the patient population such that although mean oesophageal acid clearance times are greater for reflux patients than normal controls, only about half of the reflux patients demonstrate abnormal oesophageal acid clearance. (296) Similar heterogeneity in reflux patients has also been demonstrated in a large review of 24 hour oesophageal pH data which showed that patients with hiatus hernia had the most prolonged acid clearance times. (297) Little et al showed that patients with oesophagitis have more reflux episodes of greater than five minutes' duration than those without oesophagitis or normal subjects as measured during 24 hour oesophageal pH monitoring. (271) Therefore, a subset of reflux patients exists that demonstrates impaired acid clearance. As discussed above, abnormal oesophageal acid clearance may be caused by abnormal oesophageal motor function or abnormal salivation.

<u>Oesophageal peristalsis</u>

Primary peristalsis is the main type of oesophageal motor event that clears the oesophagus of refluxed acid in patients with gastro-oesophageal reflux disease. (259) In a study by Kahrilas et al, oesophageal motor function in normal controls and patients with mild to severe oesophagitis was compared. (260) It was shown that the frequency of peristaltic

dysfunction increases with the severity of reflux, rising from 25% in individuals with mild oesophagitis to 48% in patients with severe oesophagitis. Patients with Barrett's oesophagus have the highest frequency of peristaltic dysfunction. (279, 298)

In patients with gastro-oesophageal reflux disease, the main types of peristaltic dysfunction which impair oesophageal volume clearance are failure of the peristaltic contraction to traverse the entire oesophageal body, and low amplitude peristaltic contractions. (38) Peristaltic failure results in minimal oesophageal volume clearance. Low amplitude peristaltic contractions achieve varying degrees of partial volume clearance depending on the severity of retrograde escape of the oesophageal contents. Using concurrent manometry and fluoroscopy, Kahrilas et al found that the peristaltic amplitude required to clear a swallowed bolus of liquid barium, varies according to the oesophageal segment, so that very weak contractions are effective in the proximal oesophagus but contraction amplitudes of at least 30 mmHg are required in the distal oesophagus for effective volume clearance. (38) Richter et al confirmed these findings using concurrent manometry and scintigraphy and showed that once peristaltic amplitude exceeds 30 mmHg, a single peristaltic sequence effectively clears the oesophagus. (37)

In addition to defects in primary peristalsis, patients with gastro-oesophageal reflux disease may have defective secondary peristalsis. Williams et al demonstrated that patients with reflux oesophagitis have a higher distension threshold for the initiation of secondary peristalsis than controls or patients without oesophagitis and these peristaltic contractions have a weaker propulsive force as measured by a strain gauge attached to an inflatable balloon. (299) Schoeman and Holloway found that patients with reflux disease have a considerably lower secondary peristaltic response rate to air or water distension compared to aged matched normal controls. (300)

Using manometry and pH measurements, Allen et al reported that in patients with gastro-oesophageal reflux disease, secondary peristalsis is a rare motor event after a reflux episode

accounting for less than 1% of oesophageal body motor responses and only one of seven such events results in oesophageal acid clearance. (301) In contrast, using ambulatory oesophageal manometry and pH, Penagini et al reported that the initial oesophageal clearance event is secondary peristalsis in 13% of cases. (249) The different findings in these two studies may reflect differences in the recording methods and manometric definitions. Whilst the role of secondary peristalsis in the clearance of oesophageal acid in reflux patients seems unimportant, it may be of significance during sleep when the rate of primary peristalsis is substantially reduced. (302)

Role of saliva

As outlined earlier, saliva is an important component of the neutralisation process of refluxed acid. Sonnenberg et al analysed the salivary function in 44 patients with reflux oesophagitis and found that there was no difference in resting salivary flow rate or buffering capacity compared to normal controls. (303) However, there was a significant difference in salivary secretion stimulated by acid perfusion of the oesophagus. Young normal controls displayed a doubling or tripling of the salivary rate, whilst reflux oesophagitis patients and age-matched controls had no increased salivary secretion after oesophageal acidification. The significance of this finding is unknown, given that the latency for increased salivary flow was 20 to 30 minutes after initial oesophageal acid perfusion, although it may potentially have an impact on the neutralisation of subsequent acid reflux episodes.

Cigarette smokers have been shown to secrete less saliva than non-smoking control subjects, have acid clearance times 50% higher than those of non-smokers and have only 60% titratable salivary base secretion of non-smokers. (304, 305) Reduced salivation of cigarette smokers has been postulated to be mediated by an anticholinergic effect, similar to that seen in patients using medications with anticholinergic effects.

Chapter 8

Transient lower oesophageal sphincter relaxations: mechanisms and control

8.1 Introduction

As discussed in chapter 7 of this thesis, for many years reflux was thought to occur as a result of a chronically weak lower oesophageal sphincter. (246) More recent studies, using concurrent pH and oesophageal motility measurements have shown that most reflux events occur during transient, brief lower oesophageal sphincter relaxations rather than due to defective lower oesophageal sphincter pressure. (95) Recognition of transient lower oesophageal sphincter relaxations provides an explanation for the occurrence of reflux events in patients who have a normal resting lower oesophageal sphincter pressure.

Lower oesophageal sphincter relaxation not related to swallowing was first described in 1964 as a mechanism of belching. (306) However, it was not until 1980 that Dent et al described the relationship between gastro-oesophageal reflux and transient episodes of lower oesophageal sphincter relaxation. (95) These lower oesophageal sphincter relaxations associated with reflux were initially termed "inappropriate" because they did not occur during a normal primary or secondary peristaltic sequence. As these relaxations became recognised as a physiological response in normal healthy subjects and are a normal mechanism of belching, (94, 307) the term inappropriate has been dropped and the term transient lower oesophageal sphincter relaxation has been accepted, and is used to describe lower oesophageal sphincter relaxations not triggered by swallowing.

8.2 Characteristics of transient lower oesophageal sphincter relaxations

8.2.1 Manometric characteristics

Transient lower oesophageal sphincter relaxations are characterised by abrupt decreases in lower oesophageal sphincter pressure that are not triggered by swallowing and typically have a duration longer than swallow-induced relaxations. The definition of transient lower oesophageal sphincter relaxation has previously been arbitrary and derived from subjective

visual pattern recognition rather than objective analysis of the variables involved. Variables that have been used include the relationship between swallowing and the onset of lower oesophageal sphincter relaxation, the rate, time interval and magnitude of the fall in sphincter pressure and the duration of sphincter relaxation. (95, 96, 116, 164, 247, 248, 259, 261, 308-311)

The most important issue in identifying transient lower oesophageal sphincter relaxations is the differentiation from swallow-induced lower oesophageal sphincter relaxations. This is done by analysing the timing between swallowing and the onset of the lower oesophageal sphincter relaxation. Using pharyngeal manometry, Holloway et al found that for dry swallows, the onset of the pharyngeal pressure wave ranges from 3.7 seconds before and 1.6 seconds after the onset of lower oesophageal sphincter relaxation. (312) Using mylohyoid electromyography, Mittal and McCallum found that the onset of the mylohyoid electromyogram complex is up to 3 seconds before the onset of swallow-induced lower oesophageal sphincter relaxation. (96)

Transient lower oesophageal sphincter relaxations are typically of longer duration than swallow-induced lower oesophageal sphincter relaxations, almost invariably lasting greater than 10 seconds and possibly up to 45 seconds. (96, 156, 313, 314) As spontaneous swallowing occurs frequently, there is a possibility that a swallow can occur at the onset of, but independently from a transient lower oesophageal sphincter relaxation. Kawahara et al found that in children with pathological reflux disease, 23% of the reflux episodes occurring during lower oesophageal sphincter relaxation were associated with swallowing and most of these lower oesophageal sphincter relaxations lasted greater than 5 seconds suggesting that these lower oesophageal sphincter relaxations are fundamentally different from swallow-induced lower oesophageal sphincter relaxations. (315) In normal healthy subjects, Sifrim et al demonstrated that prolonged (greater than 9.7 seconds duration) swallow-induced lower oesophageal sphincter relaxations are different from transient lower oesophageal sphincter relaxations as they are of longer duration and acid reflux occurs later after complete lower

oesophageal sphincter relaxation and therefore, do not simply represent a chance association between a swallow and a transient lower oesophageal sphincter relaxation. (316)

The rate of lower oesophageal sphincter relaxation is an important variable when characterising transient lower oesophageal sphincter relaxations. An arbitrary relaxation rate of at least 1 mmHg/s has been used in some studies (116, 261) and this has been shown to be a good discriminator between transient lower oesophageal sphincter relaxations and lower oesophageal sphincter pressure drifts. (313) The time from onset to complete lower oesophageal sphincter relaxation always occurs in less than 10 seconds and this is a reasonable cut-off in distinguishing transient lower oesophageal sphincter relaxations from linear lower oesophageal sphincter drifts starting from a high lower oesophageal sphincter pressure. (313)

During a transient lower oesophageal sphincter relaxation, reflux usually only occurs if the nadir pressure is at least within 2 mmHg of intragastric pressure. Some studies have classified transient lower oesophageal sphincter relaxations as either complete or incomplete, based on the nadir lower oesophageal sphincter pressure. (91, 164, 247, 310) However, the significance of incomplete transient lower oesophageal sphincter relaxations, as defined as a nadir pressure of greater than 4 mmHg, is indeterminate, as reflux does not usually occur during these events. (164) Therefore, it is best to avoid the term, incomplete transient lower oesophageal sphincter relaxations and to restrict the term, transient lower oesophageal sphincter relaxations, to those events in which nadir pressure falls to at least within 2 mmHg of intragastric pressure.

The criteria that have proved optimal for the definition of transient lower oesophageal sphincter relaxations are best described by Holloway et al: "1) absence of swallowing for 4 s before to 2 s after the onset of lower oesophageal sphincter relaxation; 2) relaxation rate of $\geq$1 mmHg/s; 3) time from the onset of relaxation to complete relaxation of $\leq$10 s; and 4) nadir pressure of $\leq$2 mmHg. Excluding lower oesophageal sphincter relaxations associated

with multiple swallows lower oesophageal sphincter pressure falls that fulfil the last three criteria but have a duration of >10 s can also be judged to be transient lower oesophageal sphincter relaxations irrespective of the timing of the onset of the lower oesophageal sphincter pressure fall to swallowing." (313)

8.2.2 Other events

Transient lower oesophageal sphincter relaxation is not an isolated event confined to the lower oesophageal sphincter only. There are a number of associated events involving the crural diaphragm and upper gastrointestinal tract.

Crural diaphragm

During a transient lower oesophageal sphincter relaxation, there is selective inhibition of crural diaphragm activity and the degree of inhibition correlates with the magnitude of lower oesophageal sphincter relaxation. (91, 238) However, during a swallow-induced lower oesophageal sphincter relaxation, there is only partial or no inhibition of the crural diaphragm. Crural diaphragm inhibition may be an important factor that facilitates flow across the oesophagogastric junction during a transient lower oesophageal sphincter relaxation. Absence of crural diaphragm inhibition with swallow-induced lower oesophageal sphincter relaxation may help to prevent reflux of gastric contents during swallowing.

Oesophageal body

At the onset of most transient lower oesophageal sphincter relaxations, a synchronous, low amplitude pressure wave is often seen in the distal oesophageal body. (96) During a prolonged, more than 15 second duration transient lower oesophageal sphincter relaxation, Dent et al found that in patients with gastro-oesophageal reflux disease, swallowing rarely triggers a normal primary peristaltic wave. (248) However, in contrast, Sifrim et al showed that in normal volunteers, during a transient lower oesophageal sphincter relaxation, swallowing triggers a contraction down the entire oesophageal body in nearly all cases.

(317) The reason for this discrepancy between these two studies may be related to the different subject populations and recording methods. Using an intra-oesophageal balloon to create an artificial high pressure zone, Sifrim et al found that transient lower oesophageal sphincter relaxations by themselves do not inhibit muscle contractility in the body of the oesophagus. However, if the transient lower oesophageal sphincter relaxations are associated with abrupt distension of the lower oesophagus by gastro-oesophageal reflux of air or acid, as indicated by the occurrence of a common cavity, partial inhibition of muscle contractility in the body of the oesophagus occurs. (317)

Pharynx

Pharyngeal and mylohyoid activity has been reported to occur at the onset of a portion of transient lower oesophageal sphincter relaxations and has been interpreted as partial or incomplete swallows. (96) However, other studies have been unable to find a consistent relationship between submental electromyogram signals and transient lower oesophageal sphincter relaxations. (314, 318)

Stomach

The effect of transient lower oesophageal sphincter relaxations on gastric fundus pressure has not been specifically studied. During a transient lower oesophageal sphincter relaxation, there is a small decrease in intragastric pressure. (319) Whether this is due to gastric relaxation or small amounts of gastric contents escaping into the oesophagus has not been adequately defined.

8.3 Triggers and modulating factors

As transient lower oesophageal sphincter relaxations are an important mechanism of gastro-oesophageal reflux, it is important to examine the factors which trigger and modulate this event. Factors that trigger transient lower oesophageal sphincter relaxations include gastric distension and possibly pharyngeal stimulation. Factors that inhibit the triggering of transient

lower oesophageal sphincter relaxations include supine posture, sleep, anaesthesia and cold stress.

8.3.1 Gastric distension

Approximately 15 ml of air enters the stomach with each swallow. (320) Without an adequate venting mechanism, this would cause uncontrolled gastric bloating with air. Gastric distension is a potent stimulus for the triggering of transient lower oesophageal sphincter relaxations. Transient lower oesophageal sphincter relaxation is an important component of belching, which prevents the stomach from becoming overdistended with swallowed air. Gastric distension with air (94, 116, 321) or an air-filled balloon (319) has been shown to increase the rate of transient lower oesophageal sphincter relaxations and this increase is proportional to the distension volume and returns to baseline levels with cessation of the distending stimulus. Gastric partitioning studies in the dog, have shown that the subcardiac region of the stomach has the lowest threshold for the triggering of transient lower oesophageal sphincter relaxations. (322) Limiting distension of the subcardiac region of the stomach by surgical reinforcement, substantially reduces eructation in dogs, and by implication, would inhibit transient lower oesophageal sphincter relaxations. (323)

Most studies have shown that after a meal, there is an increase in the rate of transient lower oesophageal sphincter relaxations in patients with gastro-oesophageal reflux disease and normal healthy volunteers. (156, 261, 308, 324) This increase can last up to 3 hours and is most likely to be due to the effect of gastric distension. Some studies have not demonstrated an increase in the rate of transient lower oesophageal sphincter relaxations after a meal. (96, 249) The reason for this discrepancy may be related to differences in the definition of transient lower oesophageal sphincter relaxation, (96) differences in posture and physical activity in the fasting and postprandial periods and meal composition. (249) Meals are associated with an increase in the proportion of transient lower oesophageal sphincter relaxations associated with reflux and this is an important contributor to the increase in reflux

events after a meal. (96, 156, 249, 261, 308, 324) In patients with gastro-oesophageal reflux disease, intraduodenal fat infusion has no effect on the rate of transient lower oesophageal sphincter relaxations, but increases the likelihood of reflux occurring during a transient lower oesophageal sphincter relaxation. (325)

8.3.2 Pharyngeal activity

In patients in whom lower oesophageal sphincter pressure is measured with a catheter via a gastrostomy tube, insertion of a separate tube into the pharynx via the nostril increases the rate of transient lower oesophageal sphincter relaxations. (310) This finding suggests that pharyngeal stimulation with a catheter may be an important stimulus for the triggering of transient lower oesophageal sphincter relaxations. However, the role of pharyngeal stimulation in unintubated subjects is questionable.

It has been suggested that transient lower oesophageal sphincter relaxations are due to subthreshold activation of the deglutition reflex. (49) Pharyngeal stimulation is able to evoke swallowing, characterised by the sequential semiautomatic discharge of muscles of the oropharyngeal, laryngeal and the oesophageal regions. (326, 327) In the opossum, high frequency electrical stimulation of the superior laryngeal nerve leads to the full deglutition sequence. (49) However, low frequency electrical stimulation of the superior laryngeal nerve or light stroking of the pharynx leads to isolated lower oesophageal sphincter relaxation. This reflex depends on afferent pathways travelling from the pharynx or larynx via the superior laryngeal nerve of the vagus and glossopharyngeal nerves (328) and project to the nucleus tractus solitarius and dorsal motor nucleus of the vagus. In humans, injection of minute amounts of water into the pharynx leads to inhibition in the body of the oesophagus (329) and prolonged lower oesophageal sphincter relaxation, not associated with swallowing. (330-332) However, there are important distinct differences between transient lower oesophageal sphincter relaxations and pharyngeal-stimulus induced lower oesophageal sphincter relaxations. Whilst both types of lower oesophageal sphincter relaxation are of

long duration, lasting up to 60 seconds, lower oesophageal sphincter relaxation induced by injecting small amounts of water into the pharynx is usually less complete than transient lower oesophageal sphincter relaxations. (330-332) Furthermore, in a recent study, Mittal et al demonstrated that in normal humans, lower oesophageal sphincter relaxation induced by injecting small amounts of water into the pharynx is usually not associated with inhibition of the crural diaphragm, oesophageal common cavity or acid reflux. (331)

8.3.3 Posture

In sheep, the supine posture inhibits eructation and by implication transient lower oesophageal sphincter relaxation produced by gastric air insufflation. (333) In dogs, (334) normal healthy humans (94) and in patients with gastro-oesophageal reflux disease, (335) the supine posture inhibits transient lower oesophageal sphincter relaxations produced by gastric air insufflation.

The mechanism of postural suppression of transient lower oesophageal sphincter relaxations is unclear. Freidin et al found that in patients with gastro-oesophageal reflux disease the rate of postprandial transient lower oesophageal sphincter relaxation was lower in the supine than sitting position and postulated that in the supine position there was less air accumulation in the gastric fundus and therefore less stimulation of mechanoreceptors located in the gastric fundus. (309) Studies in sheep showed that postural suppression of eructation may result from bathing of the gastric cardia by residual gastric fluid. (333) However, Little et al showed that in dogs, removal of the fluid did not overcome the strong postural suppression of transient lower oesophageal sphincter relaxations and concluded that this postural suppression was not due to the presence of a gastric pool of liquid in the subcardiac region. (334) Nevertheless, it is possible that not all the fluid was removed and only a small amount of residual fluid in the subcardiac region may be sufficient to suppress transient lower oesophageal sphincter relaxations. Kapur et al demonstrated that in patients with gastro-oesophageal reflux disease, the rate of postprandial transient lower oesophageal sphincter

relaxations is equally common in both the right and left lateral positions. (336) However, a larger proportion of transient lower oesophageal sphincter relaxations was associated with reflux in the right lateral position, compared to the left lateral position, suggesting that although the gastric contents lie in closer proximity to the oesophagogastric junction when in the right lateral position they do not cause inhibition of transient lower oesophageal sphincter relaxations. (336) Ireland et al investigated whether head position, acting via the vestibular system is responsible for the postural suppression of transient lower oesophageal sphincter relaxations. (337) They found that varying head position had no effect on the rate of transient lower oesophageal sphincter relaxations triggered by gas insufflation.

8.3.4 Sleep

The triggering of transient lower oesophageal sphincter relaxations is influenced by the level of consciousness. During stable sleep, transient lower oesophageal sphincter relaxations are suppressed in normal healthy humans and in patients with gastro-oesophageal reflux disease. (95, 338) Any transient lower oesophageal sphincter relaxations that do occur, are limited to brief periods of arousal during sleep lasting 5 to 10 seconds. (338) These periods of arousal are not triggered by gastro-oesophageal reflux, as the reflux events always follow the arousal period. The mechanism by which sleep influences the triggering of transient lower oesophageal sphincter relaxations is unknown. However during sleep, suppression of transient lower oesophageal sphincter relaxations would be serve as a protective mechanism in the prevention of gastro-oesophageal reflux.

8.3.5 Anaesthesia

In dogs, anaesthesia has been shown to suppress the triggering of transient lower oesophageal sphincter relaxations produced by air insufflation. (339) In anaesthetised cats, transient lower oesophageal sphincter relaxations can be triggered by acute airway

obstruction. (241) There are no data on the effect of anaesthesia on the triggering of transient lower oesophageal sphincter relaxations in humans.

8.3.6 Stress

Cold stress has been shown to decrease the rate of postprandial transient lower oesophageal sphincter relaxation in normal healthy humans. (311) In contrast, Holloway et al found that in fasting normal healthy volunteers, a 30 minute sham balloon distension of the stomach causes an increase in the rate of transient lower oesophageal sphincter relaxation, which may possibly have been related to increased stress, anxiety or level of consciousness. (319)

8.4 Neural control

Transient lower oesophageal sphincter relaxation is believed to be neurally mediated through vagal pathways. Gastric distension is believed to stimulate tension receptors in the proximal stomach which project via afferent vagal fibres to the sensory vagal nuclei in the brainstem. Pharyngeal stimulation may also send sensory signals to the brainstem. The sensory signals activate a pattern generator in the brainstem which sends intermittent signals via efferent vagal fibres to the lower oesophageal sphincter, resulting in a transient lower oesophageal sphincter relaxation. An alternative hypothesis suggests that transient lower oesophageal sphincter relaxations could be caused by a reflex lower oesophageal sphincter relaxation in response to stretching of the gastric wall via direct intramural neural connections. (340) However, suppression of transient lower oesophageal sphincter relaxations by vagal cooling suggests that direct intramural neural connections between the stomach and lower oesophageal sphincter are unlikely to play an important role in transient lower oesophageal sphincter relaxations. (116) In patients with achalasia, gastric distension is unable to provoke transient lower oesophageal sphincter relaxations, which is further evidence that transient lower oesophageal sphincter relaxations are likely to be neurally mediated. (341)

Neural control of transient lower oesophageal sphincter relaxations has been difficult to study as they can only be triggered in the awake state, except in the cat. (241) It is not clear whether neural control of transient lower oesophageal sphincter relaxation is the same as that of lower oesophageal sphincter relaxation that follows a swallow but there are similarities indicative of some common underlying neural mechanisms.

8.4.1 Vagal (efferent) pathway

The efferent pathway for transient lower oesophageal sphincter relaxation is presumably the vagus nerve, as vagal cooling in the dog inhibits transient lower oesophageal sphincter relaxations (116) and truncal vagotomy inhibits eructation. (136) The vagus also mediates swallow-induced lower oesophageal sphincter relaxation. (68, 113, 134, 137, 342) The dorsal motor nucleus of the vagus contain the nerve cell bodies that project to the lower oesophageal sphincter. (9) It is likely that transient lower oesophageal sphincter relaxation and swallow-induced lower oesophageal sphincter relaxation share a final common pathway as patients with achalasia have an absence of both transient lower oesophageal sphincter relaxation and swallow-induced lower oesophageal sphincter relaxation. (341)

8.4.2 Sensory (afferent) pathways

Gastric distension is believed to trigger transient lower oesophageal sphincter relaxations through stimulation of gastric mechanoreceptors found in the wall of the stomach, particularly in the subcardiac region. (173, 174, 319, 322) These mechanoreceptors, which are sensitive to gastric wall tension, send signals via vagal afferent fibres to the nucleus tractus solitarius and area postrema. (170, 171) Vagal afferent fibres also project to the dorsal motor nucleus of the vagus, either directly or via an interneuron. (343, 344)

Jansson demonstrated that activation of gastric receptors stimulates afferent vagal fibres, causing reflex gastric relaxation via a vago-vagal pathway. (345) A similar vago-vagal

neural pathway could potentially exist, whereby stimulation of gastric mechanoreceptors mediates transient lower oesophageal sphincter relaxations. In the opossum, gastric stretch can elicit lower oesophageal sphincter relaxation via a local intramural pathway, independent of extrinsic reflexes. (340) Whether a local pathway exists for the triggering of transient lower oesophageal sphincter relaxations is unknown. However, it is unlikely to be of significance given that triggering of transient lower oesophageal sphincter relaxation is inhibited by vagal cooling. (116) Furthermore, using retrograde labelling in the guinea pig, Brookes et al showed that inhibitory motor neurons to the lower oesophageal sphincter are located in the oesophageal body and not in the stomach. (84) In addition, a local pathway could not be involved in the selective inhibition of the crural diaphragm during a transient lower oesophageal sphincter relaxation. (91, 238)

Despite constant stimulation of gastric mechanoreceptors, transient lower oesophageal sphincter relaxations are triggered only intermittently. This is likely to occur as a result of a pattern generator in the brainstem, that responds to a constant sensory input with an intermittent motor output. (346, 347)

8.4.3 Crural diaphragm

The selective inhibition of the crural diaphragm during a transient lower oesophageal sphincter relaxation also occurs during vomiting (237) and oesophageal distension. (90) Inhibition of the crural diaphragm triggered by oesophageal distension is thought to occur at the level of the central nervous system. (90) It is possible that selective inhibition of the crural diaphragm during a transient lower oesophageal sphincter relaxation is coordinated centrally, in the brainstem. The nucleus tractus solitarius contains the dorsal respiratory group of neurons which serve as the principal rhythmic respiratory drive to diaphragmatic (phrenic) motor neurons. (235) The nucleus tractus solitarius has been proposed as the site of integrated neural control of the inhibition of respiration during swallowing. (326) Therefore, it seems plausible that during a transient lower oesophageal sphincter relaxation,

the brainstem controls the efferent output to the crural diaphragm, as well as to the lower oesophageal sphincter.

8.5 Neural receptors and therapeutic implications

There are a number of hormones and biologically active substances that may be affect the triggering of transient lower oesophageal sphincter relaxations. However, the existence of these effects does not necessarily indicate that these substances have a physiological role in the control of transient lower oesophageal sphincter relaxations. Various pharmacological agents have recently been found to influence the triggering of transient lower oesophageal sphincter relaxations. Pharmacological inhibition of the triggering of transient lower oesophageal sphincter relaxations offers the potential for a physiologically logical and therapeutically useful approach for the treatment of gastro-oesophageal reflux.

8.5.1 Cholecystokinin

Cholecystokinin (CCK) may be considered as a candidate humoral regulator for the control of transient lower oesophageal sphincter relaxations. CCK is released after a meal and the frequency of transient lower oesophageal sphincter relaxations increases after a meal. CCK-A receptors are located on smooth muscles of the gastrointestinal tract, on vagal afferents (348, 349) and in brainstem nuclei such as the nucleus tractus solitarius. (350) Different CCK receptor subtypes are present on the neurons and muscle of the cat lower oesophageal sphincter. (351) From this evidence, it seems plausible that CCK might be involved in the control of transient lower oesophageal sphincter relaxations.

Infusion of CCK-8 in dogs (352) and normal healthy humans (353) has been shown to increase the triggering of transient lower oesophageal sphincter relaxations induced by gastric distension at constant intragastric pressure using a barostat. In contrast, Ledeboer et al failed to show that CCK-33 increases the rate of transient lower oesophageal sphincter

relaxations induced by gastric distension in normal healthy volunteers possibly because a different isoform of CCK was used. (159)

In dogs, triggering of transient lower oesophageal sphincter relaxations induced by gastric distension at constant intragastric pressure using a barostat, is decreased by the CCK-A receptor antagonist devazepide but not by the CCK-B antagonist L365260. (352) Recent studies in normal healthy humans have shown that the CCK-A receptor antagonist loxiglumide decreases the triggering of transient lower oesophageal sphincter relaxations induced by gastric distension at a constant intragastric pressure (353) or fixed intragastric volume. (354) Clave et al showed that in normal human subjects, release of endogenous CCK, by stimulation with a meal and cholestyramine, increases the rate of postprandial transient lower oesophageal sphincter relaxation and gastro-oesophageal reflux episodes and this effect is counteracted by loxiglumide. (355) These studies suggest that CCK-A receptors are involved in the control of triggering of transient lower oesophageal sphincter relaxations induced by gastric distension.

The site at which CCK exerts its effect has not been defined but the weight of evidence indirectly favours an action on afferent nerves. In dogs, intracerebroventricular administration of a CCK-A receptor antagonist does not modify the rate of transient lower oesophageal sphincter relaxation induced by gastric distension, suggesting that at least in dogs, the site of action is not central. (352) It seems unlikely that CCK affects the efferent motor limb of the putative vago-vagal reflex arc that is believed to mediate transient lower oesophageal sphincter relaxations, as loxiglumide has no effect on swallow-induced lower oesophageal sphincter relaxations. (354)

It is possible that CCK and loxiglumide may have an effect on gastric compliance, thereby influencing the triggering of transient lower oesophageal sphincter relaxations. CCK has no effect on gastric tone at physiological doses. (217) However, the effect of loxiglumide on gastric compliance as measured by a gastric barostat is conflicting; one study reported no

change, (356) but another study did report a reversal by loxiglumide of the increase in gastric compliance induced by intraduodenal lipid infusion. (357) Studies examining the effect of loxiglumide on postprandial gastric relaxation have also been conflicting; two studies have reported no effect, (218, 219) but one study reported a reduction in the postprandial gastric relaxation after duodenal infusion of a liquid meal. (220) Boulant et al showed that during pressure-controlled distension, loxiglumide decreased the maximal intragastric distension volumes, compared to saline control, although the actual pressure measurements, and therefore compliance on the two study days is not mentioned. (353) Thus, a peripheral site of action on gastric mechanoreceptors cannot be entirely excluded. Using in vitro muscle strips, Clave et al were unable to demonstrate an inhibitory effect of CCK on the lower oesophageal sphincter, suggesting that the increase in transient lower oesophageal sphincter relaxations and the decrease in basal lower oesophageal sphincter pressure induced by endogenous CCK in vivo is caused by the activation of CCK receptors located on an extrasphincteric site. (355)

8.5.2 Nitric oxide

As discussed in chapter 4 of this thesis, there is convincing evidence from animal and human studies that nitric oxide is an important neurotransmitter involved in relaxation of the lower oesophageal sphincter. (17, 18, 58, 137-139) Morphological studies have shown that nitric oxide synthase activity is present in the myenteric plexus, (140) in smooth muscle cells of the lower oesophageal sphincter (141) and in the nucleus tractus solitarius and nucleus ambiguus. (142) Therefore, it seems plausible that nitric oxide might be involved in the triggering of transient lower oesophageal sphincter relaxations.

Boulant et al reported that N^G-nitro-L-arginine-methylester (L-NAME), a specific blocker of nitric oxide synthesis reduces the rate of transient lower oesophageal sphincter relaxations induced by pressure-controlled gastric insufflation of air in dogs, suggesting that nitric oxide is involved in the control of transient lower oesophageal sphincter relaxations. (352)

Similarly, Hirsch et al found that in normal healthy humans, infusion of N^G-monomethyl-L-arginine (L-NMMA), a specific inhibitor of nitric oxide synthesis, significantly inhibits the increase in transient lower oesophageal sphincter relaxations during volume-controlled gastric distension with a balloon. (358) L-NMMA has no effect on swallow-induced lower oesophageal sphincter relaxations, suggesting that the effect of nitric oxide blockade on transient lower oesophageal sphincter relaxations is not on the efferent limb of the putative vago-vagal reflex that is believed to mediate transient lower oesophageal sphincter relaxations. It is possible that L-NMMA has an effect on gastric compliance, therefore influencing the triggering of transient lower oesophageal sphincter relaxations. Animal studies have shown that inhibition of nitric oxide synthase blocks gastric relaxation. (177, 178, 359) Hirsch et al found that L-NMMA was associated with a significantly greater increase in intragastric pressure during volume-controlled gastric distension. (358) This effect would be expected to increase, rather than decrease the triggering of transient lower oesophageal sphincter relaxations. Therefore, it is unlikely that L-NMMA inhibits transient lower oesophageal sphincter relaxations by influencing the behaviour of gastric wall mechanoreceptors. It seems more likely that L-NMMA is acting either on the afferent limb of the reflex arc mediating transient lower oesophageal sphincter relaxations or centrally, in the brainstem, possibly in the nucleus tractus solitarius, a site known to contain nitric oxide synthase. (142)

8.5.3 Morphine

Endogenous opioid peptides have been shown to be present along sensory ascending neural pathways (360) and in the stomach. (361) Opioid receptors have been demonstrated to exist along afferent neural pathways and centrally in the brainstem. (362, 363) Therefore, it seems plausible that endogenous opioids could be involved in the control of transient lower oesophageal sphincter relaxations.

As morphine, an opioid agonist has been shown to increase residual lower oesophageal sphincter pressure during swallow-induced lower oesophageal sphincter relaxations, (364) Penagini and Bianchi postulated that morphine might potentially also reduce reflux by increasing residual lower oesophageal sphincter pressure during transient lower oesophageal sphincter relaxations. Indeed, they demonstrated that in patients with gastro-oesophageal reflux disease, morphine markedly reduces the rate of reflux episodes induced by gastric distension with 10% dextrose. (365) However, the mechanism is attributable to a decrease in the rate of transient lower oesophageal sphincter relaxations rather than any increase in residual lower oesophageal sphincter pressure during transient lower oesophageal sphincter relaxations, as residual pressure was not affected. Interestingly, morphine has no effect on the triggering of transient lower oesophageal sphincter relaxations in normal healthy subjects. This may be a reflection of underlying differences in the control of transient lower oesophageal sphincter relaxations between normal healthy subjects and patients with gastro-oesophageal reflux disease, as discussed later in this chapter.

It seems unlikely that the site of action of morphine is the efferent neural pathway, as transient lower oesophageal sphincter relaxation duration and residual lower oesophageal sphincter pressure are not affected. Morphine could possibly be acting on opioid receptors along the afferent pathway or centrally on the integrative mechanisms in the brainstem that are believed to mediate transient lower oesophageal sphincter relaxations.

8.5.4 Anticholinergic agents

Muscarinic cholinergic receptors are present in the brain and peripheral tissues, including the lower oesophageal sphincter. (366-368) Molecular cloning studies have identified five distinct muscarinic receptor genes (m1-m5), although the precise muscarinic receptor subtypes and their localisation within the brain and lower oesophageal sphincter remains to be fully elucidated. Selective muscarinic agonists and antagonists could potentially influence the control of transient lower oesophageal sphincter relaxations.

In normal human volunteers, the non-selective muscarinic anticholinergic agent, atropine, significantly reduces the frequency of transient lower oesophageal sphincter relaxations and reflux after a meal. (243, 332) Atropine also reduces the frequency of transient lower oesophageal sphincter relaxations induced by gastric distension with air and increases the threshold of pharyngeal stimulus required to elicit lower oesophageal sphincter relaxation. (332) In dogs, atropine has not been shown to have any effect on transient lower oesophageal sphincter relaxations induced by gastric distension with air. (116) The reason for this discrepancy is unclear, but may be due to the fact that lower oesophageal sphincter pressure was less than 2 mmHg for most of the recording time during atropine infusion in the dogs, making transient lower oesophageal sphincter relaxations more difficult to recognise. In addition, in the human studies, crural diaphragm electromyogram recordings were used to more accurately identify transient lower oesophageal sphincter relaxations during periods of atropine-induced low lower oesophageal sphincter pressure. (243, 332)

The site at which atropine exerts its effect on the triggering of transient lower oesophageal sphincter relaxations is discussed in chapters 10-12 of this thesis.

8.5.5 Sumatriptan

Most of the 5-hydroxytryptamine (5-HT) present in the human body is located in the gastrointestinal tract. (369) 5-HT may be involved in gastrointestinal motility by direct actions on smooth muscle, by affecting the release of transmitters within the myenteric plexus, or by activation of intrinsic and extrinsic reflexes. (370) Receptors for 5-hydroxytryptamine-1 (5-HT1) are present in the brainstem, spinal cord and nucleus tractus solitarius. (371) Sumatriptan, a 5-HT1 agonist, increases the rate of postprandial reflux and transient lower oesophageal sphincter relaxations in normal healthy volunteers. (372) The site of action is possibly the gastric wall, as sumatriptan has been shown to cause fundic relaxation, as measured by an increase in gastric volume during isobaric gastric distension,

and therefore increases gastric wall tension. (373) It is also possible that sumatriptan is acting centrally on 5-HT1 receptors in the brainstem.

8.5.6 Other agents

Several other pharmacological agents have been shown to influence the triggering of transient lower oesophageal sphincter relaxations. Most of these studies have only been published only in abstract form.

Somatostatin inhibits the meal-induced increase in the triggering of transient lower oesophageal sphincter relaxations in normal healthy volunteers. (374)

The 5-HT3 antagonists, ondansetron and granisetron reduce the rate of transient lower oesophageal sphincter relaxations induced by pressure-controlled gastric distension in dogs. (375)

8.6 Transient lower oesophageal sphincter relaxations in reflux disease

Whilst normal healthy volunteers reflux almost exclusively during transient lower oesophageal sphincter relaxations, patients with gastro-oesophageal reflux disease exhibit a greater degree of heterogeneity of reflux mechanisms. As discussed in chapter 7 of this thesis, in patients with gastro-oesophageal reflux disease, most reflux episodes occur during transient lower oesophageal sphincter relaxations, (247-249, 259) although low basal lower oesophageal sphincter pressure is an important mechanism in some patients. (248, 249, 260)

The manometric appearance of a transient lower oesophageal sphincter relaxation in patients with gastro-oesophageal reflux disease is indistinguishable from that seen in normal healthy volunteers. The criteria that have proved optimal for the definition of transient lower

oesophageal sphincter relaxations in normal healthy volunteers, also apply to patients with reflux disease. (313)

Patients with gastro-oesophageal reflux disease have been reported to have a higher rate of transient lower oesophageal sphincter relaxations than normal healthy subjects (164, 261) although the data are inconsistent. Several studies have reported frequencies of transient lower oesophageal sphincter relaxations, ranging from 3-8 per hour in reflux patients and 2-6 per hour in normal healthy human volunteers. (247, 261, 309, 311, 324) However, in some studies, the frequency of transient lower oesophageal sphincter relaxations is reported to be equal in reflux patients compared to normal subjects. (247, 376, 377) This discrepancy may be due to differences in the definition of transient lower oesophageal sphincter relaxation, experimental setting, posture and subject population.

It is possible that the increase in transient lower oesophageal sphincter relaxations seen in reflux disease is due to alterations in the mechanisms by which gastric distension triggers transient lower oesophageal sphincter relaxations. Whether patients with reflux disease have a greater gastric wall tension in response to distension or an exaggerated afferent response to gastric wall tension is unknown. Gastric wall tension could possibly be altered by a delay in gastric emptying, as seen in a proportion of patients with gastro-oesophageal reflux disease. (252, 272, 273) A recent study published in abstract form suggested that in patients with hiatus hernia and gastro-oesophageal reflux symptoms, the higher rate of transient lower oesophageal sphincter relaxations is due to deranged gastric anatomy, rather than any alteration in the gastric distension threshold for the triggering of transient lower oesophageal sphincter relaxations. (378)

A more consistent feature in patients with gastro-oesophageal reflux disease is a higher incidence of reflux during a transient lower oesophageal sphincter relaxation compared with normal subjects. In patients with reflux disease, 60-70% of transient lower oesophageal

sphincter relaxations are accompanied by reflux compared with 40-50% in normal subjects. (156, 164, 247, 249, 261)

The factors that determine the likelihood of reflux during a transient lower oesophageal sphincter relaxation are incompletely understood and have not been studied systematically. Important determinants of reflux during a transient lower oesophageal sphincter relaxation include abdominal straining, the presence of a hiatus hernia, degree of oesophageal shortening and proximal stomach mechanics, which have all been covered in chapter 7 of this thesis. In patients with gastro-oesophageal reflux disease, instillation of fat directly into the duodenum has been shown to increase the likelihood of reflux during a transient lower oesophageal sphincter relaxation, possibly by causing relaxation of the proximal stomach and thereby increased pooling of liquid in the proximal stomach, although this remains unproven. (325)

In patients with gastro-oesophageal reflux disease, the presence or absence of endoscopically proven erosive or reflux oesophagitis has no influence on the rate of postprandial transient lower oesophageal sphincter relaxations. (247, 248, 261) However, the effect of healing of oesophagitis on the rate of transient lower oesophageal sphincter relaxations is controversial. In one study in patients with severe reflux oesophagitis, the rate of postprandial transient lower oesophageal sphincter relaxations was unchanged after 6-8 weeks of omeprazole therapy, despite healing of oesophagitis. (256) In another study, however, in patients receiving a 3-6 month course of a H2 receptor antagonist, healing of oesophagitis was reported to lead to a reduction in postprandial transient lower oesophageal sphincter relaxations, although in that study, the definition and classification of transient lower oesophageal sphincter relaxations is questionable and the actual rates of transient lower oesophageal sphincter relaxations were not mentioned. (379) A 3 day course of cisapride, in patients with gastro-oesophageal reflux disease has no effect on the rate of postprandial transient lower oesophageal sphincter relaxations. (380)

Despite the large number of studies that have looked at the effect of fundoplication on lower oesophageal sphincter pressure, there are few studies examining the effect of fundoplication on transient lower oesophageal sphincter relaxations. In patients with gastro-oesophageal reflux disease, fundoplication causes a reduction in the frequency of transient lower oesophageal sphincter relaxations induced by gastric distension with air (381) or a meal. (382) In addition, fundoplication has been shown to reduce the proportion of transient lower oesophageal sphincter relaxations associated with reflux. (382) The mechanism whereby fundoplication influences transient lower oesophageal sphincter relaxations may be by its effects on the oesophagogastric junction or proximal stomach. Kiroff et al demonstrated that fundoplication produces an artificial high pressure zone at the oesophagogastric junction which fails to relax during transient lower oesophageal sphincter relaxations. (383) The fundic wrap may reduce the distensibility of the proximal stomach, (323) thereby reducing the stimulation of gastric mechanoreceptors known to influence gastric distension-induced transient lower oesophageal sphincter relaxations. Vu et al reported that in patients with gastro-oesophageal reflux disease, Nissen fundoplication reduces the volume and duration of postprandial proximal gastric relaxation as measured with an electronic barostat. (384) In a recent study published in abstract form, Scheffer et al demonstrated that fundoplication does not impair gastric accommodation, but does attenuate the transient lower oesophageal sphincter relaxation response to gastric distension, suggesting that the afferent response to gastric wall tension may be affected.

8.7 Summary

Current pharmacological treatment of gastro-oesophageal reflux disease is based on antacids, acid suppression or prokinetic therapy. However, these agents do not address the principal mechanism of reflux, which is transient lower oesophageal sphincter relaxation. Inhibition of transient lower oesophageal sphincter relaxations is a more physiologically attractive approach to the treatment of gastro-oesophageal reflux disease. There has recently been much progress in the understanding of the control of transient lower oesophageal sphincter

relaxations. Various pharmaceutical agents have been shown to influence the triggering of transient lower oesophageal sphincter relaxations. However, most of these agents are not clinically useful, either because of unacceptable side effects or because an orally effective formulation is not available. Better understanding of the neural control of transient lower oesophageal sphincter relaxations and the transmitters involved, may give rise to better targeted pharmacological therapy for the treatment of gastro-oesophageal reflux disease by controlling the triggering of transient lower oesophageal sphincter relaxations.

Chapter 9

Recording methods and data analysis

9.1 Perfusion manometry

9.1.1 Perfusion pump

All studies were performed in a custom built manometric laboratory, using standardised, validated perfusion manometry techniques. A low compliance pneumohydraulic capillary infusion pump, with a pump pressure of one atmosphere (100 kPa) was used. (385) Degassed, distilled water was used as a perfusate for each recording channel. The pump drives the perfusate out of a reservoir through a filter to remove any small particles and then along a manifold which has multiple side arms, one for each manometric line. The flow rate of the perfusate into the manometric line is controlled by a narrow bore hydraulic resistor, calibrated to provide a specific flow rate which is matched to the characteristics of the manometric channel. After leaving the resistors, the perfusate then passes through a pressure transducer and then into the lumina of the manometric catheter.

9.1.2 Manometric assemblies

All studies were performed using multilumen manometric assemblies constructed of silicone rubber. Each study used a slightly different, custom designed manometric assembly although they did share a number of similar features. The outer diameter of the catheters varied according to their design specifications but was less than or equal to 4.2 mm.

The manometric catheters had a number of side holes, to measure pressure in the pharynx, oesophageal body and stomach. The oesophageal body side holes were spaced either at 3 or 4 cm intervals, to obtain adequate mapping of oesophageal body motility.

A sleeve sensor was used to continuously measure pressure at the oesophagogastric junction. The sleeve sensor was reverse perfused to maximise recording fidelity. (386) The

catheter was fixed in position so that the middle of the sleeve sensor straddled the lower oesophageal sphincter.

The side holes and sleeve sensor were perfused with degassed distilled water. The perfusion rate varied between 0.15 ml/minute and 0.6 ml/minute, depending on the type of manometric assembly used.

9.1.3 Manometric technique

Each subject gave written informed consent and the protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital. All subjects were studied after an overnight fast. A cannula was inserted into the forearm vein for drug administration (in the studies described in chapters 10, 11 and 12) or for blood sampling (in the study described in chapter 13). With the subjects in a sitting position, the nasal mucosa was anaesthetised with xylocaine spray and the manometric assembly and pH electrode were passed via the nose. During intubation, subjects were instructed to swallow small sips of water, to facilitate passage of the assembly tip past the pharynx. Once the manometric assembly was inserted, it was adjusted to the correct position according to standard manometric criteria. Subjects were then allowed to accommodate to the tube before recordings were commenced.

At the start of each study, the hydraulic resistors were checked for correct flow rate. The pressure transducers were calibrated using atmospheric pressure as 0 mmHg and to a pressure of 80 mmHg and the baseline checked for baseline drift at 30 minute intervals during the study. Manometric lines were checked for air bubbles and routine debubbling was performed by mechanically flicking all connectors and water flushing. In the studies described in chapters 12 and 13, carbon dioxide flushing of the manifold and manometric lines was performed. With this procedure, carbon dioxide displaces the air and water in the lines and any residual carbon dioxide then dissolves in the water perfusate, leaving no bubbles.

At the end of the study, the manometric assembly was removed and perfusion baselines and transducer baselines checked, to calculate pressure offset between channels and to detect the presence of any signal drift.

9.2 Oesophageal pH

Oesophageal pH was measured with an antimony electrode (Synectics medical AB, Stockhom, Sweden) positioned 5 cm above the proximal margin of the lower oesophageal sphincter. Calibration of the pH electrode was performed using pH1 and pH7 buffer and then rechecked at the end of the study. If any signal drift was detected, it was assumed to have occurred in a linear fashion over the duration of the study and the pH value was appropriately corrected during subsequent analysis. However, if the signal drift was greater than 1 pH unit, then the pH tracing was excluded from the analysis.

9.3 Gastric barostat

Proximal gastric function was measured with an electronic barostat (Distender Series II G&J Electronics Inc., Willowdale, Ontario, Canada) using strict, standardised, barostat procedure guidelines. (387) The barostat consisted of a rigid cylinder that introduced or withdrew air from a polyethylene bag positioned in the proximal stomach. The electronic barostat maintains a constant pressure in the bag by inflation or deflation of air. (388, 389) When the stomach dilates, air is injected into the bag to maintain the pressure. When the stomach contracts, the barostat aspirates air from the bag and the intrabag volume decreases. Thus, the barostat measures gastric contraction and relaxation as changes in intragastric volume. The barostat is also able to inject a fixed volume of air into the bag and measure the corresponding intrabag pressure.

The lumina that were used by the barostat for delivery of air into the bag and sensing of pressure within the bag were incorporated within the manometric assembly. The polyethylene bag had a capacity of approximately 1100 ml. The proximal portion of the polyethylene bag was tied to the manometric assembly 60 mm distal to the sleeve sensor. The cylinder introduced or withdrew air from the bag at 30 ml/second via an oval channel measuring 1.9 x 2.4 mm internal diameter and 1570 mm in length. Pressure in the bag was sensed via a lumen of 0.6 mm internal diameter that opened directly into the bag.

9.4 Data acquisition

In the studies described in chapter 10 and 11, pressures sensed by the external pressure transducers and pH measurements were recorded by a 12 channel polygraph recorder (model 7D; Grass Instruments, Quincy, Massachusetts, USA) at a paper speed of 100 mm/minute. In the study described in chapter 12, pressure and pH data were digitised at 10 Hz using a NBMI016 A-D board (National Instruments, Austin, Texas). Barostat data were acquired at 1 Hz via a serial interface and together with the pH and manometric data, displayed and stored in a Macintosh computer (Apple Computer Inc., Cupertino, California, USA) using a custom written program in Labview (National Instruments) and then transcribed into AcqKnowledge (Biopac Systems, Santa Barbara, California, USA) for subsequent analysis. In the study described in chapter 13, pressure and pH data were digitised and then displayed, stored and analysed in a Macintosh computer using AcqKnowledge software.

9.5 Data analysis

9.5.1 Oesophageal manometry

Basal lower oesophageal sphincter pressure was measured at end expiration and referenced to intragastric pressure. A visual mean was taken at one minute intervals and a grand mean calculated for the recording period epochs.

Transient lower oesophageal sphincter relaxations were defined and counted separately according to published criteria. (313) Transient lower oesophageal sphincter relaxation was defined as an abrupt (≥ 1 mmHg/sec) fall in lower oesophageal sphincter pressure to a nadir pressure of ≤ 2 mmHg in ≤ 10 seconds not associated with swallowing within 4 seconds before or 2 seconds after the onset of lower oesophageal sphincter relaxation. In addition, abrupt lower oesophageal sphincter relaxations to a nadir pressure of ≤ 2 mmHg lasting ≥ 10 seconds were classified as transient lower oesophageal sphincter relaxations, irrespective of the timing of swallowing (excluding multiple rapid swallows). During this part of the analysis, the pH recording was masked. Subsequently, the pH recording was unmasked so that the occurrence of acid reflux during transient lower oesophageal sphincter relaxations could be determined.

For primary peristalsis, the duration of oesophageal pressure waves at each recording site was determined. The amplitude of oesophageal pressure waves at each recording site and the latency of wave onset between adjacent recording sites were determined for both primary and secondary peristalsis. (63) Amplitude was measured from basal end-expiratory intra-oesophageal pressure to the peak of the pressure wave. The onset of the major upstroke of the pressure wave was used as the reference point for determination of the wave duration and latency. Amplitude was calculated for all responses irrespective of whether they were classified as peristaltic or not, whereas velocity was calculated only for responses classified as being peristaltic.

Primary peristalsis was classified as successful if a propagated pressure wave of ≥ 12 mmHg at the proximal two or three (depending on the manometric catheter used) oesophageal sites and ≥ 25 mmHg in the distal oesophageal sites traversed all of the oesophageal recording sites and peristaltic velocity between adjacent recording sites was less than 6 cm/second. (38, 40) Criteria for failed peristalsis were either failure of a pressure wave ≥ 12 mmHg in the proximal oesophageal sites and ≥ 25 mmHg at the distal sites to

traverse each of the oesophageal recording sites, or synchronous pressure waves occurring at two or more recording sites. Secondary oesophageal motor responses were analysed according to the criteria for primary peristalsis. The rate of spontaneous swallowing was determined by counting the pharyngeal pressure waves.

9.5.2 pH data analysis

Acid reflux was defined as a fall in oesophageal pH below 4 for at least four seconds or, if basal oesophageal pH was already below 4, a further fall in pH of at least 1 pH unit. The onset of the drop in oesophageal pH was used as the reference time for analysis of the motor events associated with reflux. For each reflux episode the mechanism of reflux was determined from the pattern of lower oesophageal sphincter pressure and oesophageal body activity and their relationship to swallowing, and the occurrence of abdominal straining. (248, 249)

9.5.3 Barostat data analysis

During pressure-controlled and volume-controlled distensions, mean values for intrabag volume and pressure, respectively, were calculated for the second minute of each two minute distension step, thereby allowing one minute for equilibration. Minimum distending pressure (MDP) was defined as the first intrabag pressure at which the mean intrabag volume was greater than 30 ml and continuous respiratory fluctuations were first detected. Pressures were expressed as mmHg above MDP. Measured volume was corrected for the effects of air compressibility using an experimentally derived constant which also included a component related to internal compliance of the barostat unit. Gastric compliance was calculated as the ratio of change of pressure to change of volume in the barostat bag (dV/dP) for each individual subject during pressure-controlled distension.

Fasting tone was defined as the mean intrabag volume during a preprandial observation period. Postprandial changes in gastric tone were determined as the change in intrabag volume from mean fasting volume. Maximal postprandial relaxation was defined as the maximal and uniform (variations less than 30 ml) increase in intrabag volume observed after the meal. (390)

9.5.4 Symptom assessment

In the studies described in chapters 12 and 13, perception of the sensations of fullness, nausea and hunger were quantified using a validated 100 mm visual analogue scale. (391) In the barostat study described chapter 12, the additional sensation of abdominal discomfort was assessed because an intragastric barostat bag was used. In the study described in chapter 13, the additional sensations of dizziness, sleepiness and tiredness were assessed because of potential drug side effects. The visual analogue scales are lines without divisions on them, but with clearly delineated end points used to define the two ends of the scale. (eg, "no fullness at all" vs "extreme fullness") Visual analogue scales were used to assess symptoms as they show greater sensitivity and less psychological bias than asking subjects to simply indicate the presence or absence of a sensation. (387)

Chapter 10

Effect of atropine on gastro-oesophageal reflux and transient lower oesophageal sphincter relaxations in patients with gastro-oesophageal reflux disease

10.1 Introduction

Mittal et al have shown recently that atropine substantially inhibits gastro-oesophageal reflux in normal subjects by inhibition of transient lower oesophageal sphincter relaxations. (243, 332) Previous studies on the effects of atropine on reflux in patients with reflux disease, however, are few and the results conflicting. Atropine has been reported to increase the incidence of reflux during abdominal compression, (117) but decrease the rate of reflux episodes during postprandial oesophageal pH monitoring. (392) Neither of these studies, however, examined reflux mechanisms. Because gastro-oesophageal reflux occurs during absent basal lower oesophageal sphincter pressure in a significant minority of patients, the effects of atropine may not be the same as that seen in normal healthy subjects. The aim of this study, therefore, was to investigate the effects of atropine on the rate and mechanisms of reflux in patients with gastro-oesophageal reflux disease.

10.2 Methods

10.2.1 Subjects

Studies were performed in fifteen patients (11 males, 4 females) with gastro-oesophageal reflux disease defined by either erosive or ulcerative oesophagitis (Grade 2 - 10 patients, Grade 4 - 1 patient) proven at endoscopy, (258) or excessive oesophageal acid exposure (pH<4 for >5% of total time) on 24 hour ambulatory pH monitoring (4 patients). The median age of the patients was 48 years (range 32 to 69 years). Eight patients had a hiatus hernia proven endoscopically or during oesophageal manometry; in two patients the hernia was > 5 cm in length. Patients with a history of previous gastric surgery or other systemic disease known to influence reflux such as scleroderma were excluded from the study. None of the patients was taking medications known to influence oesophageal motor function, and acid suppression medications were stopped at least 72 hours before the study.

10.2.2 Recording methods

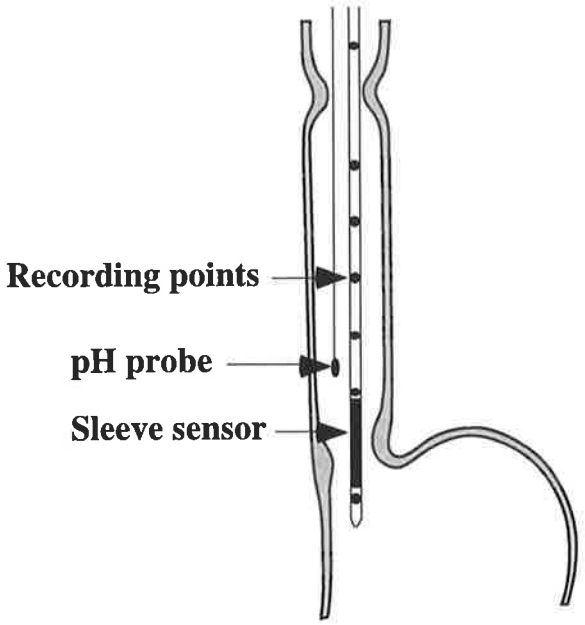

Figure 10.1 *Diagrammatic illustration of the manometric assembly and pH probe.*

An eight lumen manometric assembly incorporating a 6 cm sleeve sensor was used (Figure 10.1). A side hole in the pharynx recorded swallowing and a side hole located 1 cm distal to the sleeve sensor recorded gastric pressure. Side holes spaced at 4 cm intervals starting at the proximal margin of the sleeve sensor, monitored pressures at 5 sites along the oesophageal body. The gastric and oesophageal side holes and sleeve sensor were perfused with degassed distilled water at 0.6 ml/minute and the pharyngeal side hole at 0.3 ml/minute. Oesophageal pH was measured with an antimony electrode positioned 5 cm above the proximal margin of the lower oesophageal sphincter.

10.2.3 Study protocol

The protocol is summarised in Figure 10.2. Subjects were studied after an overnight fast. The manometric assembly and pH electrodes were passed via the nose. A cannula was inserted into the forearm vein for subsequent administration of saline or atropine. The subjects were allowed to accommodate to the assembly for 10 minutes whilst in the right

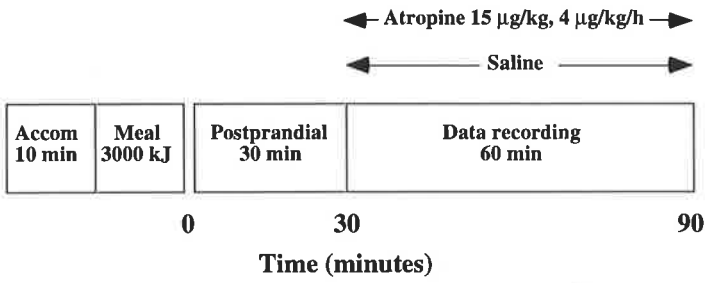

Figure 10.2 *Protocol for each study day.*

lateral position. The subjects then sat up and ate a 3000 kJ (750 kcal) soft mixed nutrient meal, consisting of savoury minced meat, mashed vegetables, 150 ml of milk and ice cream. Thirty minutes after the meal the subjects lay on a bed in the right lateral position. On separate days at least one week apart, i.v. atropine (15 µg/kg bolus, 4 µg/kg/h infusion) or saline were then given in randomised order and maintained for the subsequent 60 minute recording period. During the infusion period, the pulse and blood pressure were recorded every 15 minutes and the subjects were asked about symptoms such as blurred vision and dry mouth. Because of the potential for atropine to impair oesophageal acid clearance, thereby making it difficult to identify reflux episodes, a 5 ml oral bolus of 1.4 % bicarbonate was given if oesophageal pH did not return to a level of pH>4 within two minutes of an acid reflux event in order to aid oesophageal clearance of the acid refluxate. Ten water swallows of a 5 ml bolus were performed at the end of the 60 minute recording period.

10.2.4 Data analysis

Manometric data were analysed as described in section 9.5.1. A visual mean of the basal lower oesophageal sphincter pressure was taken at one minute intervals and a grand mean for the 60 minute recording period was calculated. Acid reflux episodes were defined, and for each reflux episode, the mechanism of reflux was determined. Transient lower oesophageal sphincter relaxations were defined and counted separately according to criteria described in section 9.5.1. In addition, when basal lower oesophageal sphincter pressure was 2 mmHg

or less, the lower oesophageal sphincter pressure tracing was analysed for episodes of transient inhibition of the crural diaphragm as evidenced by loss of respiratory increases in lower oesophageal sphincter pressure recorded by the reverse perfused sleeve sensor. (91, 243) Primary peristalsis in response to the ten water swallows was analysed for success rate, mean amplitude, and peristaltic velocity. The rate of spontaneous swallowing was determined by counting the pharyngeal pressure waves.

10.2.5 Statistical analysis

Data for reflux episodes and transient lower oesophageal sphincter relaxations were analysed using the Wilcoxon signed rank test and are presented as median (interquartile range). All other data were analysed using the paired *t* test and are presented as mean ± SEM. A p value of <0.05 was accepted as indicating statistical significance.

10.3 Results

All patients reported symptoms and exhibited signs of cholinergic blockade during atropine infusion. The mean pulse rate during atropine (98±2 beats/min) was significantly higher than that during saline infusion (74±2 beats/min, $p<0.001$). The rate of swallowing during atropine infusion (84±2 / h) was similar to that during saline (77±9 / h, $p=0.5$)

Basal lower oesophageal sphincter pressure

Atropine significantly reduced mean basal lower oesophageal sphincter pressure from 7.1±2.2 mmHg to 2.9±1.3 mmHg ($p<0.01$) and increased the proportion of time that lower oesophageal sphincter pressure was ≤ 2 mmHg from 40±9 % to 69±9 % ($p<0.05$) (Figure 10.3).

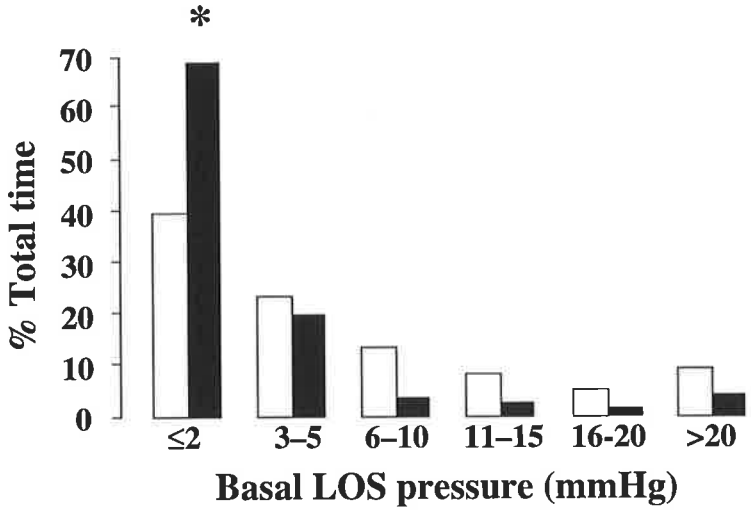

Figure 10.3 *Effect of atropine on basal lower oesophageal sphincter (LOS) pressure. Data have been grouped into intervals of pressure and expressed as a percentage of the total recording time. Atropine significantly increased the proportion of time that lower oesophageal sphincter pressure was ≤2 mmHg. Open bar = control; solid bar = atropine.*
** $p<0.05$ vs control (saline).*

Gastro-oesophageal reflux

Atropine significantly reduced the frequency of reflux episodes from a median of 5.0 (2.0-8.75) per hour to 1.0 (0-6.25) per hour ($p<0.05$), (Figure 10.4). During the saline infusion 92 reflux episodes were scored in the fifteen subjects. Overall, the predominant reflux mechanisms were transient lower oesophageal sphincter relaxations (40 episodes) and absent basal lower oesophageal sphincter pressure (35 episodes); 16 reflux episodes occurred during swallow-induced lower oesophageal sphincter relaxation and one during straining (Figure 10.5). However, there was heterogeneity among the patients with regard to the predominant mechanism of reflux; 8 patients refluxed almost exclusively during transient lower oesophageal sphincter relaxations and 4 patients almost exclusively during periods of absent basal lower oesophageal sphincter pressure (Figure 10.6).

During atropine infusion 42 reflux episodes were scored in the 15 subjects; 40 were due to absent lower oesophageal sphincter pressure and 2 were associated with abdominal

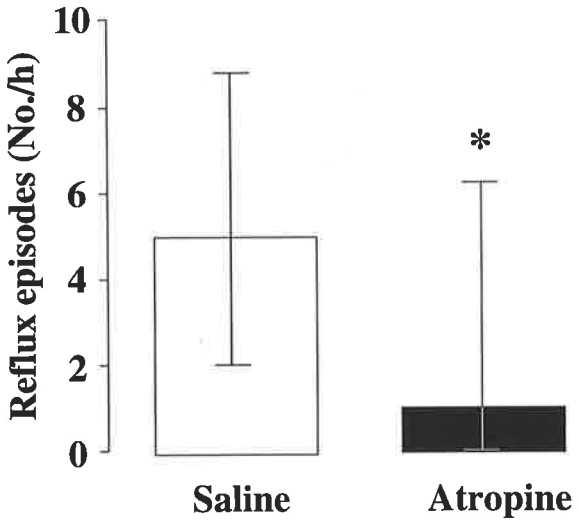

Figure 10.4 *The effect of atropine on the number of reflux episodes. Data are depicted as median (interquartile range). * p<0.05 vs control (saline).*

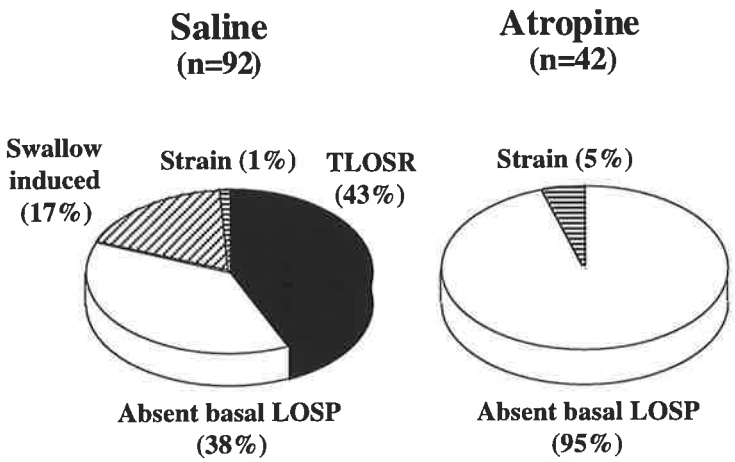

Figure 10.5 *Mechanism of reflux during saline and atropine infusion. The data for each group have been pooled and the numbers in parentheses indicate the percentage of reflux episodes. LOSP, lower oesophageal sphincter pressure; TLOSR, transient lower oesophageal sphincter relaxation.*

straining. None of the reflux episodes during atropine infusion could be attributed to transient lower oesophageal sphincter relaxations or swallow-induced lower oesophageal sphincter relaxation (Figures 10.5 and 10.6). Of the 8 patients who, during saline, refluxed mostly during transient lower oesophageal sphincter relaxations, 4 patients exhibited no

reflux and 4 patients continued to reflux because of absent basal lower oesophageal sphincter pressure as did the patient who refluxed predominantly during swallow-induced lower oesophageal sphincter relaxation during saline infusion. However, atropine inhibited reflux in 2 of the 4 patients who refluxed predominantly during periods of absent basal lower oesophageal sphincter pressure.

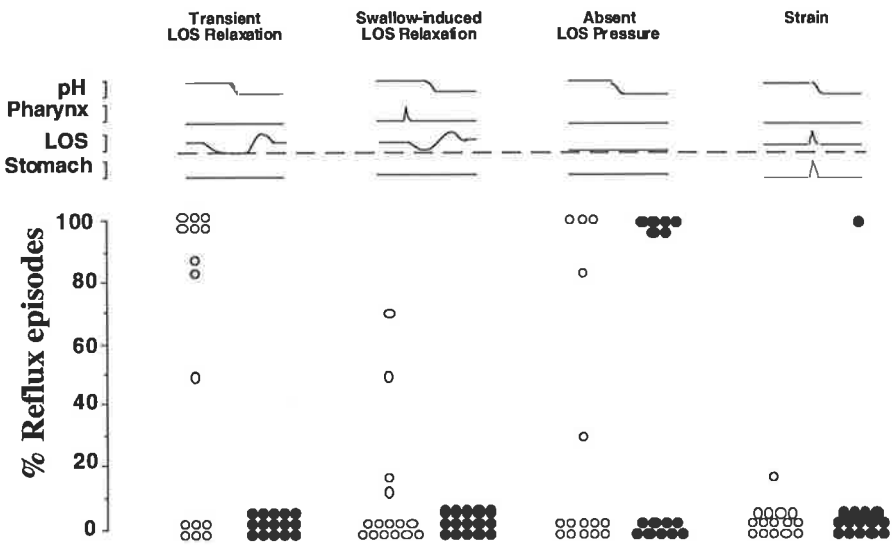

Figure 10.6 *Effect of atropine on the mechanisms of reflux. Each point represents the proportion of reflux episodes in an individual patient that is attributable to a particular mechanism. LOS, lower oesophageal sphincter. Open circles, saline; closed circles, atropine.*

Transient lower oesophageal sphincter relaxation

Atropine significantly reduced the frequency of transient lower oesophageal sphincter relaxations from a median of 2.0 (0-4.75) per hour to 0 (0-0) per hour ($p<0.01$), (Figure 10.7). This effect remained significant even after the time during which basal lower oesophageal sphincter pressure was ≤2 mmHg was excluded from the analysis. During saline infusion there were 49 transient lower oesophageal sphincter relaxations, of which 40 (82%) were associated with reflux. During atropine infusion there were only two transient lower oesophageal sphincter relaxations, both of which occurred in the one patient, neither of which was associated with a reflux event. No episodes of transient inhibition of the crural

diaphragm were seen during periods of low (≤2 mmHg) basal lower oesophageal sphincter pressure.

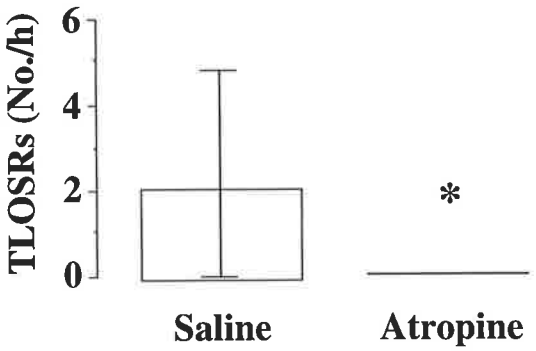

Figure 10.7 *The effect of atropine on the number of transient lower oesophageal sphincter relaxations. The data are depicted as median and interquartile range. TLOSRs, transient lower oesophageal sphincter relaxations. * p<0.01 vs control (saline).*

Primary peristalsis

Atropine had no effect on the degree of swallow-induced lower oesophageal sphincter relaxation with water swallows. Nadir lower oesophageal sphincter pressure during saline (1.2±0.3 mmHg) was similar to that seen during atropine administration (0.5±0.3 mmHg p=0.31). However, atropine significantly affected primary peristalsis; it reduced the rate of peristaltic success and wave amplitude, and increased peristaltic velocity in the distal oesophagus (Table 10.1) .

	Saline	**Atropine**
Peristaltic success (%)	48 (9)	9 (6) *
Distal Amplitude (mmHg)	55 (8)	27 (5) *
Distal Velocity (cm/sec)	2.6 (0.2)	3.6 (0.4) *

Table 10.1 *Effect of atropine on primary peristalsis . Data expressed as mean (SEM), *p<0.05 vs saline.*

10.4 Discussion

Mittal et al have shown recently that atropine substantially inhibits reflux in normal subjects by inhibition of transient lower oesophageal sphincter relaxations. (243, 332) Whether or not atropine would also inhibit reflux in patients with reflux disease, however, was not clear. Whilst normal subjects reflux almost exclusively during transient lower oesophageal sphincter relaxations, patients with reflux disease exhibit a greater degree of heterogeneity of reflux mechanisms. Although most patients reflux predominantly during transient lower oesophageal sphincter relaxations, (164, 247, 248) reflux during periods of absent basal lower oesophageal sphincter pressure is the most common mechanism of reflux in a minority of patients. (249) A low basal lower oesophageal sphincter pressure induced by atropine is insufficient to promote free reflux in healthy subjects. (243) However, atropine could conceivably aggravate reflux in some patients by reduction of an already low basal lower oesophageal sphincter pressure to a level below the threshold for gastro-oesophageal competence, or merely alter the mechanism of reflux from transient lower oesophageal sphincter relaxation to absent basal lower oesophageal sphincter pressure without changing significantly the overall rate of reflux episodes. The present study has shown that, similar to the effect in normal subjects, atropine also inhibits reflux in patients with reflux disease largely by inhibition of transient lower oesophageal sphincter relaxations as well as by inhibition of reflux during swallow-induced lower oesophageal sphincter relaxation.

Atropine almost completely inhibited transient lower oesophageal sphincter relaxations and totally abolished reflux during both transient and swallow-induced lower oesophageal sphincter relaxations. Although atropine also reduced basal lower oesophageal sphincter pressure, the effect of atropine on transient lower oesophageal sphincter relaxations cannot be explained merely on the basis of a reduction in basal lower oesophageal sphincter pressure to a level at which transient lower oesophageal sphincter relaxations could not be scored or recognised as the effect was significant even when the time during which basal lower oesophageal sphincter pressure was absent was excluded from the analysis.

Moreover, even during periods of low basal lower oesophageal sphincter pressure, there were no episodes of transient inhibition of the crural diaphragm that are useful markers for transient lower oesophageal sphincter relaxations. (243) Likewise, the unexpected reduction in reflux during swallow-induced lower oesophageal sphincter relaxation was not due to a reduction in the rate of swallowing.

The inhibitory effect of atropine on reflux in patients with reflux disease was less marked than that seen previously in normal subjects. (243) The most plausible explanation is that transient lower oesophageal sphincter relaxations were not the only mechanism of reflux in the patients. As patients with reflux oesophagitis reflux through a variety of mechanisms, one would not expect atropine to reduce reflux in all patients. Atropine might only be expected to reduce the frequency of reflux in those patients who reflux predominantly during transient lower oesophageal sphincter relaxations and not be of benefit in those patients who have a very low resting lower oesophageal sphincter pressure and high degree of free reflux. In keeping with this notion, there was no change in the number of reflux episodes occurring during absent basal lower oesophageal sphincter pressure although reflux during transient and swallow-induced lower oesophageal sphincter relaxations was totally abolished. The lack of apparent effect on reflux during absent basal lower oesophageal sphincter pressure was the result of a change of reflux mechanism from that of transient lower oesophageal sphincter relaxation to that of absent basal lower oesophageal sphincter pressure in 4 patients, balanced partly by the abolition of reflux during absent basal lower oesophageal sphincter pressure in two patients.

The effect of atropine on basal lower oesophageal sphincter pressure was more marked in patients with reflux disease than in the previous study in normal subjects. The reasons for this are not clear but it may relate to defective vagal cholinergic innervation to the oesophagus. Experimental oesophagitis is associated with impaired cholinergic innervation to the lower oesophageal sphincter in cats. (393) About 40% of patients with reflux disease have abnormal parasympathetic cardiovascular reflexes (251, 252) and the gastric secretory

response to insulin is impaired in some patients with reflux disease (250) suggesting abnormal vagal function in these patients.

Reflux was not seen during periods of low basal lower oesophageal sphincter pressure induced by atropine in normal subjects. (243) This contrasts with the findings in this study in patients with reflux disease, in some of whom atropine provoked reflux by this mechanism. This difference might have resulted from the greater effect of atropine on basal lower oesophageal sphincter pressure in the reflux patients. However, half of the patients in this study had hiatus hernias. Loss of the extrinsic diaphragmatic support could potentially aggravate the effects of low or absent lower oesophageal sphincter pressure on gastro-oesophageal competence. (267)

Anticholinergic agents have previously been regarded as being contra-indicated in patients who are prone to gastro-oesophageal reflux disease, because of their effect on reducing lower oesophageal sphincter pressure. (59, 119) Reports on the effect of anticholinergic agents on reflux, however, have been conflicting. Atropine has been reported not to increase reflux in normal volunteers (394) or in pregnant women. (395) In normal volunteers, the anticholinergic agent, dicyclomine has been reported to decrease reflux episodes in the early postprandial upright body position, but increase reflux in the supine position. (396) Information on the effect of anticholinergic agents on reflux in patients with gastro-oesophageal reflux disease is limited. In a scintigraphic study, atropine has been reported to increase reflux provoked by abdominal compression. (117) In contrast, a single subcutaneous dose of atropine was found to decrease the frequency of reflux as measured by pH monitoring during the first postprandial hour. (392) These inconsistencies are probably a result of differences in methodology, dose and route of atropine administration among the studies.

The mechanism of action of atropine in reducing gastro-oesophageal reflux and transient lower oesophageal sphincter relaxations is speculative and is further addressed in the studies

described in chapters 11 and 12 of this thesis. It is possible that atropine reduces gastric tone and thereby alters the stimulus to the gastric mechanoreceptors. Atropine decreases proximal gastric tone in the fasting state. (211) However, although atropine has been reported to have no effect on postprandial proximal gastric tone (210) the dose of atropine used was substantially lower than that used in the present study. The study described in chapter 12 of this thesis was designed to investigate the effect of atropine on the proximal gastric motor and sensory function, in order to gain further insight into the mechanism by which atropine inhibits transient lower oesophageal sphincter relaxations. It is also possible that atropine is acting centrally on the integrative mechanisms in the brainstem that are believed to mediate transient lower oesophageal sphincter relaxations. In support of this hypothesis is the recent observation that atropine also inhibits lower oesophageal sphincter relaxations induced by pharyngeal stimulation. (332) The study described in chapter 11 of this thesis was designed to further explore the role of central and peripheral cholinergic mechanisms involved in the triggering of transient lower oesophageal sphincter relaxations. It seems unlikely, however, that atropine affects the motor limb of the putative vago-vagal reflex arc that is believed to mediate transient lower oesophageal sphincter relaxations (135) as, similar to findings of previous studies, (59) atropine had no effect on swallow-induced lower oesophageal sphincter relaxation. The reduction in reflux during swallow-induced lower oesophageal sphincter relaxation, however, may indicate a more subtle interference with the motor pathway controlling lower oesophageal sphincter relaxation.

Pharmacological reduction in the rate of transient lower oesophageal sphincter relaxations is a physiologically attractive approach to the treatment of reflux disease. Although the reduction in reflux episodes was less substantial than that seen in normal subjects, consistent with the previous study in normal subjects there was complete abolition of reflux during transient lower oesophageal sphincter relaxations. This finding suggests the possibility of pharmacological control of reflux by inhibition of transient lower oesophageal sphincter relaxations. In patients with reflux disease, the effect of such an approach is likely to be influenced by the mix of reflux mechanisms in individual subjects. However, as the majority

of patients with reflux disease have no macroscopic oesophagitis and are therefore likely to reflux predominantly during transient lower oesophageal sphincter relaxations (248) effective pharmacological control of transient lower oesophageal sphincter relaxations is likely to be useful in the majority of patients. Atropine or other non-selective anticholinergic agents are not themselves appropriate potential therapeutic agents in this regard because of their deleterious effects on basal lower oesophageal sphincter pressure and oesophageal clearance. However, the findings point the way to development of other drugs that have more selective effects on triggering of transient lower oesophageal sphincter relaxations.

Chapter 11

Effect of peripheral cholinergic blockade on gastro-oesophageal reflux and transient lower oesophageal sphincter relaxations in normal subjects

11.1 Introduction

Data presented in chapter 10 of this thesis show that atropine substantially inhibits gastro-oesophageal reflux in patients with gastro-oesophageal reflux disease, predominantly by inhibition of transient lower oesophageal sphincter relaxations. The site at which atropine exerts this effect, however, is not known.

The major stimulus for triggering of transient lower oesophageal sphincter relaxations appears to be gastric distension. (319, 322) Current concepts hold that gastric distension activates a vago-vagal reflex pathway that is integrated in the brainstem possibly by the central pattern generator that controls swallowing. (135) Atropine is a non-specific muscarinic antagonist which crosses the blood-brain barrier (397) and all five of the muscarinic receptor subtypes (m1-m5) have been detected in the brain. (366-368) Atropine could therefore potentially act at two sites: peripherally, by altering the mechanical properties of the proximal stomach and thereby the activity of the gastric mechanoreceptors; and centrally, by inhibiting the central integrating mechanisms in the brainstem that are believed to mediate transient lower oesophageal sphincter relaxations.

Hyoscine butylbromide is a quaternary ammonium anticholinergic agent which does not cross the blood brain barrier (397, 398) (Boehringer-Ingelheim, personal communication). Such a compound should exert its effects almost entirely peripherally and have relatively little effect on central neural control mechanisms. The aim of this study, therefore, was to investigate the effects of selective peripheral muscarinic cholinergic inhibition by hyoscine butylbromide on the triggering of transient lower oesophageal sphincter relaxations in normal subjects.

11.2 Methods

11.2.1 Subjects

Studies were performed in ten healthy subjects (9 males, 1 female) aged 18 to 39 years (median 24 years). Subjects were free of gastrointestinal symptoms, had no history of upper gastrointestinal surgery and were not taking regular antacids or medications known to influence oesophageal motor function.

11.2.2 Recording methods

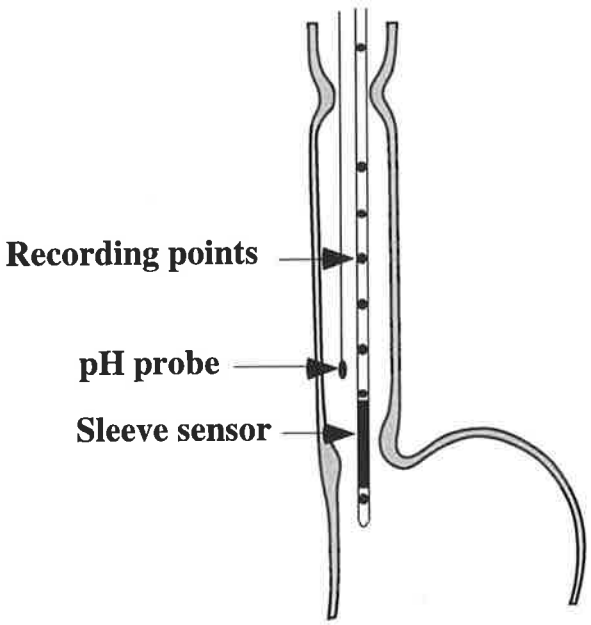

Figure 11.1 *Diagrammatic illustration of the manometric assembly and pH probe.*

A nine lumen manometric assembly incorporating a 6 cm sleeve sensor was used (Figure 11.1). A side hole in the pharynx recorded swallowing and a side hole located 1 cm distal to the sleeve sensor recorded gastric pressure. Side holes spaced at 3 cm intervals starting at the proximal margin of the sleeve sensor, monitored pressures at 6 sites along the oesophageal body. The gastric and oesophageal side holes and sleeve sensor were perfused with degassed distilled water at 0.6 ml/minute and the pharyngeal side hole at 0.3 ml/minute.

Oesophageal pH was measured with an antimony electrode positioned 5 cm above the proximal margin of the lower oesophageal sphincter.

11.2.3 Study protocol

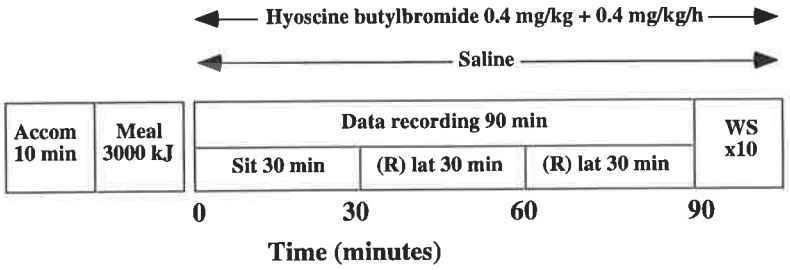

Figure 11.2 *Protocol for each study day. WS, water swallow.*

The protocol is summarised in Figure 11.2. Subjects were studied after an overnight fast. The manometric assembly and pH electrodes were passed via the nose. A cannula was inserted into the forearm vein for subsequent administration of saline or hyoscine butylbromide. The subjects were allowed to accommodate to the assembly for 10 minutes whilst in the right lateral position. The subjects then sat up and ate a 3000 kJ (750 kcal) soft mixed nutrient meal, consisting of savoury minced meat, mashed vegetables, 150 ml of milk and ice cream. Immediately after the meal, i.v. hyoscine butylbromide (0.4 mg/kg bolus, 0.4 mg/kg/h infusion) or saline were then given in randomised order on separate days at least one week apart and maintained for the subsequent 90 minute recording period. This dosage regimen was calculated using pharmacokinetic software (MacKinetics, Leon Moore, Department of Pharmacology, Uniformed Services University, Bethesda MD), and designed to rapidly achieve and maintain a high steady state serum concentration. Recordings were obtained for three 30 minute periods. Subjects sat upright for the first 30 minutes, and then lay on a bed in the right lateral position for a further two 30 minute periods. During the infusion period, the pulse and blood pressure were recorded every 15 minutes and the subjects were asked about symptoms such as blurred vision and dry mouth. Ten water swallows of a 5 ml bolus were performed at the end of the 90 minute recording period.

11.2.4 Data analysis

Manometric data were analysed as described in section 9.5.1. A visual mean of the basal lower oesophageal sphincter pressure was taken at one minute intervals and a grand mean was calculated for each 30 minute recording period. Acid reflux episodes were defined, and for each reflux episode, the mechanism of reflux was determined. Transient lower oesophageal sphincter relaxations were defined and counted separately according to criteria described in section 9.5.1. Primary peristalsis in response to the ten water swallows was analysed for success rate and wave amplitude. The rate of spontaneous swallowing was determined by counting the pharyngeal pressure waves.

11.2.5 Statistical analysis

Data for reflux episodes and transient lower oesophageal sphincter relaxations were analysed using the Wilcoxon signed rank test and are presented as median (interquartile range). All other data were analysed using the paired *t* test and are presented as mean ± SEM. A p value of <0.05 was accepted as indicating statistical significance.

11.3 Results

All subjects reported symptoms and exhibited signs of cholinergic blockade during hyoscine butylbromide infusion. The mean pulse rate during hyoscine butylbromide (92±2 beats/min) was significantly higher than that during saline infusion (71±2 beats/min, $p<0.0001$) (Figure 11.3). The overall mean rate of swallowing during hyoscine butylbromide infusion (40±5 swallows/30 min) was similar to that during saline (38±6 swallows/30 min, $p=0.49$).

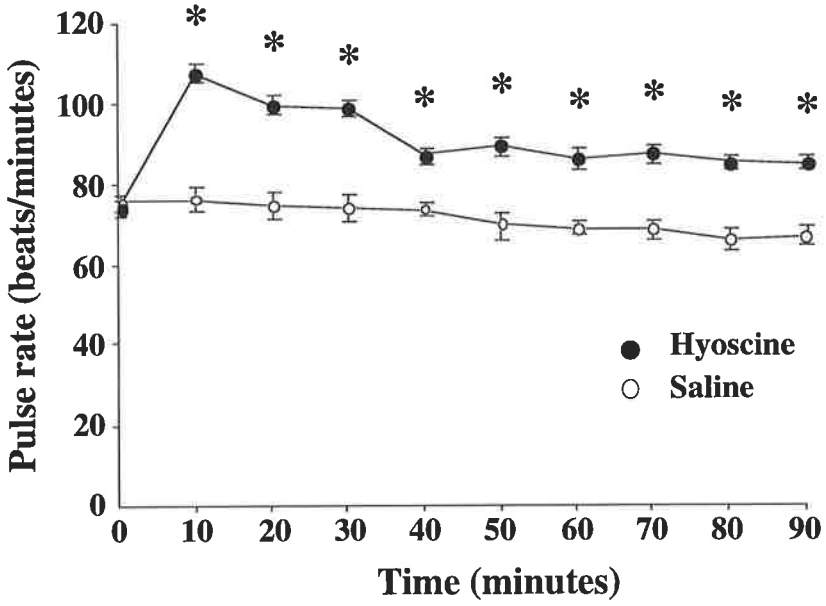

Figure 11.3 *Effect of hyoscine butylbromide on heart rate. Data are presented as mean±SEM. *p<0.001 vs control (saline)*

Basal lower oesophageal sphincter pressure

Hyoscine butylbromide significantly reduced mean basal lower oesophageal sphincter pressure during the first 30 minute postprandial period, from 8.4±0.8 mmHg to 4.1±0.7 mmHg ($p<0.001$). Although basal lower oesophageal sphincter pressure remained lower than that during saline for the remainder of the recording period, this difference was not statistically significant (Figure 11.4).

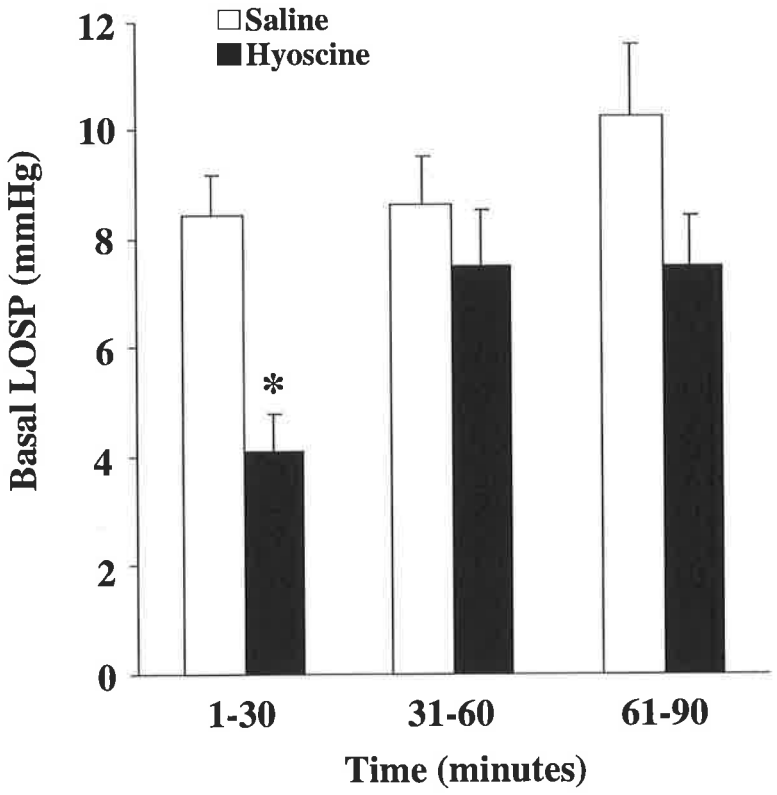

Figure 11.4 *Effect of hyoscine butylbromide on basal lower oesophageal sphincter pressure (LOSP). Data have been grouped into 30 minute periods. Data are presented as mean±SEM. *p<0.001 vs control (saline)*

Gastro-oesophageal reflux

On both study days, most acid reflux episodes occurred in the second and third half hour period, when the subjects were in the right lateral position in contrast to the first half hour period, when the subjects were sitting upright (Figure 11.5). The overall median rate during hyoscine butylbromide (1.5 episodes (1.0-2.0) /30 min) was not statistically different from that during placebo (1.8 episodes (1.0-2.5) /30 min).

During the saline infusion 53 reflux episodes were scored in the ten subjects (Figure 11.6). Overall, the predominant mechanism of reflux was transient lower oesophageal sphincter relaxations (50 episodes). Two reflux episodes occurred during swallow-induced lower oesophageal sphincter relaxation and one during straining. During hyoscine butylbromide

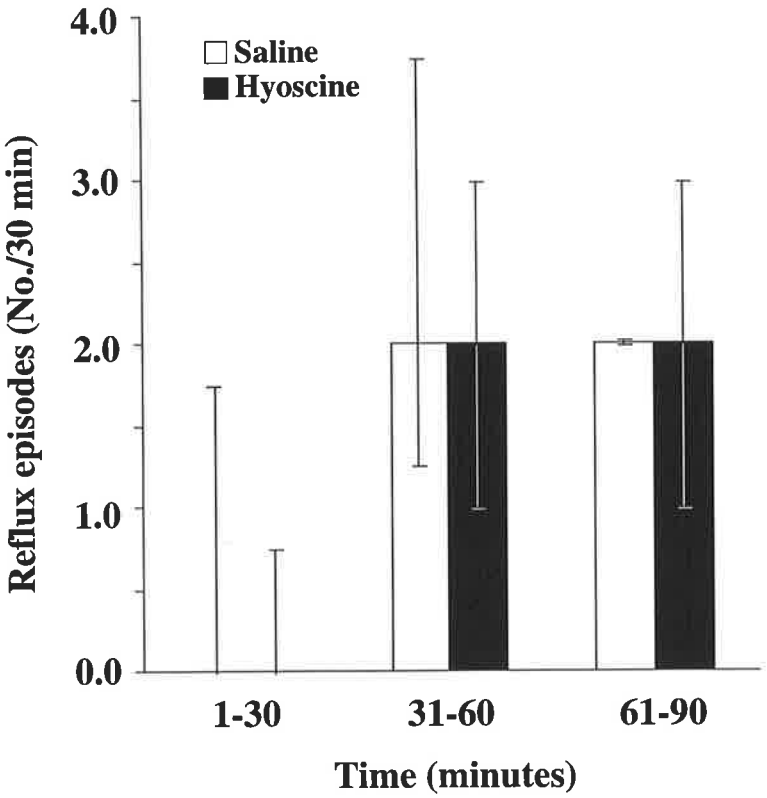

Figure 11.5 *Effect of hyoscine butylbromide on reflux episodes. Data have been grouped into 30 minute periods and are presented as median (interquartile range). The rate of reflux episodes was not influenced by hyoscine butylbromide.*

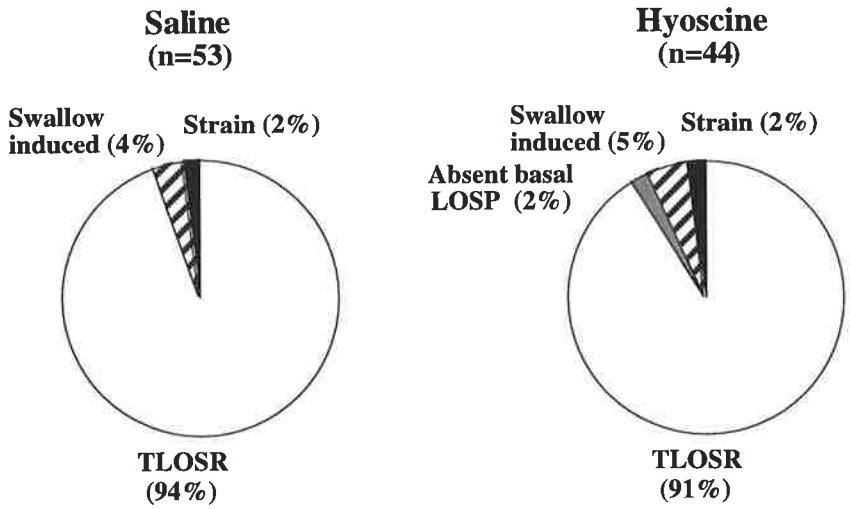

Figure 11.6 *Mechanism of reflux during saline and hyoscine butylbromide. The data are presented as a percentage of total number of reflux episodes for each group. LOSP, lower oesophageal sphincter pressure; TLOSR, transient lower oesophageal sphincter relaxation.*

infusion 44 reflux episodes were scored in the ten subjects; 40 were due to transient lower oesophageal sphincter relaxations, two occurred during swallow-induced lower oesophageal sphincter relaxation and one each was attributed to abdominal straining and to absent basal lower oesophageal sphincter pressure.

Transient lower oesophageal sphincter relaxation

Hyoscine butylbromide significantly inhibited the rate of transient lower oesophageal sphincter relaxations during the first 30 minute postprandial period from 3.5 (2.3-5.8) to 1.5 (1.0-2.0) /30 min ($p<0.02$) (Figure 11.7). However, the rates of transient lower oesophageal sphincter relaxations during hyoscine butylbromide and saline were similar during the remaining two 30 minute periods of the infusion.

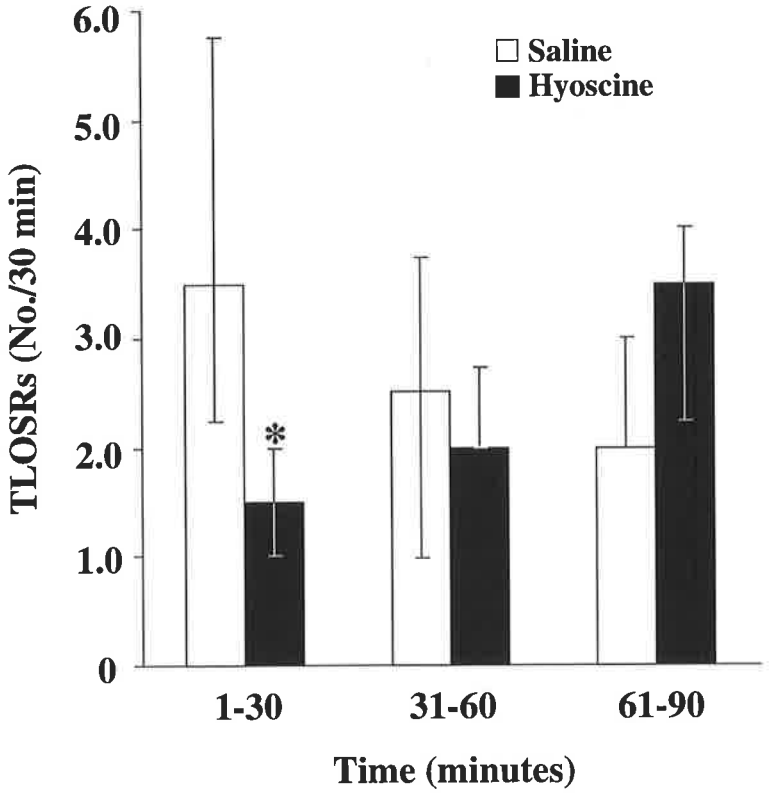

Figure 11.7 *Effect of hyoscine butylbromide on the number of transient lower oesophageal sphincter relaxations (TLOSRs). Data have been grouped into 30 minute periods and are presented as median (interquartile range). *p<0.02 vs control (saline)*

Hyoscine butylbromide had no effect on the proportion of transient lower oesophageal sphincter relaxations accompanied by acid reflux. During saline infusion there were a total of 89 transient lower oesophageal sphincter relaxations, of which 55 (62%) were associated with reflux. During hyoscine butylbromide infusion there were 71 transient lower oesophageal sphincter relaxations, of which 40 (56%) were associated with reflux.

Primary peristalsis

Hyoscine butylbromide had no effect on the degree of swallow-induced lower oesophageal sphincter relaxation with water swallows; nadir lower oesophageal sphincter pressure during saline infusion (0.3±0.4 mmHg) was similar to that seen during hyoscine butylbromide infusion (0.6±0.4 mmHg, $p=0.52$). However, hyoscine butylbromide significantly reduced the rate of primary peristaltic success (40% (30-60%) vs 95% (50-100%), $p<0.05$) and the peristaltic wave amplitude (39.3±4.9 vs 55.1±4.4 mmHg, $p<0.05$).

11.4 Discussion

This study was designed to further explore the role of peripheral cholinergic mechanisms in the triggering of transient lower oesophageal sphincter relaxations, by examining the effects of hyoscine butylbromide, a quaternary ammonium anticholinergic agent which does not cross the blood-brain barrier, on the rate and mechanisms of gastro-oesophageal reflux. In contrast to the previous findings with atropine in both normal subjects (243) and patients with reflux disease, (chapter 10 of this thesis) hyoscine butylbromide caused only a transient decrease in the rate of transient lower oesophageal sphincter relaxations and no effect on the rate of acid reflux episodes despite significant and sustained effects on primary peristalsis and heart rate.

The finding that hyoscine butylbromide had a transient inhibitory effect on the rate of transient lower oesophageal sphincter relaxations during the first 30 minutes after the meal, would lend support to the notion that the inhibitory effect of hyoscine butylbromide (and that of atropine) on transient lower oesophageal sphincter relaxations is, at least in part, mediated peripherally by altering the mechanics of the proximal stomach. However, cholinergic blockade inhibits gastric tone as shown in dogs, (208) cats, (399) and humans (chapter 12 of this thesis) and inhibition of gastric tone with sumatriptan has been shown to increase, rather than decrease the postprandial triggering of transient lower oesophageal sphincter relaxations, (372) presumably by stimulation of gastric mechanoreceptors. Therefore, the transient inhibitory effect of hyoscine butylbromide on the triggering of transient lower oesophageal sphincter relaxations is contrary to what might be expected if hyoscine butylbromide is exerting a peripheral effect on the proximal stomach. It is possible that hyoscine butylbromide is able to exert a central effect by gaining access to the brainstem centres controlling transient lower oesophageal sphincter relaxations. The vagal nuclei and the regions of the brainstem that contain the central pattern generator that are believed to control swallowing and transient lower oesophageal sphincter relaxations (135) lie close to the area postrema which lies outside the blood-brain barrier. (171) It is possible that

hyoscine butylbromide could have diffused through this area to the regions of the brainstem that control triggering of transient lower oesophageal sphincter relaxations, an effect perhaps facilitated by transient and substantially higher blood levels after the initial bolus injection of hyoscine butylbromide, as reflected by the significant initial reduction in basal lower oesophageal sphincter pressure, despite the use of an infusion in order to maintain steady state levels.

In contrast to the earlier findings with atropine, hyoscine butylbromide had no effect on the triggering of transient lower oesophageal sphincter relaxations beyond the first 30 minutes postprandially. These findings are consistent with those recently published by Fang et al using orally administered methscopolamine bromide, another peripherally acting anticholinergic agent (400) and lends indirect support for the notion that atropine is not acting peripherally to inhibit transient lower oesophageal sphincter relaxations. There are, however, other explanations as to why the effect of hyoscine butylbromide on the triggering of transient lower oesophageal sphincter relaxations was not sustained beyond the first 30 minutes postprandially. Hyoscine butylbromide has been shown to delay gastric emptying as measured by a radiolabelled semisolid test meal. (401) In this study, during hyoscine butylbromide infusion, a progressive increase in the rate of transient lower oesophageal sphincter relaxations was seen during the second and third 30 minute time periods compared to placebo infusion (Figure 11.7). Therefore, whilst gastric emptying and gastric wall tension were not measured, it is possible that the reduction in transient lower oesophageal sphincter relaxations during hyoscine butylbromide infusion was not observed beyond the first 30 minute time period, because of its deleterious effect on gastric emptying. It is also possible that the blood levels of hyoscine butylbromide were higher during the first 30 minutes than during the subsequent 60 minutes. Whilst hyoscine butylbromide plasma levels were not measured, the dosing regimen was calculated to give a steady state plasma level approximately 75% of the maximal level that would be achieved after a bolus intravenous dose at the recommended maximum dose (40 mg). The increased heart rate did persist throughout the hyoscine butylbromide infusion and at the end of the study there was

impaired oesophageal peristaltic function. Therefore, it is likely that hyoscine butylbromide concentration remained at therapeutic levels throughout the duration of the study.

In summary, this study has shown that, in contrast to the effects of atropine, peripheral cholinergic blockade with hyoscine butylbromide has only a transient inhibitory effect on transient lower oesophageal sphincter relaxations and no effect on reflux episodes. It is possible that anticholinergic agents reduce the rate of transient lower oesophageal sphincter relaxations by acting centrally on the putative regulatory centres in the brainstem and that hyoscine butylbromide only had a transient central effect in this study because of higher blood levels after the initial bolus injection, despite the use of an infusion to maintain steady state levels. However, the possibility remains, that inhibition of proximal gastric tone by anticholinergic agents might influence the triggering of transient lower oesophageal sphincter relaxations. Further exploration of the effect of atropine on the mechanics of the proximal stomach and triggering of transient lower oesophageal sphincter relaxations is outlined in chapter 12 of this thesis.

Chapter 12

Effect of atropine on proximal gastric motor and sensory function in normal subjects

12.1 Introduction

Data presented in chapter 11 of this thesis suggest that anticholinergic agents reduce the rate of transient lower oesophageal sphincter relaxations by acting centrally on the putative regulatory centres in the brainstem although the possibility remains, that inhibition of proximal gastric tone by anticholinergic agents might influence the triggering of transient lower oesophageal sphincter relaxations.

Data on the effect of atropine on the motor and sensory function of the proximal stomach are limited and findings have varied among the studies. In dogs and cats, atropine reduces fasting proximal gastric tone. (187, 208, 399) In humans, however, relatively low doses of atropine have not been shown to have any significant effect on fasting or postprandial proximal gastric tone. (210, 211)

The aim of this study was to investigate the effect of atropine on the proximal gastric motor and sensory function using an electronic barostat in normal healthy subjects in both the fasted and postprandial states, in order to gain further insight into the mechanism by which atropine inhibits transient lower oesophageal sphincter relaxations.

12.2 Methods

12.2.1 Subjects

Studies were performed in ten healthy subjects (8 males, 2 females) aged 19 to 39 years (median 25 years). Subjects were free of gastrointestinal symptoms, had no history of upper gastrointestinal surgery and were not taking regular antacids or medications known to influence oesophageal or gastric motor function.

12.2.2 Recording methods

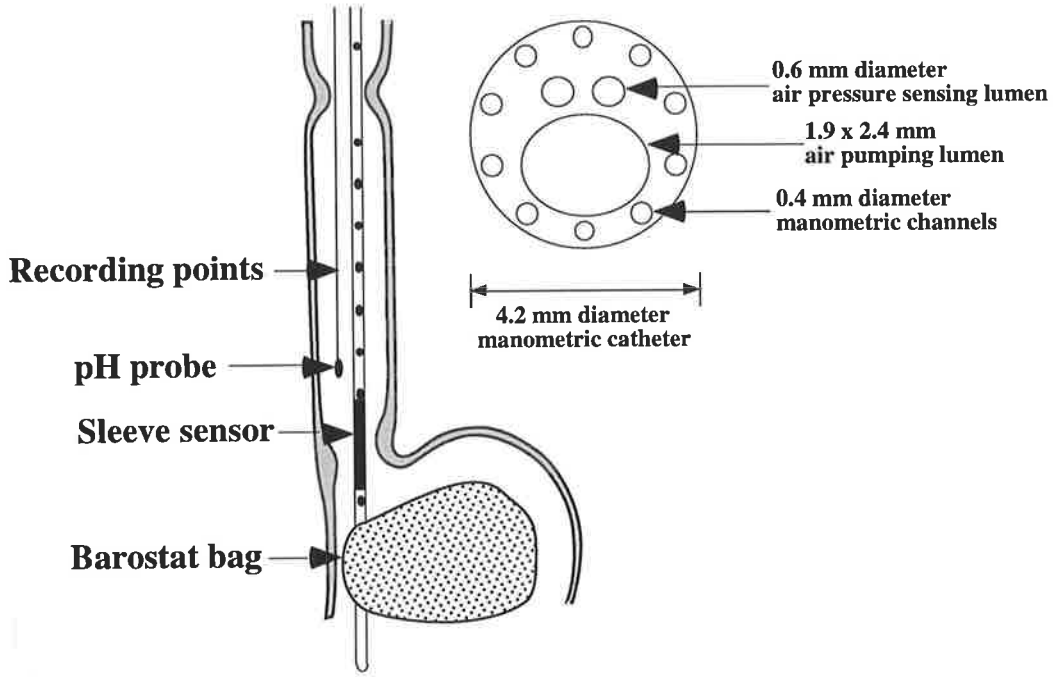

Figure 12.1 *Diagrammatic illustration of the combined manometric assembly used in the barostat study and a cross-sectional view of the assembly.*

A multilumen manometric assembly incorporating a 6 cm sleeve sensor was used (Figure 12.1). A side hole in the pharynx recorded swallowing and a side hole located 1 cm distal to the sleeve sensor recorded gastric pressure. Side holes spaced at 3 cm intervals starting at the proximal margin of the sleeve sensor, monitored pressures at 7 sites along the oesophageal body. All the side holes and sleeve sensor were perfused with degassed distilled water at 0.15 ml/minute. In addition, two lumina that were used by the barostat were incorporated into the manometric assembly. Oesophageal pH was measured with an antimony electrode positioned 5 cm above the proximal margin of the lower oesophageal sphincter.

Proximal gastric function was measured with an electronic barostat as described in detail in section 9.3 of this thesis.

Perception of the sensations of fullness, nausea, abdominal discomfort and hunger was quantified using a validated 100 mm visual analogue scale, as described in section 9.5.4 of this thesis.

12.2.3 Study protocol

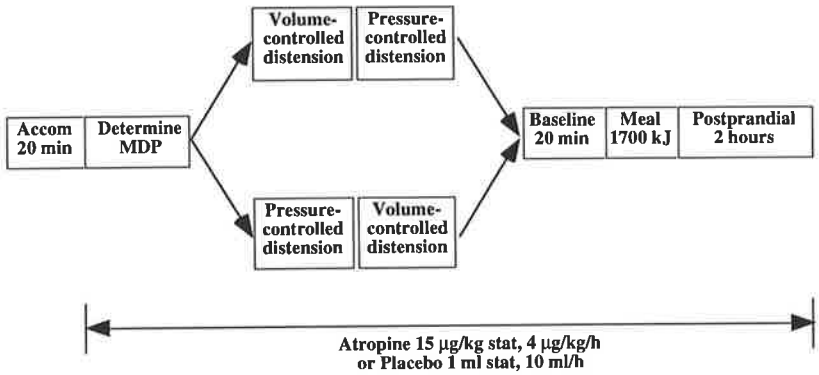

Figure 12.2 *Protocol for each study day.*

The protocol is summarised in Figure 12.2. Subjects were studied after an overnight fast. A cannula was inserted into the forearm for administration of saline or atropine. The manometric assembly, with the barostat bag folded around it, and the pH electrode were passed via an anaesthetised nostril into the proximal stomach. The barostat bag was initially unfolded by inflation with 500 ml of air under controlled conditions (pressure <20 mmHg). The bag was then deflated and the manometric assembly positioned so that the sleeve sensor straddled the lower oesophageal sphincter, thereby positioning the barostat bag in the proximal stomach. The pH electrode was then positioned 5 cm above the proximal margin of the lower oesophageal sphincter. All studies were performed with the subjects sitting upright in an ergonomic chair, designed to minimise abdominal compression. Subjects were allowed to accommodate to the manometric assembly for 20 minutes. Intravenous atropine (15 µg/kg bolus, 4 µg/kg/h infusion) or saline was then given in randomised order on separate days and maintained for the duration of the study. During the infusion period, the subject's pulse and blood pressure were recorded every 15 minutes.

Minimum distending pressure (MDP), defined as the first intrabag pressure at which the mean intrabag volume was greater than 30 ml and continuous respiratory fluctuations were first detected, was determined. Pressure-controlled (isobaric) and volume-controlled, (isovolumetric) distensions were then performed in randomised order separated by a 10 minute rest period. During pressure-controlled distensions, intrabag pressure was increased in 1 mmHg increments in a stepwise fashion every 2 minutes starting at a pressure 2 mmHg below the previously determined MDP and continued until either an intrabag volume of 800 ml or a pressure of 12 mmHg above MDP, or the threshold for subject discomfort was reached. During volume-controlled distensions, intrabag volume was increased in 100 ml increments in a stepwise fashion every 2 minutes to either 800 ml or the threshold for discomfort. Sensations of fullness, nausea, abdominal discomfort and hunger were recorded at the end of each distension step.

Following the distensions, subjects rested for 10 minutes. The barostat was then set to maintain a pressure of 2 mmHg above MDP and fasting recording of baseline intrabag volume was made for 20 minutes. The bag was then deflated and subjects consumed a 1700 kJ (400 kcal) 45% fat, soft mixed nutrient meal, consisting of savoury minced meat, mashed vegetables, milk and ice cream. The bag was then reinflated to 2 mmHg above MDP and recordings made for a further 2 hours. Sensations were assessed before and every 15 minutes after the meal.

12.2.4 Data analysis

Oesophageal manometric data were analysed as described in section 9.5.1. Acid reflux episodes were defined, and for each reflux episode, the mechanism of reflux was determined. Transient lower oesophageal sphincter relaxations were defined and counted separately according to criteria described in section 9.5.1. The rate of spontaneous swallowing was determined by counting the pharyngeal pressure waves.

Barostat data were analysed as described in detail in section 9.5.3.

12.2.5 Statistical analysis

Data for intrabag volume and pressure, and sensation scores were made using repeated measures analysis of variance (SuperAnova, Abacus Concepts Inc., Berkley, California, USA) followed by paired comparisons where appropriate. Data for reflux episodes and transient lower oesophageal sphincter relaxations were analysed using the Wilcoxon signed rank test and are presented as median (interquartile range). All other data were analysed using the paired *t* test and are presented as mean (SEM). A p value of <0.05 was accepted as indicating statistical significance.

12.3 Results

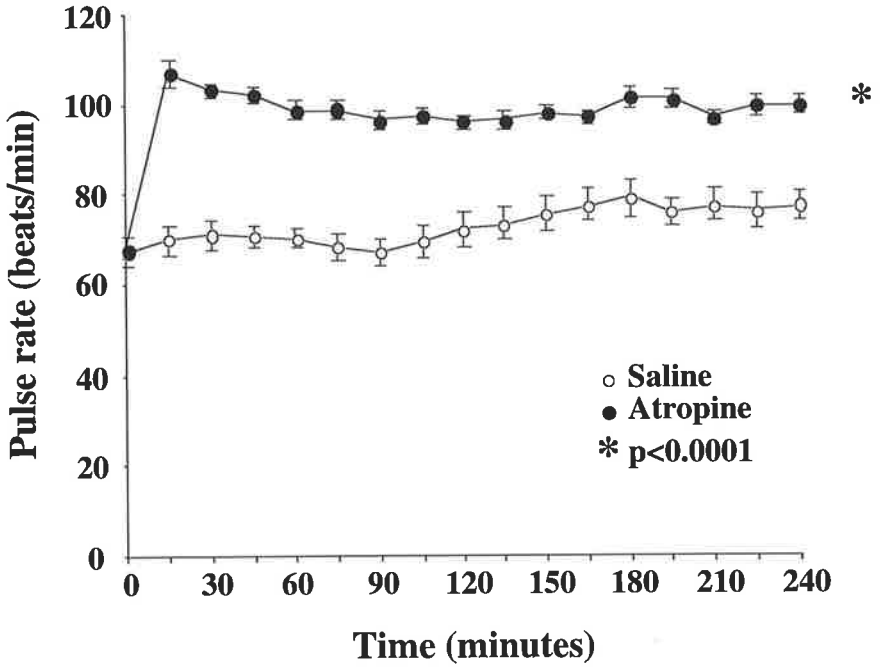

Figure 12.3 *Effect of atropine on heart rate. Data are expressed as mean (SEM)*

All subjects tolerated the study well and reported symptoms and exhibited signs of cholinergic blockade during atropine infusion. The mean pulse rate during atropine infusion (99±2 beats/min) was significantly higher than that during saline infusion (73±3 beats/min; $p<0.0001$) and remained elevated for the duration of the infusion period. (Figure 12.3) The rate of swallowing in the postprandial period during atropine infusion (104±17 per hour) was similar to that during saline infusion (104±15 per hour).

Fasting Recordings

Atropine significantly reduced fasting minimum distending pressure (MDP) from 5.5±0.4 mmHg to 4.5±0.4 mmHg ($p<0.005$). However, mean fasting basal intrabag volume at 2 mmHg above MDP during atropine (234±12 ml) was not significantly different from that during saline (240±20 ml). Atropine significantly altered the pressure-volume relationship of the proximal stomach. During pressure-controlled distension, at each distension pressure from 1 mmHg above MDP, greater volumes were observed during atropine infusion than

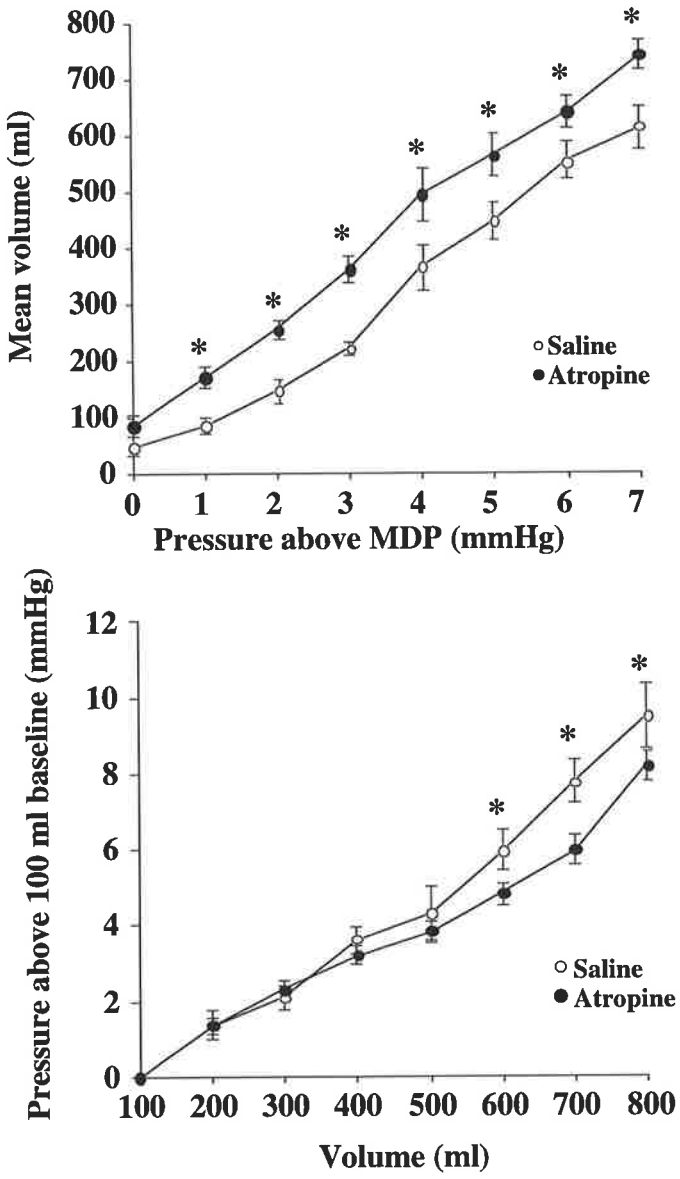

Figure 12.4 *Effect of atropine on the pressure/volume relationship of the proximal stomach during pressure-controlled (upper panel) and volume-controlled (lower panel) distension. Data are expressed as mean (SEM); *p<0.05 vs control (saline).*

during saline infusion (Figure 12.4). Consequently, atropine significantly increased the calculated compliance (*dV/dP*) of the proximal stomach from 81.3±5.3 ml/mmHg to 102.1±8.7 ml/mmHg ($p<0.05$). Similarly, during volume-controlled distension, at intrabag volumes of 600 ml and above, intrabag pressure was significantly lower during atropine compared to saline infusion (Figure 12.4).

During pressure-controlled and volume-controlled distensions, the sensations of fullness and abdominal discomfort increased in parallel with the changes in intrabag volume and pressure. The controlled distensions had no effect on nausea or hunger. Atropine had no significant effect on the level of any of the sensations tested.

Postprandial recordings

Postprandial intrabag volume

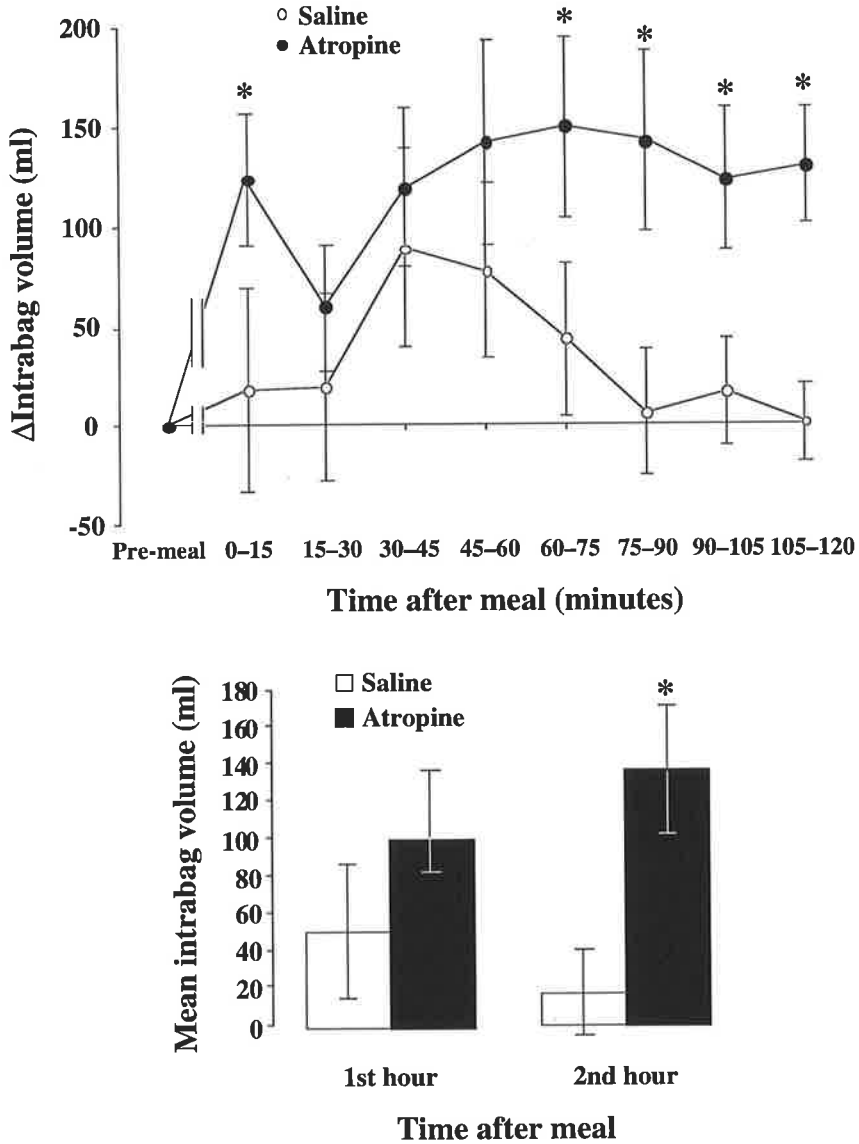

Figure 12.5 *Effect of atropine on postprandial change in proximal gastric volume. Data are expressed as mean (SEM); *p<0.05 vs control (saline).*

Ingestion of the meal was followed by an increase in intrabag volume on both study days. Maximal postprandial volumes achieved during atropine (244±46 ml) and saline infusions (187±48 ml) were not significantly different. During saline infusion, however, there was almost complete recovery of proximal gastric tone after 90 minutes. In contrast, during atropine infusion, there was no recovery of proximal gastric tone during the two hour postprandial observation period so that in the second hour, mean intrabag volume remained elevated during atropine infusion compared to saline infusion. (Figure 12.5).

Sensation scores

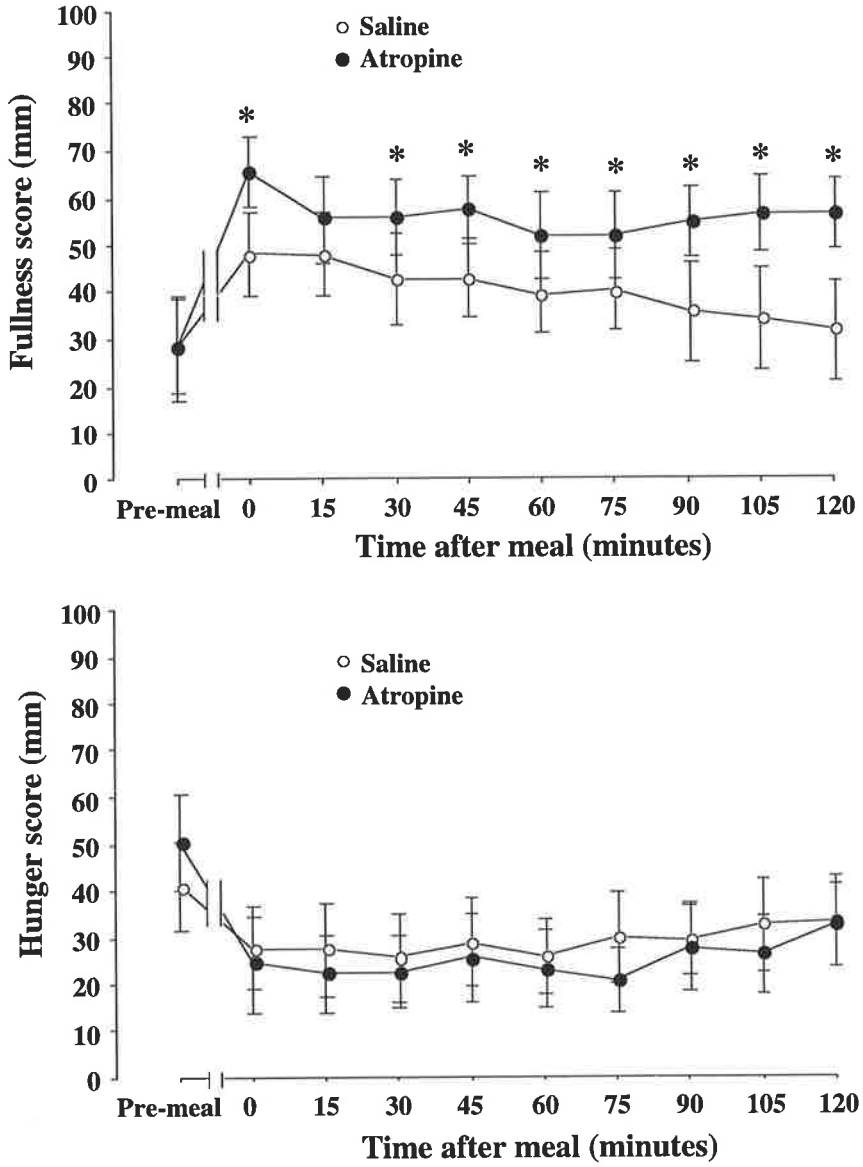

Figure 12.6 *Effect of atropine on postprandial fullness and hunger scores. Data are expressed as mean (SEM); *p<0.05 vs control (saline).*

Consumption of the meal was associated with increased fullness scores and reduced hunger scores with both atropine and saline (Figure 12.6). Fullness scores paralleled the changes in intrabag volume and hunger scores were inversely related to intrabag volume. During atropine infusion, fullness scores were higher than during saline whereas levels for hunger were similar. No significant nausea or abdominal discomfort was reported after the meal.

Transient lower oesophageal sphincter relaxations

In the postprandial period, atropine significantly reduced the frequency of transient lower oesophageal sphincter relaxations in the first and second hour: (first hour; 7.0 (5.3-10.0) per hour vs 3.0 (1.0-4.0) per hour, ($p<0.02$), second hour; 5.0 (3.3-5.8) per hour vs 1.0 (0-3.0) per hour, ($p<0.05$)) (Figure 12.7). However, atropine had no effect on the proportion of transient lower oesophageal sphincter relaxations associated with reflux. In the 8 subjects, whom had adequate pH recordings on both study days, during saline infusion there were a total of 92 transient lower oesophageal sphincter relaxations, of which 23 (25%) were associated with reflux. During atropine infusion there were 41 transient lower oesophageal sphincter relaxations, of which 5 (12%) were associated with reflux.

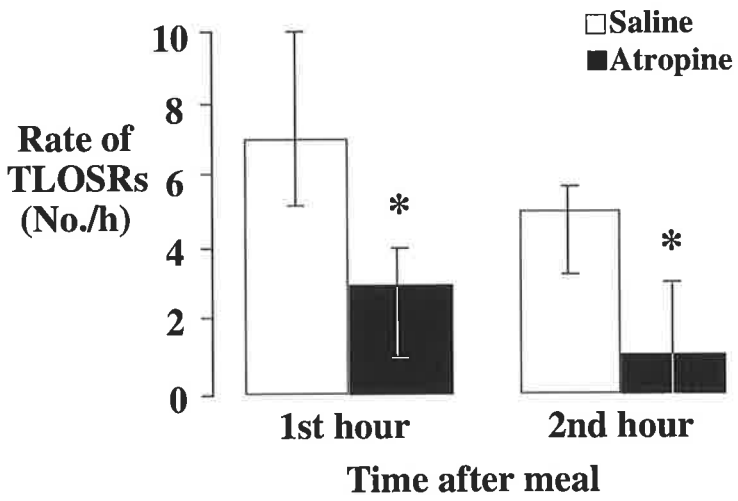

Figure 12.7 *Effect of atropine on the number of transient lower oesophageal sphincter relaxations (TLOSRs). Data are expressed as median (interquartile range); *p<0.05 vs control (saline).*

Reflux

Oesophageal pH data were available in only 8 subjects because of technical problems with the pH recording system on at least one of the study days in 2 subjects. In the postprandial period, atropine significantly reduced the rate of reflux episodes in the first postprandial hour (1.5 (0-3.0) per hour vs 0 (0-0) per hour, $p<0.05$) but not in the second hour (0 (0-1.3) per hour vs 0.5 (0-2.0) per hour, $p=0.89$) (Figure 12.8). During the saline infusion, 25 reflux episodes were scored in the 8 subjects; 23 were attributed to transient lower oesophageal sphincter relaxations, one was due to swallow-induced lower oesophageal sphincter relaxation and one was during straining. During atropine infusion, 7 reflux episodes were scored; 5 were attributed to transient lower oesophageal sphincter relaxations and 2 were due to absent basal lower oesophageal sphincter pressure.

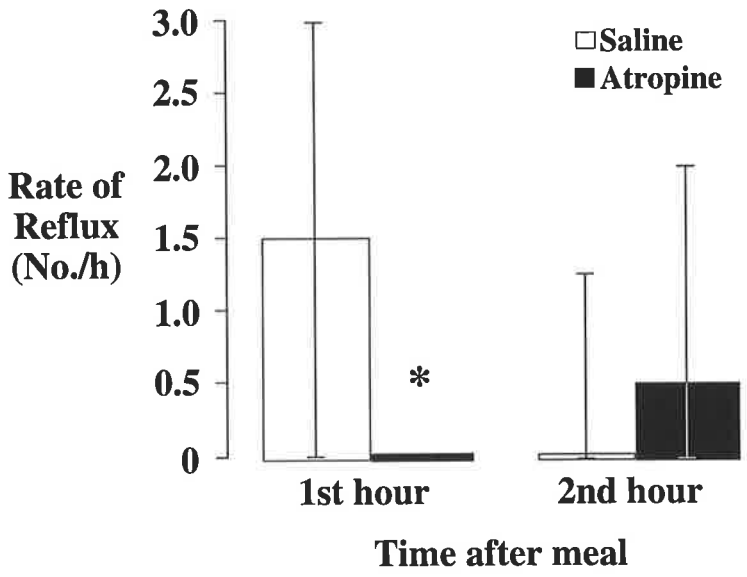

Figure 12.8 *Effect of atropine on the number of postprandial reflux episodes. Data are expressed as median (interquartile range); *p<0.05 vs control (saline).*

12.4 Discussion

Using a gastric barostat, the effects of cholinergic blockade on the function of the proximal stomach in normal human subjects were investigated. The findings indicate that cholinergic blockade with atropine significantly inhibits both fasting proximal gastric compliance and postprandial proximal gastric tone.

The reduction in MDP and fasting compliance are consistent with previous findings of the effects of atropine on fasting gastric tone in dogs (187, 208) and cats (399) and suggest that there is tonic cholinergic input to the proximal stomach. Findings from other studies in dogs suggest that this input is vagal. (208) These previous studies have also reported a reduction in fasting proximal gastric tone as evidenced by an increased basal fasting intrabag volume. (208) This study did not observe such an effect. This apparent discrepancy may be due to a methodological difference. In the previous study, MDP was determined before administration of atropine and intrabag pressure set in relation to this pressure. In the current study, however, MDP was determined after administration of atropine. Because atropine decreased MDP, basal intrabag pressure during atropine was lower than that during placebo and may have artificially reduced fasting basal intrabag volume relative to that during placebo.

These findings, however, differ from those of previous studies in normal humans which found no significant effects of atropine on fasting proximal gastric function. (210, 211) This discrepancy, may be due to differences in the doses of atropine used, which were substantially lower in the previous studies.

Atropine also had a pronounced effect on postprandial motility and effectively inhibited recovery of postprandial proximal gastric tone. Similar effects on insulin-induced proximal gastric relaxation have been reported after proximal gastric vagotomy. (402) It appears, therefore, that return of proximal gastric tone is under cholinergic control. The findings in

this study are at variance with those of the previous studies in humans, (210) again, probably because of differences on the dose of atropine.

Atropine, however, had no effect on the degree of postprandial relaxation, a finding consistent with previous findings in dogs (187) and humans. (210) Gastric relaxation is mediated by vagal nonadrenergic noncholinergic inhibitory nerves releasing nitric oxide. (206) The failure of atropine to influence this response suggests that either the inhibitory stimulus produces maximal relaxation or that cholinergic receptors are not important in the inhibitory pathway. Analogous vagal inhibitory pathways to the lower oesophageal sphincter are similarly not affected by atropine alone but can be almost completely inhibited by a combination of atropine and hexamethonium. (111)

The major impetus to this study was to address the issue of whether atropine might inhibit triggering of transient lower oesophageal sphincter relaxations by altering proximal gastric tone. The data do not support this concept. Gastric distension, either by balloons, air or meals increases the rate of transient LOS relaxations by activation of mechanoreceptors in the proximal stomach, particularly in the region adjacent to and involving the cardia. It is not known whether these mechanoreceptors respond to tension or stretch, although current evidence favours the former. Whatever the stimulus, however, under the conditions of this study in which intragastric pressure was maintained constant and slightly above MDP, relaxation of the proximal stomach by atropine would be expected to increase the rate of transient lower oesophageal sphincter relaxations. Sifrim et al have reported a similar finding recently with sumatriptan, a potent inhibitor of proximal gastric tone. (372)

In this study, as well as the study described in chapter 10 and in previous studies, (243, 332) atropine decreased, rather than increased the rate of transient lower oesophageal sphincter relaxations. The findings in this study are more consistent, therefore, with the notion that atropine reduces the rate of transient lower oesophageal sphincter relaxations via a central effect on the central pattern generator in the brainstem that is believed to mediate

transient lower oesophageal sphincter relaxations. (135) Atropine is a non-specific muscarinic antagonist which crosses the blood brain barrier (397) and all five of the muscarinic receptor subtypes (m1-m5) have been detected in the brain. (366-368) A central effect of atropine on reducing the rate of transient lower oesophageal sphincter relaxations possibly outweighs the potential peripheral effects of atropine on the mechanical properties of the proximal stomach which would be expected to increase the rate of transient lower oesophageal sphincter relaxations.

In a recently reported abstract, Massey et al demonstrated that atropine increases the rate of transient lower oesophageal sphincter relaxations, as triggered by gastric air insufflation. (403) However, the subjects were not studied in the postprandial state and the intragastric volume was not controlled or measured. It is possible that under the conditions of that study, the peripheral effects of atropine on the proximal stomach in increasing the rate of transient lower oesophageal sphincter relaxations may have outweighed any potential central effect of atropine on reducing the rate of transient lower oesophageal sphincter relaxations.

A recent study has shown that perception of gastric distension is a result of increases in gastric wall tension. (404) Glucagon-induced relaxation of the proximal stomach and increased gastric compliance increases sensitivity to pressure-controlled distension and decreases sensitivity to volume-controlled distension. (405) In the present study, however, the atropine induced increase in gastric compliance had no effect on the perception scores of fullness, hunger or abdominal discomfort during the controlled distensions during either the pressure-controlled or volume-controlled distensions. This lack of effect on the sensitivity to distension probably reflects the relatively small effects of atropine on the pressure-volume relationship of the proximal stomach compared to those seen after glucagon administration. (405) In the postprandial period, fullness scores paralleled the changes in intrabag volume. The higher level of postprandial fullness scores during atropine is most likely to reflect a higher intrabag volume, rather than any difference in visceral sensitivity.

In summary, using a gastric barostat, this experiment has demonstrated, for the first time, that in humans, fasting and postprandial proximal gastric motor function is under cholinergic control. Inhibition of proximal gastric tone by anticholinergic agents might influence the triggering of transient lower oesophageal sphincter relaxations. However, under the conditions of the study, inhibition of gastric tone by atropine might be expected to increase rather than decrease the rate of transient lower oesophageal sphincter relaxations, supporting the notion that atropine inhibits transient lower oesophageal sphincter relaxations by a central action on the integrating mechanisms in the brainstem. Further exploration of the central integrating mechanism that triggers transient lower oesophageal sphincter relaxations is required, to help identify future potential pharmacological target sites in the central nervous system.

Chapter 13

Pharmacological control of transient lower oesophageal sphincter relaxations and gastro-oesophageal reflux by the $GABA_B$ agonist baclofen in normal human subjects

13.1 Introduction

Reducing the frequency of gastro-oesophageal reflux episodes by inhibiting the triggering of transient lower oesophageal relaxations offers the potential for a physiologically logical approach for the treatment of reflux disease. Current notions hold that triggering of transient lower oesophageal sphincter relaxations is controlled by medullary brainstem centres, in particular the nucleus tractus solitarius (NTS), that integrate sensory information from the stomach and pharynx. (135) Several pharmacological agents have been shown to inhibit transient lower oesophageal sphincter relaxations and gastro-oesophageal reflux including cholecystokinin-A antagonists, (352-354) atropine, (243, 332) morphine (365) and nitric oxide synthase inhibitors (352, 358) but these agents are not clinically useful either because of unacceptable side effects or because an orally effective formulation is not available. Evidence suggests that these agents have their action primarily on the central integrating mechanisms.

Gamma-amino-butyric acid (GABA) is an important inhibitory neurotransmitter within the central nervous system. (406) $GABA_B$ receptors are abundant on vagal afferent terminals in the NTS (406) and also mediate post-synaptic inhibition. (84) In cats, GABA receptors in the vagal nuclei have been shown to play a role in the control of lower oesophageal sphincter pressure (407) and gastric motor function. (408, 409) The $GABA_B$-receptor agonist, baclofen, exerts an antitussive effect in cats, guinea pigs and humans. (410-412) The medullary centres that control swallowing, oesophageal motility and respiration are closely associated; respiration is suspended during swallowing (413) and there is inhibition of the crural diaphragm during transient lower oesophageal sphincter relaxations. (91) These observations suggest the potential for $GABA_B$ agonists to inhibit the triggering of transient lower oesophageal sphincter relaxations.

Recently baclofen has been shown to inhibit transient lower oesophageal sphincter relaxations and gastro-oesophageal reflux in dogs (414) and ferrets. (415) To date,

however, there are no data on the role of $GABA_B$ receptors or the effect of baclofen on oesophageal motor function in humans. The aim of this study was to investigate the effects of the $GABA_B$ receptor agonist, baclofen, on gastro-oesophageal reflux and lower oesophageal sphincter function in normal healthy volunteers.

13.2 Methods

13.2.1 Subjects

Studies were performed in 20 healthy subjects (14 males, 6 females) aged 18 to 39 years (median 24 years) with a mean weight of 72±2 kg (mean±SEM). Subjects had no gastrointestinal symptoms, no history of upper gastrointestinal surgery and were not taking regular antacids or medications known to influence oesophageal motor function.

13.2.2 Recording methods

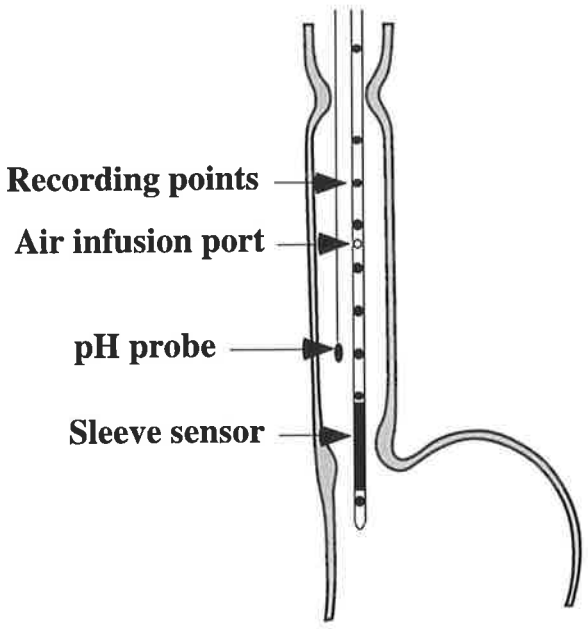

Figure 13.1 *Diagrammatic illustration of the manometric assembly and pH probe.*

An eleven lumen manometric assembly incorporating a 6 cm sleeve sensor was used (Figure 13.1). A side hole in the pharynx recorded swallowing and a side hole located 1 cm distal to the sleeve sensor recorded gastric pressure. Side holes spaced at 3 cm intervals starting at the proximal margin of the sleeve sensor, monitored pressures at 7 sites along the oesophageal body. All the side holes and sleeve sensor were perfused with degassed distilled water at 0.15 ml/minute. In addition, an infusion port was located 11 cm above the sleeve sensor for the rapid injection of air boluses into the mid oesophagus, to evaluate secondary oesophageal peristalsis. Oesophageal pH was measured with an antimony electrode positioned 5 cm above the proximal margin of the lower oesophageal sphincter.

Perception of the sensations of fullness, nausea, hunger, dizziness, sleepiness and tiredness was quantified using a validated 100 mm visual analogue scale as described in section 9.5.4 of this thesis. Sensations were recorded before drug administration as well as before and at 60 minute intervals after consumption of the meal.

13.2.3 Study protocol

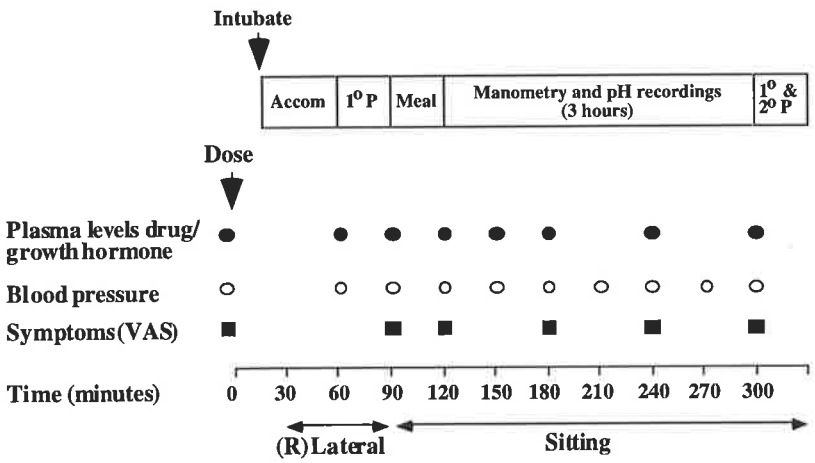

Figure 13.2 *Protocol for each study day. 1°P, primary peristalsis; 2°P, secondary peristalsis; VAS, visual analogue score.*

The protocol is summarised in Figure 13.2. Subjects were studied after an overnight fast. A baseline blood sample was taken for the measurement of growth hormone and plasma baclofen levels. Oral baclofen (40 mg) or placebo were then given double-blind in randomised order on separate days at least one week apart. The manometric assembly and pH electrode were then passed via the nose and positioned so that the sleeve straddled the lower oesophageal sphincter and the pH electrode was 5 cm above the lower oesophageal sphincter. Subjects were allowed 30 minutes to accommodate to the assembly whilst in the right lateral position.

Sixty minutes after the drug administration, primary peristalsis was assessed in response to ten 5 ml water swallows. The subjects then sat up and ate a 3000 kJ (750 kcal) soft mixed nutrient meal, consisting of savoury minced meat, mashed vegetables, 150 ml milk and ice cream. After the meal, with the subjects in the sitting position, oesophageal manometry and pH were recorded for 3 hours. Primary peristalsis was then reassessed as before, and secondary peristalsis was recorded in response to ten 20 ml boluses of air injected rapidly into the mid oesophagus.

The pulse and blood pressure were recorded every 30 minutes during the study period. Blood samples were taken before dosing and at 30-60 minute intervals after dosing for the measurement of plasma levels of baclofen and growth hormone, the latter being used as an indicator of the effect of baclofen on the central nervous system. Symptoms were assessed as described above.

13.2.4 Data analysis

Manometric data were analysed as described in section 9.5.1. A visual mean of the basal lower oesophageal sphincter pressure was taken at one minute intervals and a grand mean for the 180 minute recording period was calculated. Acid reflux episodes were defined, and for each reflux episode, the mechanism of reflux was determined. Transient lower oesophageal

sphincter relaxations were defined and counted separately according to criteria described in section 9.5.1. Primary peristalsis in response to the ten water swallows was analysed for success rate, mean amplitude, mean wave duration and peristaltic velocity. Secondary peristalsis in response to the ten 20 ml boluses of air was analysed for success rate and mean amplitude. The rate of spontaneous swallowing was determined by counting the pharyngeal pressure waves.

For the analysis of acid reflux episodes, acid reflux was defined as a drop in oesophageal pH below 4 for at least 4 seconds, or if basal oesophageal pH was already below 4, a further drop in pH of at least one unit. For the analysis of the occurrence of acid reflux during transient lower oesophageal sphincter relaxations, acid reflux was defined as a drop in pH of at least 1 pH unit. (249)

Plasma levels of baclofen were measured by Astra Hassle AB. Plasma growth hormone levels were measured by radioimmunoassay.

13.2.5 Statistical analysis

Data for reflux episodes, transient lower oesophageal sphincter relaxations and peristaltic success were analysed using the Wilcoxon signed-rank test and are presented as median (interquartile range). Paired comparisons of sensation scores were made using repeated measures analysis of variance (SuperAnova, Abacus Concepts Inc., Berkley, California, USA). All other data were analysed using the paired *t* test and are presented as mean±SEM. A p value of <0.05 was accepted as indicating statistical significance.

13.3 Results

Data on oesophageal pH were available in only 17 subjects because of technical problems with the pH recording system on at least one of the study days in 3 subjects.

Basal lower oesophageal sphincter pressure

On baclofen study days, basal lower oesophageal sphincter pressure was significantly higher (10.8±0.8 mmHg) than that on control study days (8.7±1.4 mmHg, p=0.001, Figure 13.3).

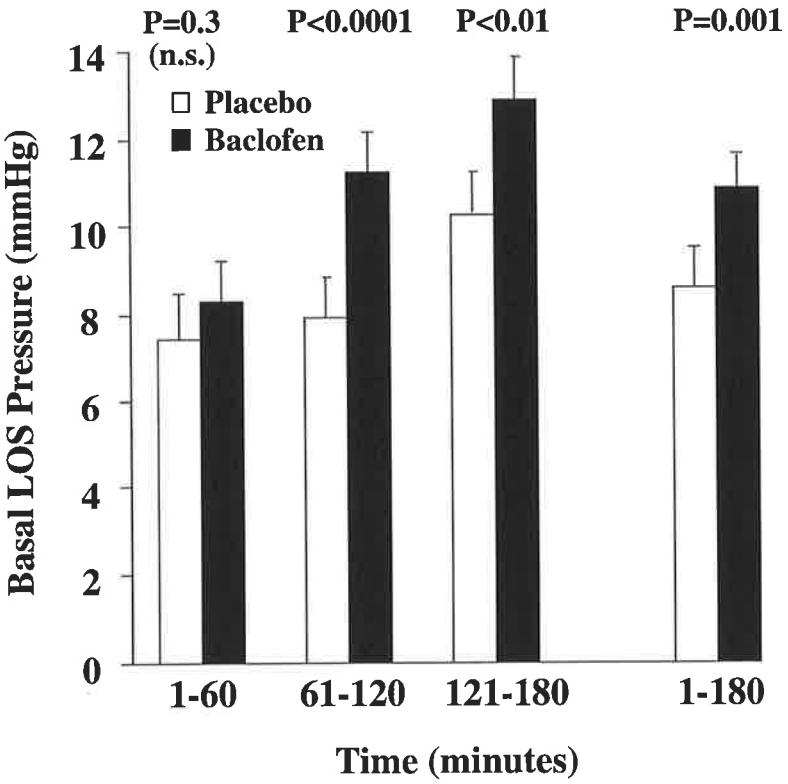

Figure 13.3 *Effect of baclofen on basal lower oesophageal sphincter pressure. Data are depicted as mean±SEM for each postprandial hour as well as for the whole three hour postprandial period. LOS, lower oesophageal sphincter; n.s., not significant.*

Transient lower oesophageal sphincter relaxations

Baclofen significantly reduced the rate of transient lower oesophageal sphincter relaxations from a median of 5.7 (4.9-7.8) per hour to 2.2 (1.3-3.8) per hour (p<0.0001). This reduction was sustained for each of the three postprandial hours (Figure 13.4). Baclofen had no effect on the likelihood of acid reflux occurring during a transient lower oesophageal sphincter relaxation. In the 17 subjects who had technically satisfactory paired oesophageal pH recordings, acid reflux occurred with 119 of 331 (36%) transient lower oesophageal sphincter relaxations during placebo, compared with 38 of 140 (27%) transient lower

oesophageal sphincter relaxations during baclofen. Baclofen had no effect on the duration of transient lower oesophageal sphincter relaxations (baclofen 15.3±0.5 seconds, placebo 15.3±0.3 seconds) or on residual pressure during transient lower oesophageal sphincter relaxations (baclofen -0.3±0.1 mmHg, placebo -0.1±0.1 mmHg).

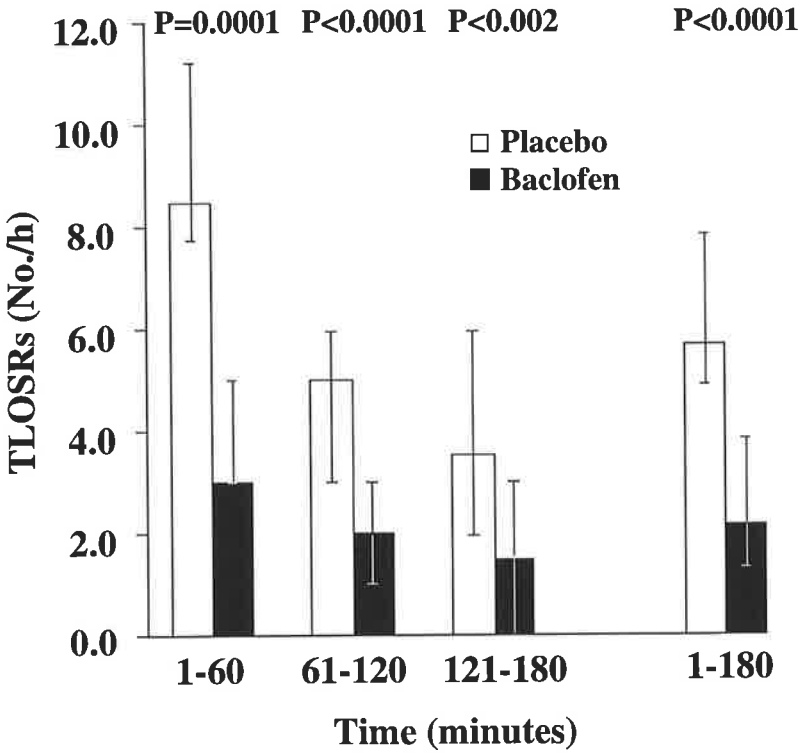

Figure 13.4 *Effect of baclofen on the rate of transient lower oesophageal sphincter relaxations. Data are depicted as median (interquartile range) for each postprandial hour as well as for the whole three hour postprandial period. TLOSRs, transient lower oesophageal sphincter relaxations.*

Gastro-oesophageal reflux

Baclofen significantly reduced the rate of reflux episodes from a median of 1.0 (0.3-2.7) per hour to 0.3 (0-1) per hour ($p<0.02$). This effect was significant only in the first postprandial hour (Figure 13.5). On the placebo study day, 76 acid reflux episodes were scored in the 17 subjects (39 - hour 1, 23 - hour 2, 14 - hour 3); 73 were due to transient lower oesophageal sphincter relaxations, 2 occurred during swallow-induced lower oesophageal sphincter relaxation and 1 was due to absent basal lower oesophageal sphincter pressure. On the

baclofen study day, 28 acid reflux episodes were scored in the 17 subjects (13 - hour 1, 11 - hour 2, 4 - hour 3); all occurred during transient lower oesophageal sphincter relaxations.

Despite the significant reduction in reflux episodes, baclofen had no significant effect on oesophageal acid exposure. The duration that oesophageal pH was <4 during baclofen (0.6% (0.2-2.3%)) was not statistically different from that during placebo (0.2% (0.1-2.0%)). Similarly, the acid clearance time was not significantly different during baclofen (14 s (7-72 s)) from that during placebo (17 s (7-51 s)).

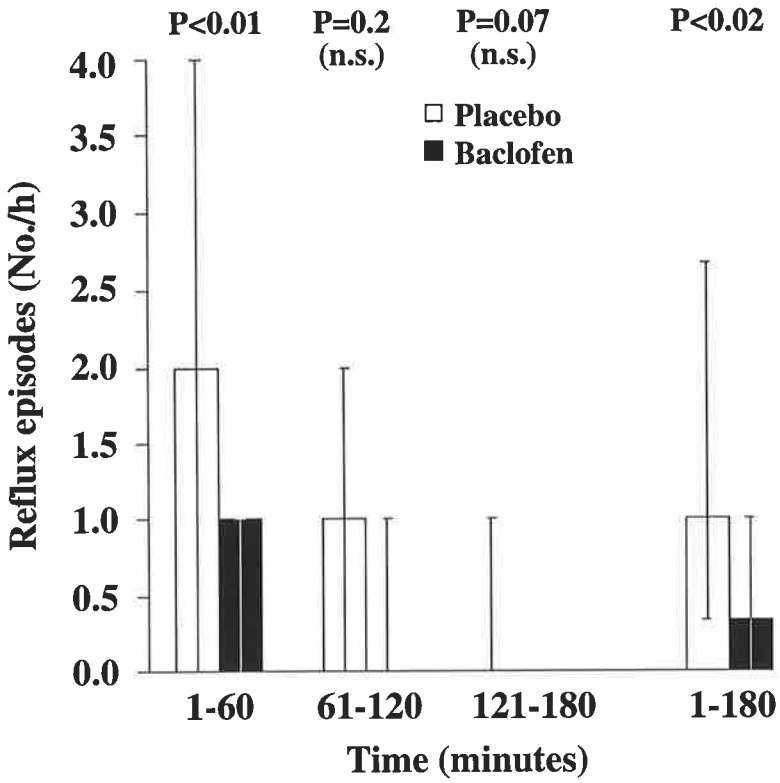

Figure 13.5 *Effect of baclofen on the rate of reflux episodes. Data are depicted as median (interquartile range) for each postprandial hour as well as for the whole three hour postprandial period. n.s., not significant.*

Swallowing

Baclofen significantly reduced the rate of swallowing from 111±14 per hour to 81±8 per hour ($p<0.01$). This reduction was sustained for each of the three postprandial hours (Figure 13.6). However, baclofen had no effect on the residual lower oesophageal sphincter pressure during water swallows (baclofen -0.1±0.1 mmHg, placebo -0.3±0.1 mmHg).

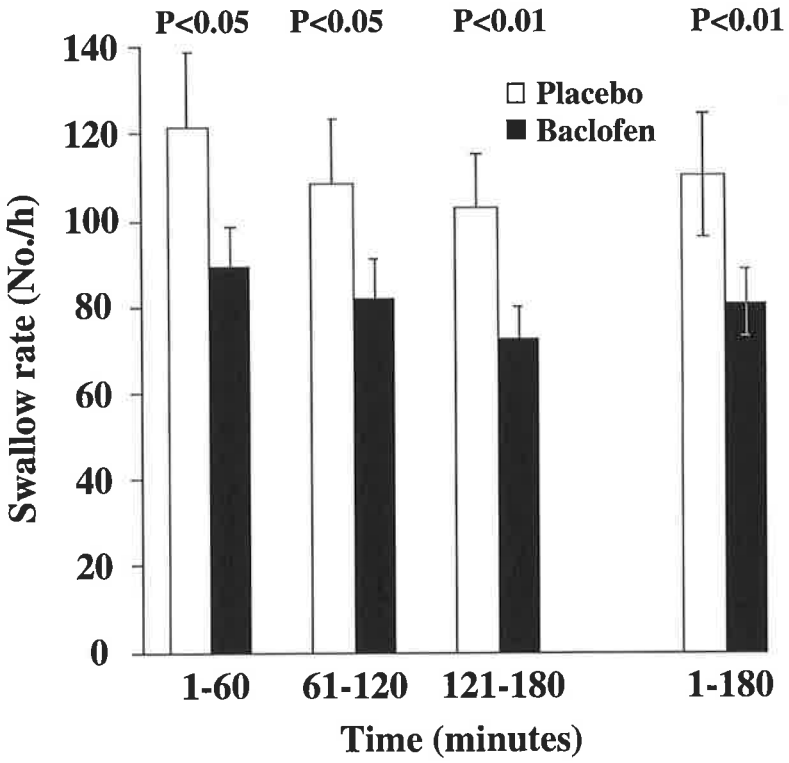

Figure 13.6 *Effect of baclofen on swallow rate. Data are depicted as mean±SEM for each postprandial hour as well as for the whole three hour postprandial period.*

Oesophageal peristalsis

Before the meal, with the subjects in the right lateral position, baclofen had small, but statistically significant effects on primary peristalsis; wave amplitude was higher and wave duration longer in the distal oesophagus, and peristaltic velocity was greater over the whole oesophagus (Table 13.1). These effects were not evident at the end of the study when peristaltic velocity was slower during baclofen. Baclofen had no effect on primary peristaltic success either before the meal or at the end of the study. Baclofen had no significant effects on secondary peristalsis when this was tested at the end of the measurement period (Table 13.1).

	Placebo	Baclofen	
Primary peristalsis			
Pre-meal			
Success (%)[a]	90 (85-100)	95 (70-100)	
Amplitude (mmHg)			
Proximal	42.8±3.9	42.2±4.6	
Distal	66.3±5.3	74.7±6.4	*
Total	56.1±4.4	60.8±5.3	
Duration (sec)			
Proximal	3.3±0.1	3.3±0.1	
Distal	3.7±0.1	4.0±0.1	*
Total	3.6±0.1	3.7±0.1	
Velocity (cm/sec)			
Proximal	3.1±0.3	3.5±0.5	
Distal	3.0±0.2	3.2±0.2	
Total	3.0±0.1	3.3±0.1	*
At end of study			
Success (%)[a]	85 (50-100)	85 (40-100)	
Amplitude (mmHg)			
Proximal	36.9±3.5	31.5±3.4	
Distal	49.6±4.8	53.6±5.4	
Total	44.2±4.1	44.4±4.5	
Duration (sec)			
Proximal	3.4±0.2	3.4±0.1	
Distal	3.5±0.1	3.5±0.1	
Total	3.5±0.1	3.4±0.1	
Velocity (cm/sec)			
Proximal	3.1±0.3	2.8+0.2	
Distal	3.3±0.2	2.8+0.1	*
Total	3.1±0.2	2.8±0.1	*
Secondary peristalsis			
At end of study			
Success (%)	55.3±6.6	52.1±7.2	
Amplitude (mmHg)			
Proximal	30.0±2.2	29.0±3.6	
Distal	52.4±5.4	49.9±6.2	
Total	42.9±4.0	40.9±4.9	

Table 13.1 *Effect of baclofen on primary and secondary peristalsis.* [a] *Values are median (interquartile range); all other values are mean ±SEM; * p<0.05 vs placebo.*

Plasma growth hormone and baclofen levels

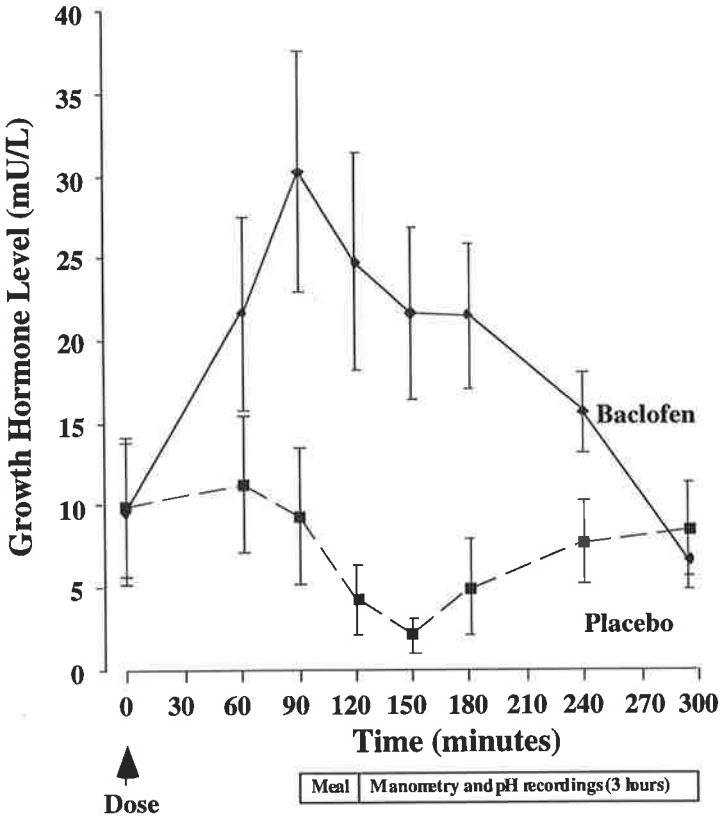

Figure 13.7 *Effect of a single oral dose of baclofen (40 mg) on plasma levels of growth hormone. Data are depicted as mean±SEM*

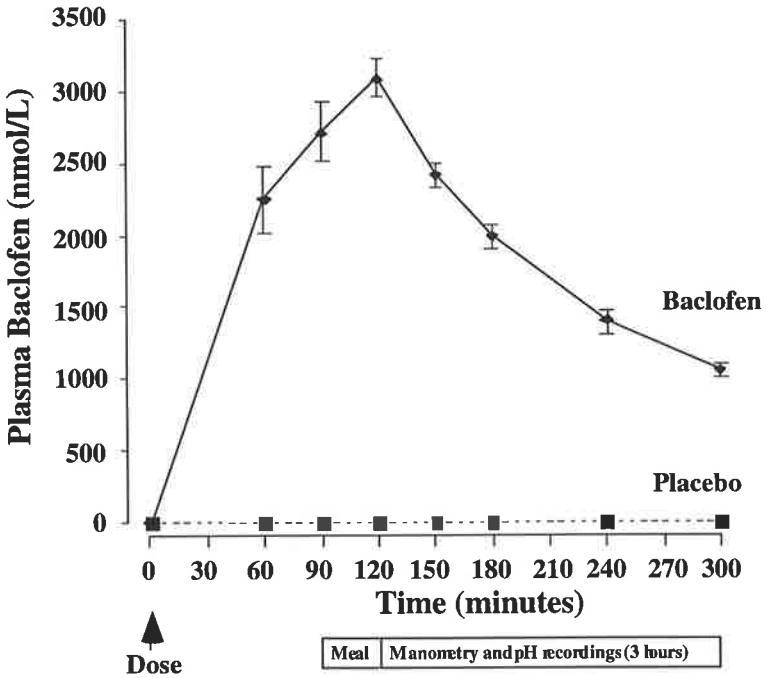

Figure 13.8 *Effect of a single oral dose of baclofen (40 mg) on plasma levels of baclofen. Data are depicted as mean±SEM*

Baclofen caused a significant increase in growth hormone level with a peak occurring 90 minutes after dosing (Figure 13.7). There was no increase in growth hormone level on the placebo day. The peak plasma level of baclofen occurred 120 minutes after dosing, which corresponded to the start of the postprandial recording (Figure 13.8).

Symptoms and side effects

Symptom scores were analysed as changes from pre-dose levels. There were no overall significant differences in the change in symptom scores between the two study days. Two subjects, both female with relatively low body weights (52.5 and 62 kg respectively), experienced significant sleepiness and tiredness after baclofen that persisted for almost 24 hours. Two subjects withdrew from the study because of vomiting, one after baclofen administration and one after placebo. Baclofen had no effect on blood pressure (baclofen 115±1/70±1 mmHg, placebo 114±1/70±1 mmHg) or heart rate (baclofen 72±1 beats/min, placebo 73±1 beats/min).

13.4 Discussion

Current pharmacological treatment of reflux disease is based on acid suppression, prokinetic agents, or both. These approaches, however, do not address the major mechanism underlying reflux of gastric contents into the oesophagus, transient lower oesophageal sphincter relaxations. Both static (164, 247, 248) and ambulatory (249) manometric studies have shown that transient lower oesophageal sphincter relaxation is clearly the most prevalent mechanism in the majority of patients with reflux disease, particularly those with either no mucosal breaks or with only mild erosive oesophagitis who comprise the bulk of patients with reflux disease. Therefore, control of reflux by controlling the rate of transient lower oesophageal sphincter relaxations is not only pathophysiologically attractive but also an eminently applicable approach in most patients with reflux disease. Previous studies with anticholinergic agents, (243, 332) opiates, (365) and CCK-A (352-354) and nitric oxide synthase antagonists (352, 358) have shown that it is feasible to reduce the rate of transient lower oesophageal sphincter relaxations pharmacologically.

Baclofen is a $GABA_B$ agonist currently available for the management of spasticity. The present study has shown that a single oral dose of baclofen inhibits the rate of transient lower oesophageal sphincter relaxations by over 60% in normal human subjects. These findings are consistent with other recent findings in dogs (414) and ferrets. (415) As in previous studies in normal humans, (95) transient lower oesophageal sphincter relaxation was the major mechanism underlying reflux episodes. Accordingly, baclofen also reduced the number of reflux episodes by over 60%. This effect, however, was significant for only the first postprandial hour, probably because of the low rate of reflux episodes in the subsequent two hours even on the placebo treatment day. Baclofen also caused a modest but significant rise in postprandial basal lower oesophageal sphincter pressure. A similar effect has been noted in dogs (414) and ferrets. (415) This may be of functional and therapeutic value in an important subgroup of patients with gastro-oesophageal reflux disease in whom absent basal lower oesophageal sphincter pressure is the predominant mechanism of reflux. (248, 249)

Despite the reduction in the rate of reflux episodes, there was no reduction in oesophageal acid exposure. This may also be a result of a type II statistical error arising from the small number of acid reflux episodes overall and the absence of any reflux episodes in four subjects. Importantly, and in contrast to the effects of atropine (243) and morphine, (365) baclofen had no detrimental effect on oesophageal acid clearance.

Baclofen had only small and inconsistent effects on primary peristalsis, and no effect on secondary peristalsis. These effects, however, did not alter peristaltic success. The reasons for the inconsistent effects observed at the start and end of the study are unclear and may possibly reflect differences in plasma levels of baclofen or the influence of posture. (44)

The site at which baclofen acts to inhibit the triggering of transient lower oesophageal sphincter relaxations has yet to be determined. However, evidence exists to support actions both centrally, within the central nervous system, and peripherally, on vagal afferents. Transient lower oesophageal sphincter relaxations are believed to be mediated by vagal pathways and integrated by a pattern generator in the brainstem. (135) GABA, via $GABA_B$ receptors, inhibits release of neurotransmitters from visceral afferents. (406) $GABA_B$ receptors are present throughout the central nervous system, including the nucleus tractus solitarius and nucleus ambiguus (406) which are the sensory and motor nuclei respectively of the vagus nerve and which are believed to comprise the major elements of the pattern generator that controls swallowing and transient lower oesophageal sphincter relaxations. Baclofen readily crosses the blood-brain barrier and has been shown to inhibit the cough reflex in cats via a central effect. (410) The medullary centres controlling swallowing, oesophageal motility and respiration are closely associated. Therefore, baclofen could potentially inhibit the triggering of transient lower oesophageal sphincter relaxations by acting centrally on the pattern generator in the brainstem which is believed to mediate transient lower oesophageal sphincter relaxations. This concept is further supported by the recent finding that baclofen, when instilled intracerebroventricularly, inhibits vagal efferent

responses to gastric distension. (416) Inhibition of the central pattern generator may also account for the significant reduction in the rate of swallowing by baclofen.

Another possibility is that baclofen acts peripherally on the stomach. Recent in vivo and in vitro studies have shown that baclofen reduces the sensitivity of gastric mechanoreceptors. (417, 418) Interestingly, this effect occurs in the context of reduced gastric compliance, in the rat (174, 419) and ferret (173) which is thought to be mediated via a central site of action. (420) Such changes in gastric compliance, however, would be expected to increase rather than decrease the rate of transient lower oesophageal sphincter relaxations. Although preliminary evidence exists to support the presence of $GABA_B$ receptors on the preganglionic vagal motor neuron, (421) it is unlikely that baclofen exerts its action primarily on the efferent motor pathway because it had no effect on either the residual lower oesophageal sphincter pressure during water swallows or transient lower oesophageal sphincter relaxations, or on the duration of transient lower oesophageal sphincter relaxations.

The mechanism by which baclofen increases postprandial basal lower oesophageal sphincter pressure is unknown. Recent data obtained in ferrets suggest that baclofen may have two actions on the vagal innervation of the lower oesophageal sphincter. (421) Baclofen increases basal lower oesophageal sphincter pressure, an effect that is blocked by vagotomy, suggesting that baclofen may reduce the central vagal inhibitory drive to the lower oesophageal sphincter. In vagotomised ferrets, when given during stimulation of the peripheral end of the cut vagus nerve, baclofen increases basal lower oesophageal sphincter pressure suggesting that baclofen inhibits the of vagal output onto inhibitory nonadrenergic noncholinergic inhibitory neurons

In summary, the $GABA_B$ agonist, baclofen, substantially and significantly inhibited transient lower oesophageal sphincter relaxations and thereby reflux episodes in normal subjects. Baclofen also caused a modest but nevertheless significant increase in basal lower oesophageal sphincter pressure. These findings suggest a potential therapeutic role for

baclofen in the treatment of reflux disease. The efficacy of $GABA_B$ agonists in reducing the frequency of transient lower oesophageal sphincter relaxations and reflux in patients with gastro-oesophageal reflux disease, however, awaits further study.

Appendix

Publications arising

Original articles

Lidums I, Checklin H, Mittal RK, Holloway RH. Effect of atropine on gastro-oesophageal reflux and transient lower oesophageal sphincter relaxations in patients with gastro-oesophageal reflux disease. Gut 1998; 43: 12-16.

Lidums I, Lehmann A, Checklin H, Dent J, Holloway RH. Control of transient lower esophageal sphincter relaxations and gastroesophageal reflux by the $GABA_B$ agonist baclofen in normal human subjects. Accepted for publication Gastroenterology 1999.

Lidums I, Hebbard GS, Holloway RH. Effect of atropine on proximal gastric motor and sensory function in normal subjects. Submitted for publication 1999.

Lidums I, Checklin H, Holloway RH. Effect of peripheral cholinergic blockade on gastro-oesophageal reflux and transient lower oesophageal sphincter relaxations in normal subjects. Submitted for publication 1999.

Published abstracts

Lidums I, Bermingham H, Mittal RK, Holloway RH. Atropine inhibits gastroesophageal reflux and transient lower esophageal sphincter relaxations in patients with gastroesophageal reflux disease. Gastroenterology 1997; 112: A775.

Lidums I, Bermingham H, Holloway RH. Effect of hyoscine butylbromide on gastroesophageal reflux and transient lower esophageal sphincter relaxations in healthy volunteers. Gastroenterology 1997; 112: A778.

Lidums I, Hebbard GS, Holloway RH. Cholinergic control of proximal gastric motor function in normal subjects. Gastroenterology 1999; 116: A1029.

Lidums I, Lehmann A, Checklin H, Dent J, Holloway RH. The $GABA_B$ agonist baclofen inhibits transient lower esophageal sphincter relaxations and gastroesophageal reflux in normal human subjects. Gastroenterology 1999; 116: A1029.

Other articles published whilst working towards this thesis:

Lidums I, Holloway R. A GP's guide to oesophageal motility disorders. Modern Medicine of Australia 1996; 39: 80-89.

Lidums I, Holloway R. Motility abnormalities in the columnar-lined esophagus. Gastroenterology Clinics of North America 1997; 26: 519-531.

Presentations arising

Oral presentations

May 1997: American Gastroenterology Association Annual Scientific Meeting.
Effect of hyoscine butylbromide on gastroesophageal reflux and lower esophageal sphincter function in healthy volunteers.

May 1999: American Gastroenterology Association Annual Scientific Meeting.
The $GABA_B$ agonist baclofen inhibits transient lower esophageal sphincter relaxations and gastroesophageal reflux in normal human subjects.

May 1999: American Gastroenterology Association Annual Scientific Meeting.
Cholinergic control of proximal gastric motor function in normal subjects.

Poster presentations

May 1997: American Gastroenterology Association Annual Scientific Meeting.
Atropine inhibits gastroesophageal reflux and transient lower esophageal sphincter relaxations in patients with gastroesophageal reflux disease.

Bibliography

1. Skandalakis JE, Gray SW, Skandalakis LJ. Surgical anatomy of the oesophagus. In: Jamieson GG, ed. Surgery of the Oesophagus. New York: Churchill Livingston, 1988:19-35.

2. Hopwood D. The oesophageal lining. In: Whitehead R, ed. Gastrointestinal and Oesophageal Pathology. New York: Churchill Livingstone, 1989:3-12.

3. Pope CE. Normal anatomy and development anomalies. In: Sleisenger MH, Fordtran JS, ed. Gastrointestinal Disease. Pathophysiology, Diagnosis, Management. Philadelphia: W. B. Saunders Company, 1993:311-318.

4. Roman C, Gonella J. Extrinsic control of digestive tract motility. In: Johnson LR, ed. Physiology of the Gastrointestinal Tract. 2nd ed. New York: Raven Press, 1987:507-553.

5. Collman P, Tremblay L, Diamant N. The distribution of spinal and vagal sensory neurons that innervate the esophagus of the cat. Gastroenterology 1992;103:817-822.

6. Altschuler SM, Bao X, Bieger D, Hopkins DA, Miselis RR. Viscerotopic representation of the upper alimentary tract in the rat: sensory ganglia and nuclei of the solitary and spinal trigeminal tracts. J Comp Neurol 1989;283:248-268.

7. Cunningham ET, Sawchenko PE. Central neural control of esophageal motility: a review. Dysphagia 1990;5:35-51.

8. Mayer EA, Gebhart GF. Basic and clinical aspects of visceral hyperalgesia. Gastroenterology 1994;107:271-293.

9. Collman PI, Tremblay L, Diamant NE. The central vagal efferent supply to the esophagus and lower esophageal sphincter of the cat. Gastroenterology 1993;104:1430-1438.

10. Aziz Q, Thompson DG. Brain-gut axis in health and disease. Gastroenterology 1998;114:559-578.

11. Diamant NE. Physiology of esophageal motor function. Gastroenterol Clin N Am 1989;18:179-194.

12. Goyal RK, Hirano I. The enteric nervous system. New Engl J Med 1996;334:1106-1115.

13. Diamant NE, El Sharkawy TY. Neural control of esophageal peristalsis: A conceptual analysis. Gastroenterology 1977;72 :546-556.

14. Dodds WJ, Stef JJ, Stewart ET, Hogan WJ, Arndorfer RC, Cohen EB. Responses of feline esophagus to cervical vagal stimulation. Am J Physiol 1978;235:E63-E73.

15. Gilbert R, Rattan S, Goyal RK. Pharmacologic identification, activation and antagonism of two muscarine receptor subtypes in the lower esophageal sphincter. J Pharmacol Exp Ther 1984;230:284-291.

16. Gilbert RJ, Dodds WJ. Effect of selective muscarinic antagonists on peristaltic contractions in opossum smooth muscle. Am J Physiol 1986;250:G50-G59.

17. Tottrup A, Svane D, Forman A. Nitric oxide mediating NANC inhibition in opossum lower esophageal sphincter. Am J Physiol 1991;260:G385-G389.

18. Murray J, Du C, Ledlow A, Bates JN, Conklin JL. Nitric oxide: mediator of nonadrenergic noncholinergic responses of opossum esophageal muscle. Am J Physiol 1991;261:G401-G406.

19. Goyal RK, Rattan S, Said SI. VIP as a possible neurotransmitter of non-cholinergic non-adrenergic inhibitory neurones. Nature 1980;288:378-380.

20. Daniel EE, Posey-Daniel V. Neuromuscular structures in opossum esophagus: role of interstitial cells of Cajal. Am J Physiol 1984;246:G305-G315.

21. Berezin I, Daniel EE, Huizinga JD. Ultrastructure of interstitial cells of Cajal in the canine distal esophagus. Can J Physiol Pharmacol 1994;72:1049-1059.

22. Faussone-Pellegrini MS, Cortesini C. Ultrastructural features and localization of the interstitial cells of Cajal in the smooth muscle coat of human esophagus. J Submicrosc Cytol 1985;17:187-197.

23. Sanders KM. A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology 1996;111:492-515.

24. Pouderoux P, Kahrilas PJ. Function of upper esophageal sphincter during swallowing: the grabbing effect. Am J Physiol 1997;272:G1057-G1063.

25. Christensen J. Motor functions of the pharynx and esophagus. In: Johnson LR, ed. Physiology of the Gastrointestinal Tract. 2nd ed. New York: Raven Press, 1987:595-612.

26. Mayrand S, Diamant NE. Measurement of human esophageal tone in vivo. Gastroenterology 1993;105:1411-1420.

27. Dodds WJ, Stewart ET, Hodges D, Zboralske FF. Movement of the feline esophagus associated with respiration and peristalsis. An evaluation using tantalum markers. J Clin Invest 1973;52:1-13.

28. Sugarbaker DJ, Rattan S, Goyal RK. Swallowing induces sequential activation of esophageal longitudinal smooth muscle. Am J Physiol 1984;247:G515-G520.

29. Edmundowicz SA, Clouse RE. Shortening of the esophagus in response to swallowing. Am J Physiol 1991;260:G512-G516.

30. Pouderoux P, Lin S, Kahrilas PJ. Timing, propagation, coordination, and effect of esophageal shortening during peristalsis. Gastroenterology 1997;112:1147-1154.

31. Kahrilas PJ, Wu S, Lin S, Pouderoux P. Attenuation of esophageal shortening during peristalsis with hiatus hernia. Gastroenterology 1995;109:1818-1825.

32. Sifrim D, Janssens J, Vantrappen G. A wave of inhibition precedes primary peristaltic contractions in the human esophagus. Gastroenterology 1992;103:876-882.

33. Clouse RE, Staiano A. Topography of the esophageal peristaltic pressure wave. Am J Physiol 1991;261:G677-G684.

34. Clouse RE, Staiano A, Bickston SJ, Cohn SM. Characteristics of the propagating pressure wave in the esophagus. Dig Dis Sci 1996;41:2369-2376.

35. Li M, Brasseur JG, Dodds WJ. Analyses of normal and abnormal esophageal transport using computer simulations. Am J Physiol 1994;266:G525-G543.

36. Brasseur JG. Mechanical studies of the esophageal function. Dysphagia 1993;8:384-386.

37. Richter JE, Blackwell JN, Wu WC, Johns DN, Cowan RJ, Castell DO. Relationship of radionuclide liquid bolus transport and esophageal manometry. J Lab Clin Med 1987;109:217-224.

38. Kahrilas PJ, Dodds WJ, Hogan WJ. Effect of peristaltic dysfunction on esophageal volume clearance. Gastroenterology 1988;94:73-80.

39. Humphries TJ, Castell DO. Pressure profile of esophageal peristalsis in normal humans as measured by direct intraesophageal transducers. Am J Dig Dis 1977;22:641-645.

40. Hewson EG, Ott DJ, Dalton CB, Chen YM, Wu WC, Richter JE. Manometry and radiology. Complementary studies in the assessment of esophageal motility disorders. Gastroenterology 1990;98:626-632.

41. Russell COH, Whelan G. Oesophageal manometry: how well does it predict oesophageal function. Gut 1987;28:940-945.

42. DeVault K, Castell JA, Castell DO. How many swallows are required to establish reliable esophageal peristaltic parameters in normal subjects? An on-line computer analysis. Am J Gastroenterol 1987;82:754-757.

43. Dodds WJ, Hogan WJ, Reid DP, Stewart ET, Arndorfer RC. A comparison between primary esophageal peristalsis following wet and dry swallows. J Appl Physiol 1973;35:851-857.

44. Kaye MD. Alteration of esophageal peristalsis by body position. Dig Dis Sci 1981;26:897-901.

45. Dent J. Normal oesophageal function. In: Jamieson GG, ed. Surgery of the Oesophagus. New York: Churchill Livingston, 1988:37-49.

46. Ask P, Tibbling L. Effect of time interval between swallows on esophageal peristalsis. Am J Physiol 1980;238:G485-G490.

47. Vanek AW, Diamant NE. Responses of the human esophagus to paired swallows. Gastroenterology 1987;92:643-650.

48. Meyer GW. Human esophageal response to rapid swallowing: muscle refractory period or neural inhibition. Am J Physiol 1981;241:G129-G136.

49. Paterson WG, Rattan S, Goyal RK. Experimental induction of isolated lower esophageal sphincter relaxation in anesthetized opossums. J Clin Invest 1986;77:1187-1193.

50. Conklin JL. Control of esophageal motor function. Dysphagia 1993;8:311-317.

51. Dodds WJ, Christensen J, Dent J, Arndorfer RC, Wood JD. Pharmacologic investigation of primary peristalsis in smooth muscle portion of opossum esophagus. Am J Physiol 1979;237:E561-E566.

52. Dodds WJ, Christensen J, Dent J, Wood J, Arndorfer RC. Esophageal contractions induced by vagal stimulation in the opossum. Am J Physiol 1978;235:E392-E401.

53. Janssens J, DeWever I, Vantrappen G, Hellemans J. Peristalsis in smooth muscle esophagus after transection and bolus deviation. Gastroenterology 1976;71:1004-1009.

54. Rattan S, Gidda JS, Goyal RK. Membrane potential and mechanical responses of the opossum esophagus to vagal stimulation and swallowing. Gastroenterology 1983;85:922-928.

55. Biancani P, Hillemeier C, Bitar KN, Makhlouf GM. Contraction mediated by Ca2+ influx in esophageal muscle and by Ca2+ release in the LES. Am J Physiol 1987;253:G760-G766.

56. Conklin JL, Christensen J. Neuromuscular control of the oropharynx and esophagus in health and disease. Annu Rev Med 1994;45:13-22.

57. Sarna SK, Daniel EE, Waterfall WE. Myogenic and neural control systems for esophageal motility. Gastroenterology 1977;73:1345-1352.

58. Du C, Murray J, Bates JN, Conklin JL. Nitric oxide: mediator of NANC hyperpolarization of opossum esophageal smooth muscle. Am J Physiol 1991;261:G1012-G1016.

59. Dodds W, Dent J, Hogan W, Arndorfer R. Effect of atropine on esophageal motor functions in humans. Am J Physiol 1981;240:G290-G296.

60. Yamato S, Spechler SJ, Goyal RK. Role of nitric oxide in esophageal peristalsis in the opossum. Gastroenterology 1992;103:197-204.

61. Konturek JW, Thor P, Lukaszyk A, Gabryelewicz A, Konturek SJ, Domschke W. Endogenous nitric oxide in the control of esophageal motility in humans. J Physiol Pharmacol 1997;48:201-209.

62. Diamant NE. Physiology of the esophagus. In: Sleisenger MH, Fordtran JS, ed. Gastrointestinal Disease. Pathophysiology, Diagnosis, Management. Philadelphia: W. B. Saunders Company, 1993:319-330.

63. Schoeman MN, Holloway RH. Stimulation and characteristics of secondary oesophageal peristalsis in normal subjects. Gut 1994;35:152-158.

64. Enzmann DR, Harell GS, Zboralske FF. Upper esophageal responses to intraluminal distension in man. Gastroenterology 1977;72:1292-1298.

65. Yamamoto Y, Liu J, Smith TK, Mittal RK. Distension-related responses in circular and longitudinal muscle of the human esophagus: an ultrasonographic study. Am J Physiol 1998;275:G805-G811.

66. Winship DH, Zboralske FF. The esophageal propulsive force: esophageal response to acute obstruction. J Clin Invest 1967;46:1391-1401.

67. Paterson WG. Neuromuscular mechanisms of esophageal responses at and proximal to a distending balloon. Am J Physiol 1991;260:G148-G155.

68. Ryan JP, Snape WJ, Cohen S. Influence of vagal cooling on esophageal function. Am J Physiol 1977;232:E159-E164.

69. Christensen J, Lund GF. Esophageal responses to distension and electrical stimulation. J Clin Invest 1969;48:408-419.

70. Christensen J. Mechanisms of secondary esophageal peristalsis. Am J Med 1997;103:44S-46S.

71. Paterson WG. Electrical correlates of peristaltic and nonperistaltic contractions in the opossum smooth muscle esophagus. Gastroenterology 1989;97:665-675.

72. Paterson WG, Hynna Liepert TT, Selucky M. Comparison of primary and secondary esophageal peristalsis in humans: effect of atropine. Am J Physiol 1991;260:G52-G57.

73. Fyke FE, Code CF, Schlegel JF. The gastroesophageal sphincter in healthy human beings. Gastroenterologia Basel 1956;86:135-150.

74. Bombeck CT, Dillard DH, Nyhus LM. Muscular anatomy of the gastroesophageal junction and role of phrenoesophageal ligament; autopsy study of sphincter mechanism. Ann Surg 1966;164:644-654.

75. Liebermann-Meffert D, Allgower M, Schmid P, Blum AL. Muscular equivalent of the lower esophageal sphincter. Gastroenterology 1979;76:31-38.

76. Liu JB, Miller LS, Goldberg BB, Feld RI, Alexander AA, Needleman L, Castell DO, Klenn PJ, Millward CL. Transnasal US of the esophagus: preliminary morphologic and function studies. Radiology 1992;184:721-727.

77. Seelig LL Jr., Goyal RK. Morphological evaluation of opossum lower esophageal sphincter. Gastroenterology 1978;75:51-58.

78. Christensen J, Roberts RL. Differences between esophageal body and lower esophageal sphincter in mitochondria of smooth muscle in opossum. Gastroenterology 1983;85:650-656.

79. Christensen J. Anatomy of the myenteric plexus of the opossum esophagus. Gastroenterology 1982;83:1033-1042.

80. Sengupta A, Paterson WG, Goyal RK. Atypical localization of myenteric neurons in the opossum lower esophageal sphincter. Am J Anat 1987;180:342-348.

81. Yuan S, Costa M, Brookes SJH. Neuronal pathways and transmission to the lower esophageal sphincter of the guinea pig. Gastroenterology 1998;115:661-671.

82. Sohn UD, Harnett KM, De Petris G, Behar J, Biancani P. Distinct muscarinic receptors, G proteins and phospholipases in esophageal and lower esophageal sphincter circular muscle. J Pharmacol Exp Ther 1993;267:1205-1214.

83. De Man JG, Pelckmans PA, Boeckxstaens GE, Bult H, Oosterbosch L, Herman AG, Van Maercke YM. The role of nitric oxide in inhibitory non-adrenergic non-cholinergic neurotransmission in the canine lower oesophageal sphincter. Br J Pharmacol 1991;103:1092-1096.

84. Brookes SJ, Chen BN, Hodgson WM, Costa M. Characterization of excitatory and inhibitory motor neurons to the guinea pig lower esophageal sphincter. Gastroenterology 1996;111:108-117.

85. Welch RW, Drake ST. Normal lower esophageal sphincter pressure: A comparison of rapid vs slow pull-through techniques. Gastroenterology 1980;78:1446-1451.

86. Liu J, Parashar VK, Mittal RK. Asymmetry of lower esophageal sphincter pressure: is it related to the muscle thickness or its shape? Am J Physiol 1997;272:G1509-G1517.

87. Kahrilas PJ, Lin S, Chen J, Manka M. The effect of hiatus hernia on gastro-oesophageal junction pressure. Gut 1999;44:476-482.

88. Mittal RK, Rochester DF, McCallum RW. Electrical and mechanical activity in the human lower esophageal sphincter during diaphragmatic contraction. J Clin Invest 1988;81:1182-1189.

89. Boyle JT, Altschuler SM, Nixon TE, Tuchman DN, Pack AI, Cohen S. Role of the diaphragm in the genesis of lower esophageal sphincter pressure in the cat. Gastroenterology 1985;88:723-730.

90. Altschuler SM, Boyle JT, Nixon TE, Pack AI, Cohen S. Simultaneous reflex inhibition of lower esophageal sphincter and crural diaphragm in cats. Am J Physiol 1985;249:G586-G591.

91. Mittal RK, Fisher MJ. Electrical and mechanical inhibition of the crural diaphragm during transient relaxation of the lower esophageal sphincter. Gastroenterology 1990;99:1265-1268.

92. Penagini R, Picone A, Bianchi PA. Quantification of the oesophageal peristaltic reflex in man. J Neurogastroenterol Mot 1996;8:89-94.

93. Paterson W, Rattan S, Goyal R. Esophageal responses to transient and sustained esophageal distension. Am J Physiol 1988;255:G587-G595.

94. Wyman JB, Dent J, Heddle R, Dodds WJ, Toouli J, Downton J. Control of belching by the lower oesophageal sphincter. Gut 1990;31:639-646.

95. Dent J, Dodds WJ, Friedman RH, Sekiguchi T, Hogan WJ, Arndorfer RC, Petrie DJ. Mechanism of gastroesophagal reflux in recumbent asymptomatic subjects. J Clin Invest 1980;65:256-267.

96. Mittal RK, McCallum RW. Characteristics of transient lower esophageal sphincter relaxation in humans. Am J Physiol 1987;252:G636-G641.

97. Conklin JL, Christensen J. Motor functions of the pharynx and esophagus. In: Johnson LR, ed. Physiology of the Gastrointestinal Tract. 3rd ed. New York: Raven Press, 1994:903-928.

98. Schulze-Delrieu K, Crane SA. Oxygen uptake and mechanical tension in esophageal smooth muscle from opossums and cats. Am J Physiol 1982;242:G258-G262.

99. Christensen J. Oxygen dependence of contractions in esophageal and gastric pyloric and ileocecal muscle of opossums. Proc Soc Exp Biol Med 1982;170:194-202.

100. Robison BA, Percy WH, Christensen J. Differences in cytochrome c oxidase capacity in smooth muscle of opossum esophagus and lower esophageal sphincter. Gastroenterology 1984;87:1009-1013.

101. Weisbrodt NW, Murphy RA. Myosin phosphorylation and contraction of feline esophageal smooth muscle. Am J Physiol 1985;249:C9-C14.

102. de Carle DJ, Christensen J, Szabo AC, Templeman DC, McKinley DR. Calcium dependence of neuromuscular events in esophageal smooth muscle of the opossum. Am J Physiol 1977;232:E547-E552.

103. Murray J, Du C, Ledlow A, Maternach P, Conklin J. Guanylate cyclase inhibitors: effect on tone, relaxation, and cGMP content of lower esophageal sphincter. Am J Physiol 1992;263:G97-G101.

104. Asoh R, Goyal RK. Electrical activity of the opossum lower esophageal sphincter in vivo. Its role in the basal sphincter pressure. Gastroenterology 1978;74:835-840.

105. Reynolds JC, Ouyang A, Cohen S. Electrically coupled intrinsic responses of feline lower esophageal sphincter. Am J Physiol 1982;243:G415-G423.

106. Zelcer E, Weisbrodt NW. Electrical and mechanical activity in the lower esophageal sphincter of the cat. Am J Physiol 1984;246:G243-G247.

107. Conklin JL, Du C, Murray JA, Bates JN. Characterization and mediation of inhibitory junction potentials from opossum lower esophageal sphincter. Gastroenterology 1993;104:1439-1444.

108. Sohn UD, Zoukhri D, Dartt D, Sergheraert C, Harnett KM, Behar J, Biancani P. Different protein kinase C isozymes mediate lower esophageal sphincter tone and phasic contraction of esophageal circular smooth muscle. Mol Pharmacol 1997;51:462-470.

109. Greenwood RK, Schegel JF, Code CF, Ellis FH J. The effect of sympathectomy, vagotomy and oesophageal interruption on the canine gastro-oesophageal sphincter. Thorax 1962;17:310-319.

110. Price LM, El-Sharkawy TY, Mui HY, Diamant NE. Effect of bilateral cervical vagotomy on balloon-induced lower esophageal sphincter relaxation in the dog. Gastroenterology 1979;77:324-329.

111. Goyal RK, Rattan S. Nature of the vagal inhibitory innervation to the lower esophageal sphincter. J Clin Invest 1975;55:1119-1126.

112. Goyal RK, Rattan S. Genesis of basal sphincter pressure: effect of tetrodotoxin on lower esophageal sphincter pressure in opossum in vivo. Gastroenterology 1976;71:62-67.

113. Rattan S, Goyal RK. Neural control of the lower esophageal sphincter: influence of the vagus nerves. J Clin Invest 1974;54:899-906.

114. Waldeck F. A new procedure for functional analysis of the lower esophageal sphincter (LES). Pflugers Arch 1972;335:74-84.

115. Zwick R, Bowes KL, Daniel EE, Sarna SK. Mechanism of action of pentagastrin on the lower esophageal sphincter. J Clin Invest 1976;57:1644-1651.

116. Martin CJ, Patrikios J, Dent J. Abolition of gas reflux and transient lower esophageal sphincter relaxation by vagal blockade in the dog. Gastroenterology 1986;91:890-896.

117. Fisher RS, Malmud LS, Roberts GS, Lobis IF. The lower esophageal sphincter as a barrier to gastroesophageal reflux. Gastroenterology 1977;72:19-22.

118. Richardson BJ, Welch RW. Differential effect of atropine on rightward and leftward lower esophageal sphincter pressure. Gastroenterology 1981;81:85-89.

119. Lind JF, Crispin JS, McIver DK. The effect of atropine on the gastroesophageal sphincter. Can J Physiol Pharmacol 1968;46 :233-238.

120. Csendes A, Oster M, Brandsborg O, Moller JT, Overgaard, Brandsborg M, Funch-Jensen P, Amdrup. The effect of vagotomy on human gastroesophageal sphincter pressure in the resting state and following increases in intra-abdominal pressure. Surgery 1979;85:419-424.

121. Fournet J, Snape WJ, Cohen S. Sympathetic control of lower esophageal sphincter function in the cat. Action of direct cervical and splanchnic nerve stimulation. J Clin Invest 1979;63:562-570.

122. Gonella J, Niel JP, Roman C. Sympathetic control of lower oesophageal sphincter motility in the cat. J Physiol 1979;287:177-190.

123. Kawahara H, Blackshaw LA, Lehmann A, Dent J. Responses of the rat lower oesophageal sphincter (LOS) to vagal efferent activation. Neurogastroenterol Motil 1997;9:85-97.

124. DiMarino AJ, Cohen S. The adrenergic control of lower oesophageal sphincter function. An experimental model of denervation sensitivity. J Clin Invest 1973;52:2264-2271.

125. Behar J. Neural control of the lower esophageal sphincter in the cat: Studies on the excitatory pathways to the lower esophageal sphincter. Gastroenterology 1982;82:680-688.

126. Zfass AM, Prince R, Allen FN, Farrar JJ. Inhibitory beta adrenergic receptors in the human distal oesophagus. Am J Dig Dis 1970;15:303-310.

127. Thorpe JAC. Effect of propranolol on the lower oesophageal sphincter in man. Curr Med Res Opin 1980;7:91-95.

128. Giles GR, Mason MC, Humphries C, Clark CG. Action of gastrin on the lower oesophageal sphincter in man. Gut 1969;10:730-734.

129. Kaye MD, Rein R, Johnson WP, Showalter JP. Responses of the competent and incompetent lower oesophageal sphincter to pentagastrin and abdominal compression. Gut 1976;17:933-939.

130. Trindade LM, Rosenberg IL, Rozycki ZJ, Giles G. The response of the lower oesophageal sphincter to maximal doses of pentagastrin. Br J Surg 1975;62:11-14.

131. Dent J, Hansky J. Relationship of serum gastrin response to lower oesophageal sphincter pressure. Gut 1976;17:144-146.

132. Dodds WJ, Hogan WJ, Miller WN, Barreras RF, Arndorfer RC, Stef JJ. Relationship between serum gastrin concentration and lower esophageal sphincter pressure. Am J Dig Dis 1975;20:201-207.

133. Henderson JM, Lidgard G, Osborne DH, Carter DC, Heading RC. Lower oesophageal sphincter response to gastrin - pharmacological or physiological? Gut 1978;19:99-102.

134. Reynolds RPE, El-Sharkawy TY, Diamant NE. Lower esophageal sphincter function in the cat: role of central innervation assessed by transient vagal blockade. Am J Physiol 1984;246:G666-G674.

135. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology 1995;109:601-610.

136. Strombeck DR, Harrold D, Ferrier W. Eructation of gas through the gastroesophageal sphincter before and after truncal vagotomy in dogs. Am J Vet Res 1987;48:207-210.

137. Yamato S, Saha JK, Goyal RK. Role of nitric oxide in lower esophageal sphincter relaxation to swallowing. Life Sci 1992;50:1263-1272.

138. Ny L, Alm P, Larsson B, Ekstrom P, Andersson KE. Nitric oxide pathway in cat esophagus: localization of nitric oxide synthase and functional effects. Am J Physiol 1995;268:G59-G70.

139. Preiksaitis HG, Tremblay L, Diamant NE. Nitric oxide mediates inhibitory nerve effects in human esophagus and lower esophageal sphincter. Dig Dis Sci 1994;39:770-775.

140. Bredt DS, Hwang PM, Snyder SH. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 1990;347:768-770.

141. Murray JA, Clark ED. Characterization of nitric oxide synthase in the opossum esophagus. Gastroenterology 1994;106:1444-1450.

142. Weidner EB, Bao X, Altschuler SM. Localization of nitric oxide synthase in the brain stem neural circuit controlling esophageal peristalsis in rats. Gastroenterology 1995;108:367-375.

143. Miller CA, Barnette MS, Ormsbee HSI, Torphy TJ. Cyclic nucleotide-dependent protein kinases in the lower esophageal sphincter. Am J Physiol 1986;251:G794-G803.

144. Mearin F, Mourelle M, Guarner F, Salas A, Riveros Moreno V, Moncada S, Malagelada JR. Patients with achalasia lack nitric oxide synthase in the gastro-oesophageal junction. Eur J Clin Invest 1993;23:724-728.

145. Murray JA, Ledlow A, Launspach J, Evans D, Loveday M, Conklin JL. The effects of recombinant human hemoglobin on esophageal motor functions in humans. Gastroenterology 1995;109:1241-1248.

146. Aggestrup S, Uddman R, Sundler F, Fahrenkrug J, Hakanson R, Sorensen HR, Hambraeus G. Lack of vasoactive intestinal polypeptide nerves in esophageal achalasia. Gastroenterology 1983;84:924-927.

147. Biancani P, Walsh JH, Behar J. Vasoactive intestinal polypeptide: a neurotransmitter for lower esophageal sphincter. J Clin Invest 1984;73:963-967.

148. Rattan S, Said SI, Goyal RK. Effect of vasoactive intestinal polypeptide (VIP) on lower esophageal sphincter pressure (LESP). Proc Soc Exp Biol Med 1977;155:40-43.

149. Behar J, Guenard V, Walsh JH. VIP and acetylcholine: neurotransmitters in esophageal circular smooth muscle. Am J Physiol 1989;257:G380-G385.

150. Parkman HP, Reynolds JC, Coy DH. (N-AC-TYR, D-PHE)-GRF: an effective antagonist of endogenous and exogenous vasoactive intestinal polypeptide (VIP) in vivo at the feline lower esophageal sphincter (LES) (abstract). Gastroenterology 1987;92:1566.

151. Rattan S, Gonnella P, Goyal RK. Inhibitory effect of calcitonin gene-related peptide and calcitonin on opossum esophageal smooth muscle. Gastroenterology 1988;94:284-293.

152. Uc A, Murray JA, Conklin JL. Effects of calcitonin gene-related peptide on opossum esophageal smooth muscle. Gastroenterology 1997;113:514-520.

153. Dent J, Dodds WJ, Sekiguchi T, Hogan W, Arndorfer RC. Interdigestive phasic contractions of the human lower esophageal sphincter. Gastroenterology 1983;84:453-460.

154. Holloway R, Blank E, Takahashi I, Dodds W, Hogan W, Dent J. Variability of lower esophageal sphincter pressure in the fasted unanesthetized opossum. Am J Physiol 1985;248:G398-G406.

155. Holloway RH, Blank E, Takahashi I, Dodds WJ, Layman RD. Motilin: A mechanism incorporating the opossum lower esophageal sphincter into the migrating motor complex. Gastroenterology 1985;89:507-515.

156. Schoeman MN, Tippett MD, Akkermans LM, Dent J, Holloway RH. Mechanisms of gastroesophageal reflux in ambulant healthy human subjects. Gastroenterology 1995;108:83-91.

157. Nebel OT, Castell DO. Lower esophageal sphincter pressure changes after food ingestion. Gastroenterology 1972;63:778-783.

158. Nebel OT, Castell DO. Inhibition of the lower oesophageal sphincter by fat - a mechanism for fatty food intolerance. Gut 1973;14:270-274.

159. Ledeboer M, Masclee AA, Batstra MR, Jansen JB, Lamers CB. Effect of cholecystokinin on lower oesophageal sphincter pressure and transient lower oesophageal sphincter relaxations in humans. Gut 1995;36:39-44.

160. Brazer SR, Borislow DS, Liddle RA. Cholecystokinin is not a major hormonal regulator of esophageal sphincter pressure. Gastroenterology 1990;99:641-645.

161. Zerbib F, Varannes SBd, Bentouimou N, Leray V, Cherbut C, Roze C, Galmiche JP. Simultaneous post-prandial assessment of lower esophageal sphincter tone, proximal gastric tone and plasma cholecystokinin in normal man (abstract). Gastroenterology 1995;4:A714.

162. Fakhry N, D'Amato M, Hirsch D, Holloway RH, Vrij V, Mathus-Vliegen EMH, Tytgat GNJ, Boeckxstaens GE. Loxiglumide inhibits meal-induced transient LES relaxations in obese patients (abstract). Gastroenterology 1997;112:A730.

163. Trudgill N, D'Amato M, Riley S. Loxiglumide inhibits post-prandial transient lower oesophageal sphincter relaxations in patients with gastro-oesophageal reflux disease (abstract). Gastroenterology 1997;112:A315.

164. Dodds WJ, Dent J, Hogan WJ, Helm JF, Hauser R, Patel GK, Egide MS. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. N Engl J Med 1982;307:1547-1552.

165. McGuigan JE, Ament ME. Anatomy and developmental anomalies. In: Sleisenger MH, Fordtran JS, ed. Gastrointestinal Disease. Pathophysiology, Diagnosis, Management. Philadelphia: W. B. Saunders Company, 1993:459-477.

166. Kelly KA. Gastric emptying of liquids and solids: roles of proximal and distal stomach. Am J Physiol 1980;239:G71-G76.

167. Torgesen J. The muscular build and movements of the stomach and duodenal bulb. Acta Radiol (Suppl) 1942;45:1-101.

168. Toner PG, Watt PCH, Boyd SM. The gastric mucosa. In: Whitehead R, ed. Gastrointestinal and Oesophageal Pathology. New York: Churchill Livingstone, 1989:13-28.

169. Prechtl JC, Powley TL. The fibre composition of the abdominal vagus of the rat. Anat Embryol 1990;181:101-115.

170. Kalia M, Mesulam MM. Brain stem projections of sensory and motor components of the vagus complex in the cat: II. Laryngeal, tracheobronchial, pulmonary, cardiac, and gastrointestinal branches. J Comp Neurol 1980;193:467-508.

171. Leslie R, Reynolds D, Lawes I. Central connections of the nuclei of the vagus nerve. In: Ritter S, Ritter R, Barnes C, ed. Neuroanatomy and Physiology of Abdominal Vagal Afferents. Boca Raton: CRC Press, 1992:81-98.

172. Sawchenko PE. Central connections of the sensory and motor nuclei of the vagus nerve. J Auton Nerv Syst 1983;9:13-26.

173. Blackshaw LA, Grundy D, Scratcherd T. Vagal afferent discharge from gastric mechanoreceptors during contraction and relaxation of the ferret corpus. J Auton Nerv Syst 1987;18:19-24.

174. Andrews PLR, Grundy D, Scratcherd T. Vagal afferent discharge from mechanoreceptors in different regions of the ferret stomach. J Physiol (Lond) 1980;298:513-524.

175. Grundy D. Mechanoreceptors in the gastrointestinal tract. J Smooth Musc Res 1993;29:37-46.

176. Mayer EA. The physiology of gastric storage and emptying. In: Johnson LR, ed. Physiology of the Gastrointestinal Tract. 3rd ed. New York: Raven Press, 1994:929-976.

177. Desai KM, Sessa WC, Vane JR. Involvement of nitric oxide in the reflex relaxation of the stomach to accommodate food or fluid. Nature 1991;351:477-479.

178. Meulemans A, Schuurkes J. Intralipid-induced gastric relaxation is mediated via NO. Neurogastroenterol Motil 1995;7:151-155.

179. Christensen J. A commentary on the morphological identification of interstitial cells of Cajal in the gut. J Auton Nerv Syst 1992;37:75-88.

180. Cannon WB. The movements of the stomach studied by means of the rontgen rays. Am J Physiol 1898;1:359-382.

181. Collins PJ, Houghton LA, Read NW, Horowitz M, Chatterton BE, Heddle R, Dent J. Role of the proximal and distal stomach in mixed solid and liquid meal emptying. Gut 1991;32:615-619.

182. Malagelada J-R, Azpiroz F, Mearin F. Gastroduodenal motor function in health and disease. In: Sleisenger MH, Fordtran JS, ed. Gastrointestinal Disease. Pathophysiology, Diagnosis, Management. Philadelphia: W. B. Saunders Company, 1993:486-508.

183. Hall KE, El-Sharkawy TY, Diamant NE. Vagal control of canine postprandial upper gastrointestinal motility. Am J Physiol 1986;250:G501-G510.

184. Abrahamsson H, Jansson G. Elicitation of reflex vagal relaxation of the stomach from pharynx and esophagus in the cat. Acta Physiol Scand 1969;77:172-178.

185. De Ponti F, Azpiroz F, Malagelada J. Relaxatory responses of canine proximal stomach to esophageal and duodenal distension. Importance of vagal pathways. Dig Dis Sci 1989;34:873-881.

186. Cannon W, Lieb C. The receptive relaxation of the stomach. Am J Physiol 1911;29:270-273.

187. Jahnberg T, Abrahamsson H, Jansson G, Martinson J. Gastric relaxatory response to feeding before and after vagotomy. Scand J Gastroenterol 1977;12:225-228.

188. Rao SSC, Schulze-Delrieu K. The stomach, pylorus and duodenum. In: Kumar D, Wingate D, ed. An illustrated guide to gastrointestinal motility. Edinburgh: Churchill Livingstone, 1993:373-392.

189. Schulze-Delrieu K, Shirazi SS. Pressure and length adaptations in isolated cat stomach. Am J Physiol 1987;252:G92-G99.

190. Stadaas J. Intragastric pressure/volume relationship in the normal human stomach. Scand J Gastroent 1975;10:135-140.

191. Wilbur BG, Kelly KA. Effect of proximal gastric, complete gastric, and truncal vagotomy on canine gastric electric activity, motility, and emptying. Ann Surg 1973;178:295-303.

192. Wilbur BG, Kelly KA, Code CF. Effect of gastric fundectomy on canine gastric electrical and motor activity. Am J Physiol 1974;226:1445-1449.

193. Moragas G, Azpiroz F, Pavia J, Malagelada J-R. Relations among intragastic pressure, post-cibal perception and gastric emptying. Am J Physiol 1993;264:G1112-G1117.

194. Paraskevopoulos JA, Houghton LA, Eyre-Brooke I, Johnson AG, Read NW. Effect of composition of gastric contents on resistance to emptying of liquids from stomach in humans. Dig Dis Sci 1988;33:914-918.

195. Dozois RR, Kelly KA, Code CF. Effect of distal antrectomy on gastric emptying of liquids and solids. Gastroenterology 1971;61:675-681.

196. Mroz CT, Kelly KA. The role of extrinsic antral nerves in the regulation of gastric emptying. Surg Gynecol Obstet 1977;145:369-377.

197. Malbert CH, Mathis C. Antropyloric modulation of transpyloric flow of liquids in pigs. Gastroenterology 1994;107:37-46.

198. Weisbrodt NW, Wiley JN, Oberholt BF, Bass P. A relation between gastroduodenal muscle contractions and gastric emptying. Gut 1969;10:543-548.

199. Stemper TJ, Cooke AR. Gastric emptying and its relationship to antral contractile activity. Gastroenterology 1975;69:649-653.

200. Camilleri M, Malagelada J-R, Brown M, Becker G, Zinsmeister AR. Relation between antral motility and gastric emptying of solids and liquids in humans. Am J Physiol 1985;249:G580-G585.

201. Anvari M, Yu P, Dent J, Jamieson GG. Role of antral intramural neural pathways in control of gastric emptying in the pig. J Physiol 1995;488:203-209.

202. Meyer JH. Motility of the stomach and gastroduodenal junction. In: Johnson LR, ed. Physiology of the Gastrointestinal Tract. 2nd ed. New York: Raven Press, 1987:613-629.

203. Bauer AJ, Sanders KM. Gradient in excitation-contraction coupling in canine gastric antral circular muscle. J Physiol 1985;369:283-294.

204. Andrews PLR, Scratcherd T. The gastric motilty patterns induced by direct and reflex excitation of the vagus nerves in the anaesthetized ferret. J Physiol 1980;302:363-378.

205. Sarna SK, Daniel EE. Vagal control of gastric electrical control activity and motility. Gastroenterology 1975;68:301-308.

206. Desai KM, Warner TD, Bishop AE, Polak JM, Vane JR. Nitric oxide, and not vasoactive intestinal peptide, as the main neurotransmitter of vagally induced relaxation of the guinea pig stomach. Br J Pharmacol 1994;113:1197-1202.

207. Grundy D, Gharib-Naseri MK, Hutson D. Role of nitric oxide and vasoactive intestinal polypeptide in vagally mediated relaxation of the gastric corpus in the anaesthetized ferret. J Auton Nerv Syst 1993;43:241-246.

208. Azpiroz F, Malagelada J-R. Importance of vagal input in maintaining gastric tone in the dog. J Physiol (Lond) 1987;384:511-524.

209. Grundy D, Gharib-Naseri MK, Hutson D. Plasticity in the gastric inhibitory innervation after immunization against VIP and vagotomy in the ferret. Am J Physiol 1993;265:G432-G439.

210. Bruley des Varannes S, Parys V, Ropert A, Chayvialle JA, Roze C, Galmiche JP. Erythromycin enhances fasting and postprandial proximal gastric tone in humans. Gastroenterology 1995;109:32-39.

211. Parys V, Bruley des Varannes S, Ropert A, Roze C, Galmiche JP. Use of an electronic barostat for measurement of motor response of the proximal stomach to feeding and different nervous stimuli in man. Gastroenterol Clin Biol 1993;17:321-328.

212. Ozaki H, Blondfield DP, Hori M, Publicover NG, Kato I, Sanders KM. Spontaneous release of nitric oxide inhibits electrical, Ca2+ and mechanical transients in canine gastric smooth muscle. J Physiol (Lond) 1992;445:231-247.

213. Konturek JW, Thor P, Domschke W. Effects of nitric oxide on antral motility and gastric emptying in humans. Eur J Gastroenterol Hepatol 1995;7:97-102.

214. Jansson G, Martinson J. Studies on the ganglionic site of action of the sympathetic outflow to the stomach. Acta Physiol Scand 1966;68:184-192.

215. Andrews PL, Grundy D, Lawes IN. The role of the vagus and splanchnic nerves in the regulation of intragastric pressure in the ferret. J Physiol 1980;307:401-411.

216. Andrews PLR, Lawes INC. Interactions between splanchnic and vagus nerves in the control of mean intragastric pressure in the ferret. J Physiol (Lond) 1984;351:473-490.

217. Chey W, Hasler W, Bhattacharyya N, Soudah H, Owyang C. Intraduodenal lipid induces isobaric gastric fundus relaxation, antral motor inhibition and pyloric contraction: Role of endogenous CCK (abstract). Gastroenterology 1993;104:A817.

218. Salet G, Thimister P, Roelofs J, Hopman W, Smout A, Jansen J, Akkermans L. Proximal gastric responses to a CCK-A receptor antagonist in man (abstract). Gastroenterology 1996;110:A750.

219. Zerbib F, Bruley des Varannes S, D'Amato M, Scarpignato C, Galmiche JP. Effect of the CCK-A receptor antagonist loxiglumide on gastric tone and transient lower esophageal sphincter relaxations in humans (abstract). Gastroenterology 1997;112:A857.

220. Zerbib F, Bruley des Varannes S, D'Amato M, Scarpignato C, Galmiche J. Effect of loxiglumide on lower esophageal sphincter motor events and gastric relaxation induced by duodenal infusion of a liquid meal in healthy subjects (abstract). Gastroenterology 1998;114:A863.

221. Lee KY, Chang TM, Chey WY. Effect of rabbit antimotilin serum on myoelectric activity and plasma motilin concentration in fasting dog. Am J Physiol 1983;8:G547-G553.

222. Poitras P, Steinbach JH, VanDeventer G, Code CF, Walsh JH. Motilin-independent ectopic fronts of the interdigestive myoelectric complex in dogs. Am J Physiol 1980;239:G215-G220.

223. Valenzuela JE, Grossman MI. Effect of pentagastrin and caerulein on intragastric pressure in dogs. Gastroenterology 1975;69:1383-1384.

224. Valenzuela JE. Effect of intestinal hormones and peptides on intragastric pressure in dogs. Gastroenterology 1976;71:766-769.

225. Valenzuela JE. Dopamine as a possible neurotransmitter in gastric relaxation. Gastroenterology 1976;71:1019-1022.

226. Morgan KG, Schmalz PF, Szurszewski JH. The inhibitory effects of vasoactive intestinal polypeptide on the mechanical and electrical activity of canine antral smooth muscle. J Physiol (Lond) 1978;282:437-450.

227. Morgan K, Schmalz P, Go V, Szurszewski J. Electrical and mechanical effects of molecular variants of CCK on antral smooth muscle. Am J Physiol 1978;235:E324-E329.

228. Sanders KM, Vogalis F. Organization of electrical activity in the canine pyloric canal. J Physiol (Lond) 1989;416:49-66.

229. Zaninotto G, DeMeester TR, Schwizer W, Johansson KE, Cheng SC. The lower esophageal sphincter in health and disease. Am J Surg 1988;155:104-111.

230. O'Sullivan GC, DeMeester TR, Joelsson BE, Smith RB, Blough RR, Johnson LF, Skinner DB. Interaction of lower esophageal spincter pressure and length of spincter in the abdomen as determinants of gastroesophageal competence. Am J Surg 1982;143:40-47.

231. DeMeester TR, Lafontaine E, Joelsson BE, Skinner DB, Ryan JW, O'Sullivan GC, Brunsden BS, Johnson LF. Relationship of a hiatal hernia to the function of the body of the esophagus and the gastroesophageal junction. J Thorac Cardiovasc Surg 1981;82:547-558.

232. Mittal RK, Fisher M, McCallum RW, Rochester DF, Dent J, Sluss J. Human lower esophageal sphincter pressure response to increased intra-abdominal pressure. Am J Physiol 1990;258:G624-G630.

233. Moore KL. The digestive system. In: Moore KL, ed. The developing human. Clinically orientated embryology. 3rd ed. Philadelphia: W B Saunders Company, 1982:227-254.

234. Delattre JF, Palot JP, Ducasse A, Flament JB, Hureau J. The crura of the diaphragm and diaphragmatic passage. Applications to gastroesophageal reflux, its investigation and treatment. Anat Clin 1985;7:271-283.

235. Berger AJ, Mitchell RA, Severinghaus JW. Regulation of respiration: (second of three parts). New Engl J Med 1977;297:138-143.

236. Easton PA, Fitting JW, Grassino AE. Costal and crural diaphragm in early inspiration: free breathing and occlusion. J App Physiol 1987;63:1622-1628.

237. Monges H, Salducci J, Naudy B. Dissociation between the electrical activity of the diaphragmatic dome and crura muscular fibers during esophageal distension, vomiting and eructation. An electromyographic study in the dog. J Physiol (Paris) 1978;74:541-554.

238. Martin CJ, Dodds WJ, Liem HH, Dantas RO, Layman RD, Dent J. Diaphragmatic contribution to gastroesophageal competence and reflux in dogs. Am J Physiol 1992;263:G551-G557.

239. Altschuler SM, Davies RO, Pack AI. Role of medullary inspiratory neurones in the control of the diaphragm during oesophageal stimulation in cats. J Physiol (Lond) 1987; 391:289-298.

240. Klein WA, Parkman HP, Dempsey DT, Fisher RS. Sphincterlike thoracoabdominal high pressure zone after esophagogastrectomy. Gastroenterology 1993;105:1362-1369.

241. Mittal RK, Sivri B, Shirmer BD, Heine KJ. Effect of crural myotomy on the incidence and mechanism of gastroesophageal refux in cats. Gastroenterology 1993;105:740-747.

242. Mittal RK, Balaban DH. The esophagogastric junction. New Engl J Med 1997;336:924-932.

243. Mittal RK, Holloway R, Dent J. Effect of atropine on the frequency of reflux and transient lower esophageal sphincter relaxation in normal subjects. Gastroenterology 1995;109:1547-1554.

244. Munzer D. Importance of the angle of Hiss in the gastroesophageal reflux disease (GERD) (abstract). Am J Gastroenterol 1993;88:1514.

245. Thor KD, Hill LD, Mercer DD, Kozarek RD. Reappraisal of the flap valve mechanism in the gastroesophageal junction: a study of a new valvuloplasty in cadavers. Acta Chir Scand 1987;153:25-28.

246. Dodds WJ, Hogan WJ, Helm JF, Dent J. Pathogenesis of reflux oesophagitis. Gastroenterology 1981;81:376-394.

247. Mittal RK, McCallum RW. Characteristics and frequency of transient relaxations of the lower esophageal sphincter on patients with reflux esophagitis. Gastroenterology 1988;95:593-599.

248. Dent J, Holloway RH, Toouli J, Dodds WJ. Mechanisms of lower oesophageal sphincter incompetence in patients with symptomatic gastroesophageal reflux. Gut 1988; 29:1020-1028.

249. Penagini R, Schoeman MN, Dent J, Tippett MD, Holloway RH. Motor events underlying gastro-oesophageal reflux in ambulant patients with reflux oesophagitis. J Neurogastroenterol Mot 1996;8:131-141.

250. Ogilvie AL, James PD, Atkinson M. Impairment of vagal function in reflux oesophagitis. Q J Med 1985;213:61-74.

251. Chakraborty TK, Ogilvie AL, Heading RC, Ewing DJ. Abnormal cardiovascular reflexes in patients with gastro-oesophageal reflux. Gut 1989;30:46-49.

252. Cunningham KM, Horowitz M, Riddel PS, G.J. M, Myers JC, Holloway RH, Wishart JM, Jamieson GG. Relations among autonomic nerve dysfunction, oesophageal motility and gastric emptying in gastro-oesophageal reflux disease. Gut 1991;32:1436-1440.

253. Grossman MI. What is physiological? (letter). Gastroenterology 1973;65:994.

254. Eastwood GL, Castell DO, Higgs RH. Experimental esophagitis in cats impairs lower esophageal sphincter pressure. Gastroenterology 1975;69:146-153.

255. Wesdorp ICE, Bartelsman J, Schipper MEI, Tytgat GN. Effect of long term treatment with cimetidine and antacids in Barrett's oesophagus. Gut 1981;22:724-727.

256. Downton J, Dent J, Heddle R, Toouli J, Buckle PJ, MacKinnon AM, Wyman JB. Elevation of gastric pH heals peptic oesophagitis - a role for omeprazole. J Gastroenterol Hepatol 1987;2:317-324.

257. Singh P, Adamopoulos A, Taylor RH, Colin-Jones DG. Oesophageal motor function before and after healing of oesophagitis. Gut 1992;33:1590-1596.

258. Hetzel DJ, Dent J, Reed WD, Narielvala FM, Mackinnon M, McCarthy JH, Mitchell B, Beveridge BR, Laurence BH, Gibson GG, Grant AK, Shearman DJC, Whitehead R, Buckle PJ. Healing and relapse of severe peptic esophagitis after treatment with omeprazole. Gastroenterology 1988;95:903-912.

259. Dodds WJ, Kahrilas PJ, Dent J, Hogan WJ, Kern MK, Arndorfer RC. Analysis of spontaneous gastroesophageal reflux and esophageal acid clearance in patients with reflux esophagitis. J Gastrointest Mot 1990;2:79-89.

260. Kahrilas PJ, Dodds WJ, Hogan WJ, Kern M, Arndorfer RC, Reece A. Esophageal peristaltic dysfunction in peptic esophagitis. Gastroenterology 1986;91:897-904.

261. Holloway RH, Kocyan P, Dent J. Provocation of transient lower esophageal sphincter relaxations by meals in patients with symptomatic gastroesophageal reflux. Dig Dis Sci 1991;36:1034-1039.

262. Vantrappen G, Janssens J. Lower esophagus: pathophysiological aspects. In: Daniel EE, Tomita T, Tsuchida S, Watanabe M, ed. Sphincters: Normal function-changes in diseases. Boca Raton: CRC Press Inc, 1992:67-82.

263. Wright RA, Hurwitz AL. Relationship of hiatal hernia to endoscopically proved reflux esophagitis. Dig Dis Sci 1979;24:311-313.

264. Ott DJ, Gelfand DW, Chen YM, Wu WC, Munitz HA. Predictive relationship of hiatal hernia to reflux esophagitis. Gastrointest Radiol 1985;10:317-320.

265. Berstad A, Weberg R, Froyshov Larsen I, Hoel B, Hauer-Jensen M. Relationship of hiatus hernia to reflux oesophagitis. Scand J Gastroenterol 1986;21:55-58.

266. Mittal RK, Rochester DF, McCallum RW. Effect of the diaphragmatic contraction on lower oesophageal sphincter pressure in man. Gut 1987;28:1564-1568.

267. Sloan S, Rademaker AW, Kahrilas PJ. Determinants of gastroesophageal junction incompetence: hiatal hernia, lower esophageal sphincter, or both? Ann Intern Med 1992;117:977-982.

268. Holloway RH, Dent J. Pathophysiology of gastroesophageal reflux. Lower esophageal sphincter dysfunction in gastroesophageal reflux disease. Gastroenterol Clin N Am 1990;19:517-535.

269. Mittal RK, Lange RC, McCallum RW. Identification and mechanism of delayed esophageal acid clearance in subjects with hiatus hernia. Gastroenterology 1987;92:130-135.

270. Behar J, Ramsby G. Gastric emptying and antral motility in reflux esophagitis. Effect of oral metoclopramide. Gastroenterology 1978;74:253-256.

271. Little AG, DeMeester TR, Kirchner PT, O'Sullivan GC, Skinner DB. Pathogenesis of esophagitis in patients with gastroesophageal reflux. Surgery 1980;88:101-107.

272. McCallum RW, Berkowitz DM, Lerner E. Gastric emptying in patients with gastroesophageal reflux. Gastroenterology 1981;80:285-291.

273. Maddern GJ, Chatterton BE, Collins PJ, Horowitz M, Shearman DJC, Jamieson GG. Solid and liquid gastric emptying in patients with gastro-oesophageal reflux. Br J Surg 1985;72:344-347.

274. Collins BJ, McFarland RJ, O'Hare MMT, Shaw C, Buchanan KD, Love AHG. Gastric emptying of a solid-liquid meal and gastro-intestinal hormone responses in patients with erosive oesophagitis. Digestion 1986;33:61-68.

275. Micali B, Albanese V, Baldari S, Cogliandolo A, Guilino FM, Scarpignato C. Gastric emptying of solids in gastroesophageal reflux. Gastroenterol Clin Biol 1986;10:656-661.

276. Shay SS, Eggli D, McDonald C, Johnson LF. Gastric emptying of solid food in patients with gastroesophageal reflux. Gastroenterology 1987;92:459-465.

277. Keshavarzian A, Bushnell DL, Sontag S, Yegelwel EJ, Smid K. Gastric emptying in patients with severe reflux esophagitis. Am J Gastroenterol 1991;86:738-742.

278. Scarpignato C, Franze A. Esophageal exposure to acid in GERD patients with and without delayed gastric emptying. Effect of cisapride. Hepato-Gastroenterol 1992;39:91-92.

279. Stein HJ, Hoeft S, DeMeester TR. Functional foregut abnormalities in Barrett's esophagus. J Thorac Cardiovasc Surg 1993;105:107-111.

280. Penagini R, Hebbard G, Horowitz M, Dent J, Bermingham H, Jones K, Holloway RH. Motor function of the proximal stomach and visceral perception in gastro-oesophageal reflux disease. Gut 1998;42:251-257.

281. Benini L, Sembenini C, Castellani G, Caliari S, Fioretta A, Vantini I. Gastric emptying and dyspeptic symptoms in patients with gastroesophageal reflux. Am J Gastroenterol 1996;91:1351-1354.

282. Urbain D, Muls V, Caucheteur B, Cadiere GB, Ham HR. Gastric emptying in patients with gastroesophageal reflux disease (letter). Am J Gastroenterol 1997;92:724.

283. Hartley MN, Walker SJ, Mackie CR. Abnormal gastric adaptive relaxation in patients with gastro-oesophageal reflux. Gut 1990;31:500-503.

284. Zerbib F, Bruley des Varannes S, Ropert A, Lamouliatte H, Galmiche J. Postprandial fundic relaxation is increased in patients gastro-esophageal reflux disease. Gastroenterology 1998;114:A346.

285. Attwood SEA, DeMeester TR, Bremner CG, Barlow AP, Hinder RA. Alkaline gastroesophageal reflux: implications in the development of complications in Barrett's columnar-lined lower esophagus. Surgery 1989;106:764-770.

286. Stein HJ, Barlow AP, DeMeester TR, Hinder RA. Complications of gastroesophageal reflux disease. Role of the lower esophageal sphincter, esophageal acid and acid/alkaline exposure, and duodenogastric reflux. Ann Surg 1992;216:35-43.

287. Champion G, Richter JE, Vaezi MF, Singh S, Alexander R. Duodenogastroesophageal reflux: relationship to pH and importance in Barrett's esophagus. Gastroenterology 1994;107:747-754.

288. Parkman HP, Harris AD, Krevsky B, Urbain JL, Maurer AH, Fisher RS. Gastroduodenal motility and dysmotility: an update on techniques available for evaluation. Am J Gastroenterol 1995;90:869-92.

289. Lidums I, Holloway R. Motility abnormalities in the columnar-lined esophagus. Gastroenterol Clin N Am 1997;26:519-531.

290. King PM, Pryde A, Heading RC. Transpyloric fluid movement and antroduodenal motility in patients with gastro-oesophageal reflux. Gut 1987;28:545-548.

291. Helm JF, Dodds WJ, Pelc LR, Palmer DW, Hogan WJ, Teeter BC. Effect of esophageal emptying and saliva on clearance of acid from the esophagus. N Engl J Med 1984;310:284-288.

292. Helm J, Dodds W, Pelc L, Palmer D, Hogan W, Teeter B. Mechanisms of oesophageal acid clearance in supine normal subjects: a unifying hypothesis (abstract). Gastroenterology 1981;80:1171.

293. Helm JF, Dodds WJ, Hogan WJ, Soergel KH, Egide MS, Wood CM. Acid neutralizing capacity of human saliva. Gastroenterology 1982;83:69-74.

294. Booth DJ, Kemmerer WT, Skinner DB. Acid clearing from the distal esophagus. Arch Surg 1968;96 :731-734.

295. DeMeester TR, Johnson LF, Joseph GJ, Toscano MS, Hall AW, Skinner DB. Patterns of gastroesophageal reflux in health and disease. Ann Surg 1976;184:459-469.

296. Stanciu C, Bennett JR. Oesophageal acid clearing: one factor in the production of reflux oesophagitis. Gut 1974;15:852-857.

297. Johnson LF. 24-hour pH monitoring in the study of gastroesophageal reflux. J Clin Gastroenterol 1980;2:378-399.

298. Stein HJ, Eypasch EP, DeMeester TR, Smyrk TC, Attwood SEA. Circadian esophageal motor function in patients with gastroesophageal reflux disease. Surgery 1990;108:769-778.

299. Williams S, Thompson D, Marples M, Heggie L, O'Hanrahan T, Mani V, Bancewicz J. Identification of an abormal esophageal clearance response to intraluminal distension in patients with esophagitis. Gastroenterology 1992;103:943-953.

300. Schoeman MN, Holloway RH. Integrity and characteristics of secondary oesophageal peristalsis in patients with gastro-oesophageal reflux disease. Gut 1995;36:499-504.

301. Allen ML, Castell JA, DiMarino AJ Jr. Mechanisms of gastroesophageal acid reflux and esophageal acid clearance in heartburn patients. Am J Gastroenterol 1996;91:1739-1744.

302. Orr WC, Robinson MG, Johnson LF. Acid clearance during sleep in the pathogenesis of reflux esophagitis. Dig Dis Sci 1981;26:423-427.

303. Sonnenberg A, Steinkamp U, Weise A, Berges W, Wienbeck M, Rohner HG, Peter P. Salivary secretion in reflux esophagitis. Gastroenterology 1982;83:889-895.

304. Kahrilas PJ, Gupta RJ. The effect of cigarette smoking on salivation and esophageal acid clearance. J Lab Clin Med 1989;114:41-48.

305. Kahrilas PJ. Cigarette smoking and gastroesophageal reflux disease. Dig Dis 1992;10:61-71.

306. McNally EF, Kelly JE, Ingelfinger FJ. Mechanism of belching: effects of gastric distension with air. Gastroenterology 1964;46:254-259.

307. Kahrilas PJ, Dodds WJ, Dent J, Wyman JB, Hogan WJ, Arndorfer RC. Upper esophageal sphincter function during belching. Gastroenterology 1986;91:133-140.

308. Cucchiara S, Bortolotti M, Minella R, Auricchio S. Fasting and postprandial mechanisms of gastroesophageal reflux in children with gastroesophageal reflux disease. Dig Dis Sci 1993;38:86-92.

309. Freidin N, Mittal RK, McCallum RW. Does body posture affect the incidence and mechanism of gastro-oesophageal reflux? Gut 1991;32:133-136.

310. Mittal RK, Stewart WR, Schirmer BD. Effect of a catheter in the pharynx on the frequency of transient lower esophageal sphincter relaxations. Gastroenterology 1992;103:1236-1240.

311. Penagini R, Bartesaghi B, Bianchi P. Effect of cold stress on postprandial lower esophageal sphincter competence and gastroesophageal reflux in healthy subjects. Dig Dis Sci 1992;37:1200-1205.

312. Holloway R, Sifrim DA. Esophageal motor disorders. Current Opinion in Gastroenterology 1998;14:334-339.

313. Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J Physiol 1995;268:G128-G133.

314. Facchini FR, Chiao GZ, Noga C, Kahrilas PJ. Swallow-induced LES relaxation vs non-swallow induced LES relaxation, what is a TLESR? (abstract). Gastroenterology 1994;106:A1025.

315. Kawahara H, Dent J, Davidson G. Mechanisms responsible for gastroesophageal reflux in children. Gastroenterology 1997;113:399-408.

316. Sifrim D, Holloway RH, Missotten T, Zelter A, Janssens J. Swallow-induced abnormally prolonged lower esophageal sphincter relaxations (SAPLESRs) (abstract). Gastroenterology 1998;114:A838.

317. Sifrim D, Janssens J, Vantrappen G. Transient lower esophageal sphincter relaxations and esophageal body muscular contractile response in normal humans. Gastroenterology 1996;110:659-668.

318. Kahrilas PJ, Gupta RR, Jacob P, McLaughlin B, Rana F. Isolated transient LES relaxations are not "subthreshold swallows" (abstract). Gastroenterology 1988;95:873.

319. Holloway RH, Hongo M, Berger K, McCallum RW. Gastric distension: a mechanism for postprandial gastroesophageal reflux. Gastroenterology 1985;89:779-784.

320. Ergun GA, Kahrilas PJ, Lin S, Logemann JA, Harig JM. Shape, volume, and content of the deglutitive pharyngeal chamber imaged by ultrafast computerized tomography. Gastroenterology 1993;105:1396-1403.

321. Blackshaw LA, Staunton E, Dent J, Holloway RH, Malbert CH. Mechanisms of gastro-oesophageal reflux in the ferret. Neurogastroenterol Mot 1998;10:49-56.

322. Franzi SJ, Martin CJ, Cox MR, Dent J. Response of canine lower esophageal sphincter to gastric distension. Am J Physiol 1990;259:G380-G385.

323. Strombeck DR, Griffin D, Harrold D. Eructation of gas through the gastroesophageal sphincter before and after limiting distension of the gastric cardia or infusion of a B-adrenergic amine in dogs. Am J Vet Res 1989;50:751-753.

324. Penagini R, Bartesaghi B, Conte D, Bianchi P. Rate of transient lower oesophageal sphincter relaxations of healthy humans after eating a mixed nutrient meal: time course and comparison with fasting. Eur J Gastroenterol Hepatol 1992;4:35-38.

325. Holloway RH, Lyrenas E, Ireland AC, Dent J. Effect of introduodenal fat on lower oesophageal sphincter function and gastro-oesophageal reflux. Gut 1997;40:449-453.

326. Miller AJ. Neurophysiological basis of swallowing. Dysphagia 1986;1:91-100.

327. Storey AT. Laryngeal initiation of swallowing. Exp Neurol 1968;20:359-365.

328. Sinclair WJ. Role of the pharyngeal plexus in initiation of swallowing. Am J Physiol 1971;221:1260-1263.

329. Trifan A, Ren J, Arndorfer R, Hofmann C, Bardan E, Shaker R. Inhibition of progressing primary esophageal peristalsis by pharyngeal water stimulation in humans. Gastroenterology 1996;110:419-423.

330. Trifan A, Shaker R, Ren J, Mittal RK, Saeian K, Dua K, Kusano M. Inhibition of resting lower esophageal sphincter pressure by pharyngeal water stimulation in humans. Gastroenterology 1995;108:441-446.

331. Mittal RK, Chiareli C, Liu J, Shaker R. Characteristics of lower esophageal sphincter relaxation induced by pharyngeal stimulation with minute amounts of water. Gastroenterology 1996;111:378-384.

332. Mittal RK, Chiareli C, Liu J, Holloway RH, Dixon W. Atropine inhibits gastric distension and pharyngeal receptor mediated lower oesophageal sphincter relaxation. Gut 1997;41:285-290.

333. Dougherty RW. Esophageal innervation and the eructation reflex in sheep. Am J Vet Res 1958;19:115-128.

334. Little AF, Cox MR, Martin CJ, Dent J. Influence of posture on transient lower oesophageal sphincter relaxation and gastro-oesophageal reflux in the dog. J Gastroenterol Hepatol 1989;4:49-54.

335. Ireland AC, Dent J, Holloway RH. Preservation of postural control of transient lower oesophageal sphincter relaxations in patients with reflux oesophagitis. Gut 1999;44:313-316.

336. Kapur KC, Trudgill NJ, Riley SA. Mechanisms of gastro-oesophageal reflux in the lateral decubitus positions. Neurogastroenterol Mot 1998;10:517-522.

337. Ireland A, Dent J, Holloway RH. The role of head position in the postural control of transient lower oesophageal sphincter relaxations and belching. Gullet 1992;2:81-84.

338. Freidin N, Fisher MJ, Taylor W, Boyd D, Surratt P, McCallum RW, Mittal RK. Sleep and nocturnal acid reflux in normal subjects and patients with reflux oesophagitis. Gut 1991;32:1275-1279.

339. Cox MR, Martin CJ, Dent J, Westmore M. Effect of general anaesthesia on transient lower oesophageal sphincter relaxations in the dog. Aust NZ J Surg 1988;58:825-830.

340. Schulze-Delrieu K, Percy WH, Ren J, Shirazi SS, Von Derau K. Evidence for inhibition of opossum LES through intrinsic gastric nerves. Am J Physiol 1989;256:G198-G205.

341. Holloway R, Wyman J, Dent J. Failure of transient lower oesphageal sphincter relaxation in response to gastric distension in patients with achalasia: evidence for neural mediation of transient lower oesphageal sphincter relaxations. Gut 1989;30:762-767.

342. Paterson WG, Anderson MAB, Anand N. Pharmacological characterization of lower esophageal sphincter relaxation induced by swallowing, vagal efferent nerve stimulation, and esophageal distension. Can J Physiol Pharmacol 1992;70:1011-1015.

343. Neuhuber WL, Sandoz PA. Vagal primary afferent terminals in the dorsal motor nucleus of the rat: are they making monosynaptic contacts on preganglionic efferent neurons? Neurosci Lett 1986;69:126-130.

344. Rinaman L, Card JP, Schwaber JS, Miselis RR. Ultrastructural demonstration of a gastric monosynaptic vagal circuit in the nucleus of the solitary tract in rat. J Neurosci 1989;9:1985-1996.

345. Jansson G. Vago-vagal reflex relaxation in the stomach in the cat. Acta Physiol Scand 1969;75:245-252.

346. Doty RW. Influence of stimulus pattern on reflex deglutition. Am J Physiol 1951;166:142-158.

347. Sumi T. Some properties of cortically-evoked swallowing and chewing in rabbits. Brain Res 1969;15:107-120.

348. Wank SA. Cholecystokinin receptors. Am J Physiol 1995;269:G628-G646.

349. Corp ES, McQuade J, Moran TH, Smith GP. Characterization of type A and B CCK receptor binding sites in rat vagus nerve. Brain Res 1993;623:161-166.

350. Moran TH, Robinson PH, Goldrich MS, McHugh PR. Two brain cholecystokinin receptors: implications for behavioral actions. Brain Res 1986;362:175-179.

351. Rattan S, Goyal RK. Structure-activity relationship of subtypes of cholecystokinin receptors in the cat lower esophageal sphincter. Gastroenterology 1986;90:94-102.

352. Boulant J, Fioramonti J, Dapoigny M, Bommelaer G, Bueno L. Cholecystokinin and nitric oxide in transient lower esophageal sphincter relaxation to gastric distension in dogs. Gastroenterology 1994;107:1059-1066.

353. Boulant J, Mathieu S, D'Amato M, Abergel A, Dapoigny M, Bommelaer G. Cholecystokinin in transient lower oesophageal sphincter relaxation due to gastric distension in humans. Gut 1997;40:575-581.

354. Boeckxstaens GE, Hirsch DP, Fakhry N, Holloway RH, D'Amato M, Tytgat GNJ. Involvement of cholecystokinin A receptors in transient lower esophageal sphincter relaxations triggered by gastric distension. Am J Gastroenterol 1998;93:1823-1828.

355. Clave P, Gonzalez A, Moreno A, Lopez R, Farre A, Cusso X, D'Amato M, Azpiroz F, Lluis F. Endogenous cholecystokinin enhances postprandial gastroesophageal reflux in humans through extrasphincteric receptors. Gastroenterology 1998;115:597-604.

356. Mesquita MA, Thompson DG, Troncon LE, D'Amato M, Rovati LC, Barlow J. Effect of cholecystokinin-A receptor blockade on lipid-induced gastric relaxation in humans. Am J Physiol 1997;273:G118-G123.

357. Feinle C, D'Amato M, Read NW. Cholecystokinin-A receptors modulate gastric sensory and motor responses to gastric distension and duodenal lipid. Gastroenterology 1996;110:1379-85.

358. Hirsch DP, Holloway RH, Tytgat GNJ, Boeckxstaens GE. Involvement of nitric oxide in human transient lower esophageal sphincter relaxations and esophageal primary peristalsis. Gastroenterology 1998;115:1374-1380.

359. Meulemans AL, Eelen JG, Schuurkes JA. NO mediates gastric relaxation after brief vagal stimulation in anesthetized dogs. Am J Physiol 1995;269:G255-G261.

360. Botticelli LJ, Cox BM, Goldstein A. Immunoreactive dynorphin in mammalian spinal cord and dorsal root ganglia. Proc Natl Acad Sci USA 1981;78:7783-7786.

361. Polak JM, Bloom SR, Sullivan SN, Facer P, Pearse AGE. Enkephalin-like immunoreactivity in the human gastrointestinal tract. Lancet 1977;1:972-974.

362. Mansour A, Fox CA, Akil H, Watson SJ. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci 1995;18:22-29.

363. Grider GR, Makhlouf GM. Identification of opioid receptors on gastric muscle cells by selective receptor protection. Am J Physiol 1991;260:G103-G107.

364. Penagini R, Picone A, Bianchi PA. Effect of morphine and naloxone on motor response of the human esophagus to swallowing and distension. Am J Physiol 1996;271:G675-680.

365. Penagini R, Bianchi PA. Effect of morphine on gastroesophageal reflux and transient lower esophageal sphincter relaxation. Gastroenterology 1997;113:409-414.

366. Brann RB, Ellis J, Jorgensen H, Hill-Eubanks D, Jones SVP. Muscarinic acetylcholine receptor subtypes: localization and structure/function. Prog Brain Res 1993;98:121-126.

367. Caulfield MP. Muscarinic receptors-Characterization, coupling and function. Pharmac Ther 1993;58:319-379.

368. Levey AI. Immunological localization of m1-m5 muscarinic acetylcholine receptors in peripheral tissues and brain. Life Sci 1993;52:441-448.

369. Read NW, Gwee KA. The importance of 5-hydroxytryptamine receptors in the gut. Pharmac Ther 1994;62:159-73.

370. Gershon MD, Wade PR, Kirchgessner AL, Tamir H. 5-HT receptor subtypes outside the central nervous system. Roles in the physiology of the gut. Neuropsychopharmacology 1990;3:385-395.

371. Castro ME, Pascual J, Romon T, del Arco C, del Olmo E, Pazos A. Differential distribution of [3H] sumatriptan binding sites (5-HT1B, 5-HT1D and 5-HT1F receptors) in human brain: focus on brainstem and spinal cord. Neuropharmacology 1997;36:535-542.

372. Sifrim D, Holloway R, Missotten T, Zelter A, Tack J, Janssens J. Sumatriptan maintains the post-prandial increase in transient lower esophageal sphincter relaxations and increases gastroesophageal reflux in normal subjects. Am J Gastroenterol 1999 (In press).

373. Tack JF, Coulie B, Wilmer A, Janssens J. Sumatriptan, a 5-HT1-receptor agonist, causes a significant relaxation of the gastric fundus in man (abstract). Gastroenterology 1995;108:A696.

374. Straathof JW, Tielman S, Lamers CBHW, Masclee AAM. Somatostatin prevents meal induced alterations in lower esophageal sphincter function (abstract). Gut 1996;39:A183.

375. Rouzade ML, Fioramonti J, Bueno L. Role of 5-HT3 receptors in the control by cholecystokinin of transient relaxations of the inferior esophageal sphincter in dogs. Gastroenterol Clin Biol 1996;20:575-580.

376. Sifrim D, Holloway RH, Tack J, Silny J, Lerut T, Janssens J. Transient LES Relaxations: Are they more frequent in patients with gastroesophageal reflux disease? (abstract). Gastroenterology 1999;116:A313.

377. Trudgill NJ, Riley SA. Transient lower oesophageal sphincter relaxations in reflux disease: not the whole story? (abstract). Gastroenterology 1999;116:A338.

378. Shi G, Manka M, Kahrilas P. Lowered threshold for TLESR and GER with hiatus hernia. (abstract). Gastroenterology 1999;116:A309.

379. Baldi F, Longanesi A, Ferrarini F, Michieletti G, Morselli-Labate AM. Oesophageal motor function and outcome of treatment with H2-blockers in erosive oesophagitis. J Gastroint Mot 1992;4:165-171.

380. Holloway R, Downton J, Mitchell B, Dent J. Effect of cisapride on postprandial gastro-oesophageal reflux. Gut 1989;30:1187-1193.

381. Johnsson F, Holloway RH, Ireland AC, Jamieson GG, Dent J. Effect of fundoplication on transient lower oesophageal sphincter relaxation and gas reflux. Br J Surg 1997;84:686-689.

382. Ireland A, Holloway R, Toouli J, Dent J. Mechanisms underlying the antireflux action of fundoplication. Gut 1993;34:303-308.

383. Kiroff GK, Maddern GJ, Jamieson GG. A study of factors responsible for the efficacy of fundoplication in the treatment of gastro-oesophageal reflux. Aust NZ J Surg 1984;54:109-112.

384. Vu MK, van der Shaar PJ, Straathof JWA, Lamers CBHW, Masclee AAM. Proximal gastric motor function in reflux disease and after laparoscopic fundoplication (abstract). Gastroenterology 1998;114:A856.

385. Arndorfer RC, Stef JJ, Dodds WJ, Linehan JH, Hogan WJ. Improved infusion system for intraluminal esophageal manometry. Gastroenterology 1977;73:23-27.

386. Sivri B, Mittal RK. Reverse-perfused sleeve: an improved device for measurement of sphincteric function of the crural diaphragm. Gastroenterology 1991;101:962-969.

387. Whitehead WE, Delvaux M. Standardization of barostat procedures for testing smooth muscle tone and sensory thresholds in the gastrointestinal tract. The Working Team of Glaxo-Wellcome Research, UK. Dig Dis Sci 1997;42:223-241.

388. Azpiroz F, Malagelada J-R. Gastric tone measured by an electronic barostat in health and postsurgical gastroparesis. Gastroenterology 1987;92:934-943.

389. Azpiroz F, Malagelada JR. Physiological variations in canine gastric tone measured by an electronic barostat. Am J Physiol 1985;248:G229-G237.

390. Ropert A, Bruley des Varannes S, Bizais Y, Roze C, Galmiche J-P. Simultaneous assessment of liquid emptying and proximal gastric tone in humans. Gastroenterology 1993;105:667-674.

391. Horowitz M, Jones K, Edelbroek MA, Smout AJ, Read NW. The effect of posture on gastric emptying and intragastric distribution of oil and aqueous meal components and appetite. Gastroenterology 1993;105:382-390.

392. Pflucke VF, Anders O, Schreiber H. The effect of neostigmine and atropine on gastro-oesophageal reflux. Deutsche Zeitschrift fur Verdauungs- und Stoffwechselkrankheiten 1981;41:270-275.

393. Salapatek AMF, Diamant NE. Assessment of neural inhibition of the lower esophageal sphincter in cats with esophagitis. Gastroenterology 1993;104:810-818.

394. Skinner DB, Camp TF. Relation of esophageal reflux to lower esophageal sphincter pressures decreased by atropine. Gastroenterology 1968;54:543-551.

395. Dow TG, Brock-Utne JG, Rubin J, Welman S, Dimopoulos GE, Moshal MG. The effect of atropine on the lower esophageal sphincter in late pregnancy. Obstet Gynecol 1978;51:426-430.

396. Koerselman J, Pursnani KG, Peghini P, Mohiuddin MA, Katzka D, Akkermans LMA, Castell DO. Different effects of an oral anticholinergic drug on gastroesophageal reflux in upright and supine position in normal, ambulant subjects: a pilot study. Am J Gastroenterol 1999;94:925-930.

397. Brown JH, Taylor P. Muscarinic receptor agonists and antagonists. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, ed. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th ed. New York: McGraw Hill, 1996:141-160.

398. Greef K, Wirth KE. Pharmakologische Beeinflussing der Cholinergen Erregungsubertragung. In: Forth W, Henschler D, Rummel W, ed. Allgemeine und Spezielle Pharmakologie und Toxikologie. Mannheim: Wissenschaftsverlag, 1987:

399. Sifrim D, Coulie B, Tack J, Andrioli A, Janssens J. Cholinergic control of the gastric fundus tone. A barostat study in lightly anaesthetized cats (abstract). Gastroenterology 1996;110:A758.

400. Fang JC, Sarosiek I, Yamamoto Y, Liu J, Mittal RK. Cholinergic blockade inhibits gastro-oesophageal reflux and transient lower oesophageal sphincter relaxation through a central mechanism. Gut 1999;44:603-607.

401. Stacher G, Bergmann H, Havlik E, Schmierer G, Schneider C. Effects of oral cyclotropium bromide, hyoscine N-butylbromide and placebo on gastric emptying and antral motor activity in healthy man. Gut 1984;25:485-490.

402. Hould F-S, Cullen JJ, Kelly KA. Influence of proximal gastric vagotomy on canine gastric motility and emptying. Surgery 1994;116:83-89.

403. Massey BT, Narayanan S, Gorney JM, Hofmann C, Arndorfer RC. Atropine increases transient lower esophageal sphincter relaxations (TLESRs) if intragastric pressure is held constant (abstract). Gastroenterology 1999;116:A4506.

404. Distrutti E, Azpiroz F, Soldevilla A, Malagelada J-R. Gastric wall tension determines perception of gastric distension. Gastroenterology 1999;116:1035-1042.

405. Notivol R, Coffin B, Azpiroz F, Mearin F, Serra J, Malagelada JR. Gastric tone determines the sensitivity of the stomach to distension. Gastroenterology 1995;108:330-336.

406. Bowery NG, Pratt GD. $GABA_B$ receptors as targets for drug action. Arzneim Forsch/Drug Res 1992;42:215-223.

407. Washabau RJ, Fudge M, Price WJ, Barone FC. GABA receptors in the dorsal motor nucleus of the vagus influence feline lower esophageal sphincter and gastric function. Brain Res Bull 1995;38:587-594.

408. Williford DJ, Ormsbee HS, Norman W, Harmon JW, Garvey TQ, DiMicco JA, Gillis RA. Hindbrain GABA receptors influence parasympathetic outflow to the stomach. Science 1981;214:193-194.

409. Feng HS, Lynn RB, Han J, Brooks FP. Gastric effects of TRH analogue and bicuculline injected into dorsal motor vagal nucleus in cats. Am J Physiol 1990;259:G321-G326.

410. Bolser DC, DeGennaro FC, O'Reilly S, Chapman RW, Kreutner W, Egan RW, Hey JA. Peripheral and central sites of action of GABA-B agonists to inhibit the cough reflex in the cat and guinea pig. Br J Pharmacol 1994;113:1344-1348.

411. Dicpinigaitis PV. Use of Baclofen to suppress cough induced by angiotensin-converting enzyme inhibitors. Ann Pharmacother 1996;30:1242-1245.

412. Dicpinigaitis PV, Dobkin JB, Rauf K. Comparison of the antitussive effects of codeine and the GABA-agonist baclofen. Clin Drug Invest 1997;14:326-329.

413. Shaker R, Dodds WJ, Dantas RO, Hogan WJ, Arndorfer RC. Coordination of deglutitive glottic closure with oropharyngeal swallowing. Gastroenterology 1990;98:1478-1484.

414. Lehmann A, Antonsson M, Bremner-Danielsson M, Flardh M, Hansson-Branden L, Karrberg L. Activation of the $GABA_B$ receptor inhibits transient lower esophageal sphincter relaxations in dogs. Gastroenterology 1999;117:1147-1154.

415. Blackshaw LA, Staunton E, Lehmann A, Dent J. Inhibition of transient LES relaxations and reflux in ferrets by GABA receptor agonists. Am J Physiol 1999;277:G867-G874.

416. Partosoedarso ER, Blackshaw LA. Central $GABA_B$ receptor inhibition of gastric mechanoreceptor inputs onto vagal motor neurones (abstract). Gastroenterology 1999; 116:A1060.

417. Partosoedarso ER, Blackshaw LA. $GABA_B$ receptor inhibition of mechano- and chemoreceptor inputs onto vagal motor neurones (abstract). Gastroenterology 1999;116:A1060.

418. Page AJ, Blackshaw LA. $GABA_B$ receptors inhibit mechanosensitivity of primary afferent endings. J Neurosci 1999;19:8597-8602.

419. Wood KL, Addae JI, Andrews PL, Stone TW. Injection of baclofen into the ventromedial hypothalamus stimulates gastric motility in the rat. Neuropharmacology 1987;26:1191-1194.

420. Andrews PL, Wood KL. Systemic baclofen stimulates gastric motility and secretion via a central action in the rat. Br J Pharmacol 1986;89:461-467.

421. Blackshaw LA, Smid SD, O'Donnell TA, Dent J. $GABA_B$ receptors inhibit vagal influence on the lower oesophageal sphincter (LOS) (abstract). Gastroenterology 1999;116:A960.