THE ROLE OF DOPAMINE IN THE CONTROL OF GONADOTROPIN AND PROLACTIN SECRETION IN THE HUMAN FEMALE.

by

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I N D E X

DECLARATION

This thesis has not been submitted for the award of any other degree or diploma in any university. Except where due reference has been made in the text, the studies presented here contain no material previously published or written by another person.

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SUMMARY

This dissertation examines the hypothesis that Dopamine (DA) neurons of the tubero-infundibular system not only control Prolactin secretion from the pituitary gland, but also inhibit the secretion of Luteinising Hormone releasing factor (LRF) from the nerve terminals in the median eminence.

There is evidence to support this contention in the experimental animal. Immuno-histofluorescence studies have demonstrated a conglomeration of both DA and LRF nerve terminals in the lateral pallisade zone of the median eminence (Lofstrom et al., 1976). Electron microscopic study has shown that nerve terminals containing granules of various sizes, presumably both LRF and DA, are closely anatomically related in this region (Kobayashi et al., 1970).

Pharmacological manipulation of brain levels of DA have also supported the concept that DA inhibits LRF secretion. Studies of the turn-over of DA in the lateral pallisade zone, which apparently reflects DA neuron activity, show that DA turn-over is increased when serum LH levels are decreased and vice versa (Fuxe et al., 1969; Fuxe et al., 1969a). Oestrogen treatment of castrated rats causes an increase in DA turn-over which correlates well with the fall of serum LH (Lofstrom et al., 1977).

Administration of DA or DA receptor agonists causes a decrease in episodic LH secretion (Drouva and Gallo, 1976) and a decrease in serum LH (Mueller et al., 1976; Wuttke et al., 1971). In vitro studies with rat pituitary gland incubations have consistently demonstrated that DA has no effect on LH secretion although it actively inhibits PRL release from the pituitary gland. However, LH secretion is

inhibited when DA is incubated with the pituitary left attached to the hypothalamus (Miyachi et al., 1973).

In the human, several studies failed to show any effect of L Dopa on serum LH levels, but a recent careful study did demonstrate a decrease in serum LH with both L Dopa and the DA receptor agonist, bromocriptine (Lachelin et al., 1977). Infusion of DA at a constant rate also caused a decrease in serum LH in normal men and women (Lachelin et al., 1976).

An attempt was made to further study a possible effect of DA on LRF secretion in the human by examining the change in serum LH and FSH, induced by DA, in women with different levels of endogenous LRF secretion. Women studied in the early or mid-follicular phases of the menstrual cycle with DA showed a small but consistent decrease in serum LH but no significant alteration in serum FSH. In contrast, there was a marked decrease in LH and also, to a lesser extent, FSH in women in the pre-ovulatory phase, at a time when endogenous secretion of LRF is believed to be high (3.31.1). Control experiments showed that the effect of DA on LH and FSH secretion could not be reproduced by infusion of saline or noradrenaline (3.33; 3.34).

The LH and FSH responses to DA are also augmented in agonadal women in whom there is a presumed increase in endogenous LRF. Administration of oestrogen, which reduces LRF secretion, impairs the effect of DA on serum LH and FSH (3.36). The LH and FSH response to DA appears to correlate better with basal serum LH reflecting the endogenous level of LRF than the circulating level of oestradiol.

In contrast, the effect of DA on PRL secretion seemed more closely correlated to circulating serum oestradiol.

An attempt was made to exclude a direct effect of DA on the pituitary secretion of LH by studying the LH and FSH responses to exogenous LRF before and during a DA infusion (3.35). These results, however, were not conclusive since it was not possible to exclude an effect on endogenous LRF secretion. In vitro studies using incubated rat hemi-pituitaries (6.32) did not show any effect of DA on the spontaneous or stimulated release of LH (6.31; 6.32).

It is concluded from these studies that DA reduces serum levels of LH and FSH by inhibiting the endogenous release of LRF. It appears that the LH response to DA is a reliable, though indirect, reflection of the rate of endogenous LRF secretion.

In view of this effect of exogenous DA on LRF secretion, it could be postulated that the tubero-infundibular DA neurons exert a similar control, in physiological situations, on the secretion of LRF. If this was so, then blockade of the action of DA on LRF neurons would be expected to lead to an increase in serum LH and FSH. The means by which DA acts on LRF neurons is not established but, on the basis that it was most likely to be through a specific receptor for DA, the acute effect of DA receptor blockade on serum LH and FSH was studied. Since most of the known DA receptor antagonists are either non-specific in their action (e.g. chlorpromazine) or else they cannot be given intravenously (e.g. pimozide), it was decided to investigate the possibility that metoclopramide, an anti-emetic drug which had been shown to cause an increase in serum prolactin

(McNeilly et al., 1974), might be a useful alternative as a DA receptor antagonist.

Initial studies strongly supported the view that metoclopramide (MCP) increased PRL by blocking the action of endogenous DA at the lactotrope receptors. Stimulation of PRL secretion by MCP could be blocked by L Dopa, bromocriptine (4.32) and DA (4.33) in normal men and women. In vitro studies excluded a direct stimulation of PRL secretion by MCP (6.33) and indicated that there was mutual antagonism between DA and MCP for lactotrope receptors.

Despite this effect on serum PRL, MCP had no significant effect on serum LH and FSH in either the 0.5 mg (4.34.2) or 2.5 mg dose (4.34.1). Hence, it was not possible to confirm that DA neurons exert a tonic inhibitory control of LRF secretion in the normal human; however, neither was it possible to exclude this. The possibility is discussed that DA may inhibit LRF secretion by a mechanism which does not involve specific receptors, or if it does then these receptors may be less sensitive to the effect of MCP than is the lactotrope receptor.

An intriguing speculation which is raised by the possibility of dopaminergic control of LRF secretion, is that certain disorders in the human associated with chronic anovulation, may be mediated by an over-activity of the tubero-infundibular neurons suppressing LRF release. One such condition, chronic anovulation associated with hyperprolactinaemia, has been investigated because animal studies have previously concluded that hyperprolactinaemia, itself, may induce excessive dopaminergic activity in the hypothalamus.

Ten women with microadenomas causing hyperprolactinaemia, were studied with infusions of DA and injections of MCP. DA infusion suppressed serum LH in hyperprolactinaemic women, but to a significantly smaller degree than normal women in the early follicular phase of the cycle (5.31). This was interpreted as being consistent with a decreased secretion of endogenous LRF. However, the possibility that this might result from over-activity of DA neurons could not be confirmed because there was again no LH or FSH response to MCP, at the dose used (5.32).

Although the PRL response to MCP is significantly reduced in hyperprolactinaemic women, the rapid PRL response to DA indicates that the lactotrope receptors are functionally active. This raises the possibility that the pathogenesis of hyperprolactinaemic chronic anovulation may be related to a failure of the short loop feed-back control of excessive PRL release at the pituitary level, while excessive DA activity in the hypothalamus inhibits LRF secretion. Possible disturbances in dopaminergic neurotransmission in other human anovulatory diseases are discussed.

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PUBLISHED WORK ARISING FROM THIS STUDY.

Original Papers

1. Judd, S.J., Lazarus, L. and Compton, P.
Hyperprolactinaemia - Its Physiology and Investigation.
Medical Journal Australia, 2, 23, 1976.

2. Judd, S.J., Lazarus, L. and Smythe, G.
Prolactin Secretion by Metoclopramide in Man.
J. Clin. Endocrinol. Metab., 43, 313, 1976.

3. Judd, S.J., Lazarus, L. and Smythe, G.
Bromoergocryptine - Medieval Medicine Revisited.
Bulletin Postgraduate Committee in Medicine, University of Sydney, 2, April, 1977.

4. Judd, S.J., Rakoff, J.S. and Yen, S.S.C.
Inhibition of Gonadotropin and Prolactin Release by Dopamine : Effect of Endogenous Oestradiol Levels.
J. Clin. Endocrinol. Metab., 1978 (in press).

5. Judd, S.J.
Bromocriptine : Rational Therapy for Some Common Disorders in Obstetrics and Gynaecology.
Current Therapeutics, 1978 (in press).

6. Judd, S.J., Rigg, L.A. and Yen, S.S.C.
Interaction between Oestrogen and Dopamine in Modulating the Release of Gonadotropin and Prolactin.
J. Clin. Endocrinol. Metab., (submitted).

7. Judd, S.J., Quigley, M.E. and Yen, S.S.C.
Disturbances of Dopaminergic Neurotransmission in Patients with Hyperprolactinaemic Chronic Anovulation.
J. Clin. Endocrinol. Metab. (in preparation).

Abstracts

1. Judd, S.J., Compton, P. and Smythe, G.
Prolactin Secretion by Metoclopramide in Man.
ANZ Journ. Med., 6, 254, 1976.

2. Judd, S.J. and Lazarus, L.
Serum Prolactin and Disorders of the Hypothalamus and Pituitary Gland.
ANZ Journ. Med., 6, 169, 1976.

3. Judd, S.J., Rakoff, J.S., Rigg, L.A. and Yen, S.S.C.
Further Delineation of the Role of Dopamine in the Regulation of LH and Prolactin Secretion in Humans.
Abstract 138. 24th Annual Meeting, Society for Gynaecologic Investigation, Tuscon. March, 1977.

4. Judd, S.J., Rakoff, J.S., Rigg, L.A. and Yen, S.S.C.
Dopamine Suppression of Pituitary Release of LH and Prolactin : Effect of Gonadal Steroids.
Abstract 157. 59th Annual Meeting, Endocrine Society, Chicago, 1977.

5. Judd, S.J., Rakoff, J.S., Rigg, L.A., Quigley, M.E. and Yen, S.S.C.
Modulation of Dopamine Sensitivity for the Release of LH and Prolactin.
25th Annual Meeting, Society for Gynaecologic Investigation, 1978 (in press).

6. Quigley, M.E., Judd, S.J. and Yen, S.S.C.
Defective Dopaminergic Regulation of Prolactin and LH Secretion in Patients with Hyperprolactinaemic Chronic Anovulation Associated with Pituitary Microadenomas.
25th Annual Meeting, Society for Gynaecologic Investigation, 1978 (in press).

CHAPTER 1

DOPAMINERGIC NEUROTRANSMISSION IN THE BRAIN.

1.10 A historical review of the concept of catecholaminergic neurotransmission in the brain.

The concept that chemicals are involved in transmission of stimuli from the brain is an ancient one and can be identified in the writings of Galen (c. 130-200 AD). "The nerves shown some analogy to water pipes ('ochetos') because they conduct the activity to muscles from some source in the brain" (Kuehn, 1821). Galen envisaged the nerves as hollow tubes containing 'pneuma', a substance which allowed transmission of impulses either by physical movement within the nerve or by causing a dynamic alteration in the nerve in the same way as "sunlight is propagated into the surrounding air while ..... the substance of the sun remains in its own place" (Siegel, 1968). The Galenic concept of mechanical movement of pneuma within nerve tissue was challenged by Francis Glisson, Regius Professor of Physics at Cambridge, who in 1640, observed that there was no change in the volume of muscle during its contraction (Brooks et al., 1962). A reasonable alternative theory was suggested in 1792 by the classic experiments of Galvani in electricity, and in 1877, Du Bois-Reymond suggested that the electrical impulses travelled along nerves and stimulated the muscles they innervated, either by local currents or by some chemical formed at the nerve ending (Brooks et al., 1962).

In 1894, it was found that aqueous, alcohol or glycerine extracts of adrenal gland produced "a most powerful action upon the blood vessels, upon the heart and upon the skeletal muscle" (Oliver and Schaeffer, 1894). This active substance was isolated from the adrenal medulla by Abel, in 1899, and called epinephrine.

In 1901, Aldrich and Takamine, independently, prepared pure crystalline adrenal extract and renamed it adrenaline.

In 1904, T.R. Elliott, on the basis of a similarity in the observed effect of sympathetic nerve stimulation and administration of adrenaline, proposed that "adrenaline might then be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery" (Elliott, 1904). This work was supported by later experiments with H.H. Dale, which demonstrated that ergotoxine selectively blocked the effect of both sympathetic stimulation and the administration of adrenaline (Dale, 1934).

The classic experiments of Loewi (1921) were a major advance in establishing the concept of chemical mediation of neural transmission in the peripheral sympathetic nervous system. He demonstrated that the rate of contraction of an isolated heart could be slowed by a diffusible chemical substance released by vagal stimulation. Acceleration of heart rate could be produced by release of a chemical after stimulation of cardiac sympathetics. In later years, these substances were shown to be acetylcholine and adrenaline, respectively.

It was recognised, however, that adrenaline did not reproduce all the effects of sympathetic nerve stimulation. This led to an elaborate theory that there were two 'sympathins' (Cannon and Rosenblueth, 1933). Bacq was the first to propose that sympathin E might be noradrenaline, the non-methylated homologue of adrenaline (Bacq, 1934), but the lack of evidence of the natural occurrence of noradrenaline in the body blunted the significance of this report.

In 1943, Raab reported that the spleen contained a catecholamine which had different properties from adrenaline (Raab, 1943), and the investigation of this substance in spleen and prostate by Von Euler led to the conclusion, in 1946, that the splenic sympathetic nerves contained noradrenaline (Von Euler, 1946). With the improvement of methods of separating and identifying catecholamines and the refutation of the electrical theory of synaptic transmission by Eccles himself (Eccles, 1974), it became accepted that noradrenaline was the mediator of sympathetic nerve transmission.

The presence of noradrenaline and adrenaline in the brain was demonstrated by Von Euler and Holtz (Holtz, 1950), but these substances were believed to be only present in cerebral vasomotor nerves. However, a detailed study of catecholamines in the brain led Vogt to propose that "sympathomimetic amines, besides their role as neurotransmitters at vasomotor endings, play a part in the function of the central nervous system itself". (Vogt, 1954).

Dopamine, first synthesised in 1909 (Mannich and Jacobsohn, 1909), was thought for many years to be merely an intermediate product in the biosynthesis of noradrenaline. However, it was concluded, in 1942, that dopamine itself had an action on the cardiovascular system which was quite distinct from the effect of noradrenaline (Holtz and Credner, 1942). In 1957, Blaschko postulated an independent action of dopamine in the peripheral circulation (Blaschko, 1957), and this was confirmed by the demonstration that dopamine causes a specific increase in coeliac and renal blood flow in both the dog and man (Allwood et al., 1963; Allwood and Ginsburg, 1964).

In 1957, Montague demonstrated the presence of a catecholamine distinct from adrenaline and noradrenaline, which he believed was dopamine, in the brains of a variety of animals (Montague, 1957). The following year, it was reported that dopamine could be distinguished fluorometrically from other catecholamines by adjustment of PH, and the presence of dopamine in rabbit brain was confirmed (Carlsson et al., 1958). An extensive study by Bertler and Rosengren (1959) demonstrated that dopamine and noradrenaline were the neurotransmitters with the highest concentration in the brain substance and that there was a non-uniform distribution of these catecholamines. This was independently reported to be the case in human brain (Sano et al., 1959). This asymmetrical distribution of dopamine and noradrenaline led to the conclusion that dopamine was a neurotransmitter, in its own right, in the brain.

1.20 The Dopamine Neuron

A dopamine (DA) neuron is one which, when stimulated by a nerve impulse, synthesises and releases DA from its nerve ending into the synaptic space. DA then reacts with a specific post synaptic receptor to cause a characteristic, usually inhibitory, response (Krnjevic, 1975).

1.20.1 Synthesis of DA (Fig. 1.1)

L tyrosine, the precursor amino-acid of all catecholamine synthesis, is derived from dietary protein, from hepatic hydroxylation of phenylalanine or from the breakdown of endogenous protein (Fernstrom and Wurtman, 1977). The initial step in DA synthesis is hydroxylation to L dihydroxyphenylalanine (L Dopa); an action

Figure 1.1

Pathways of synthesis and metabolism of dopamine.

PH - phenylalanine hydroxylase

TH - tyrosine hydroxylase

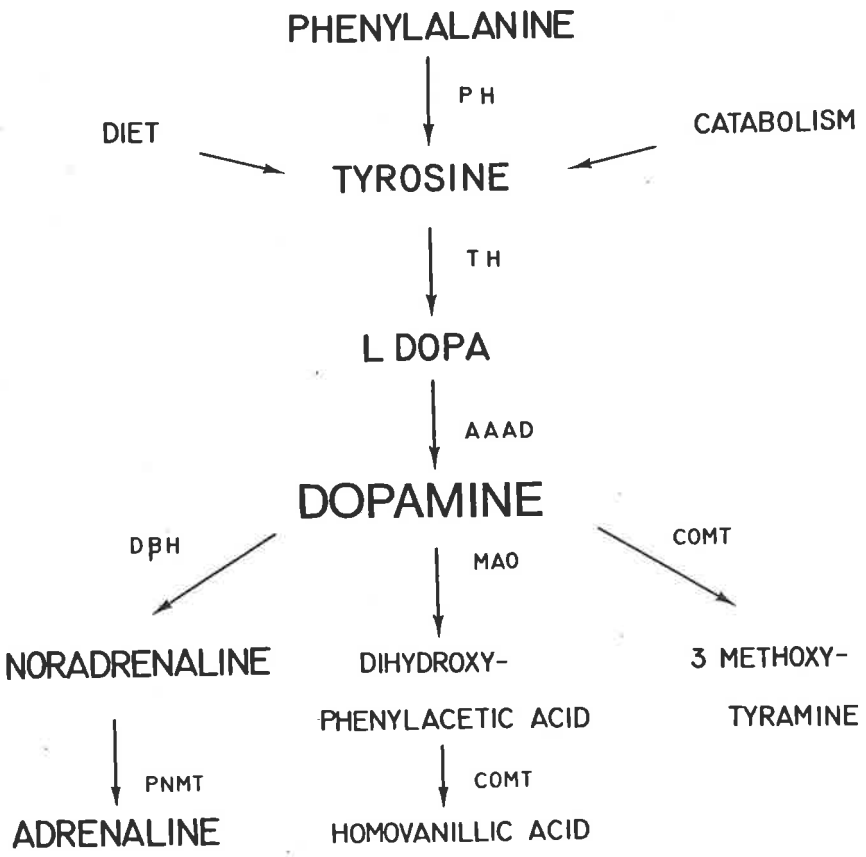
AAAD - aromatic amino-acid decarboxylase

MAO - monoamine oxidase

DBH - dopamine β hydroxylase

COMT - catechol-o-methyl transferase

PNMT - phenylethanolamine-n-methyl transferase.



catalysed in a rate limiting step by tyrosine hydroxylase (Levitt et al., 1965). L Dopa is converted to DA by the enzyme aromatic L amino-acid decarboxylase and the co-enzyme pyridoxyl phosphate.

Most of the synthesis of DA occurs in the nerve terminal although some DA is also synthesised in the cell body and is transported by axoplasmic flow to the nerve terminals. Free intraneuronal DA is rapidly metabolised by monoamine oxidase; however, vesicles in the nerve terminals store and protect DA from degradation (Geffen and Livett, 1971).

DA neurons lack the enzyme Dopamine β hydroxylase which, in other catecholaminergic neurons, allows conversion of DA to noradrenaline. A small number of brain neurons also contain phenylethanolamine-N-methyl transferase which converts noradrenaline to adrenaline.

Stimulation of the DA neuron causes the release of DA into the synaptic space and at the same time activates tyrosine hydroxylase to hasten new biochemical synthesis. Measurement of biochemical activity of DA neurons, by the rate of formation of DA from tyrosine or the rate of formation of DA metabolites, has proved a useful means of assessing neuronal activity.

1.20.2 Metabolism of DA (Figure 1.1)

DA in the synapse may interact with a post synaptic receptor to produce a specific response, or it can be removed by various routes which limit the duration of its action. DA is most commonly inactivated by re-uptake into presynaptic nerve terminals.

Here it is metabolised by intraneuronal monoamine oxidase (MAO) to form dihydroxyphenylacetic acid (Carlsson and Hillarp, 1962), or it is stored again in nerve terminal vesicles. Catechol-o-methyl transferase (COMT), an enzyme located outside the neuron, converts dihydroxyphenylacetic acid to homovanillic acid. Homovanillic acid enters the blood stream directly or via the CSF and is excreted in the urine. Small amounts of DA in the synapse can be o-methylated by COMT, without oxidative deamination by MAO, to form 3 methoxytyramine (Carlsson and Waldeck, 1964).

1.20.3 The Dopamine Receptor

The nature of the post synaptic receptor for DA has been actively investigated and there is good evidence to suggest that the receptor is a membrane bound adenyl cyclase which is specifically activated by DA to produce AMP (Kebabian et al., 1972). Increase in cyclic AMP induces hyperpolarisation of the post synaptic membrane and subsequently an inhibition of neuronal transmission (Greengard and Kebabian, 1974).

A high specificity of the DA receptor has been deduced by analysing the structure of compounds which interact with the DA receptor. DA agonists have structural configurations which resemble the 'trans' form of DA and they contain both the catechol hydroxyl group and a 2 carbon atom side chain (Iversen et al., 1975).

Several models have been used to study the DA receptor, including measurement of the inhibition of neuronal firing rate in the brain of the snail (Woodruff and Walker, 1969), and the vasodilating effect on dog renal artery (Goldberg et al., 1968).

However, the most widely used models have been the *in vitro* generation of cyclic AMP by DA in an homogenate of rat striatum (Kebabian et al., 1975) and, *in vivo*, the circling behaviour of the rat with a unilateral lesion in the nigro-striatal pathway (Costall and Naylor, 1974).

Recently it has been proposed that DA receptors may also be situated on the pre-synaptic nerve terminals and on the dendrites of the cell body itself (Aghajanian and Bunney, 1977; Bjorklund and Lindvall, 1975). These DA receptors need not behave in the same way as the post-synaptic receptors, since two functionally distinct dopamine receptors have been described in feline brain (Cools et al., 1976).

Several studies have reported that pre and post synaptic receptors may differ in their affinity for different DA receptor agonists. Apomorphine, in low doses, appears to selectively stimulate presynaptic receptors, which results in decreased synthesis and release of DA. In higher concentrations, apomorphine stimulates post-synaptic receptors to produce the same effect as would occur if DA release into the synapse was increased (Walters and Roth, 1974; Kehr et al., 1972; Baudry et al., 1977).

1.20.4 The Autoregulation of Activity in the DA Neuron

The activity of the DA neuron is controlled by feedback alteration of neuronal firing and biochemical synthesis of DA in the neuron, and by a variation of the sensitivity of the DA post-synaptic receptor. Both these actions appear to be mediated by activation of DA receptors.

Blockade of DA receptors is associated with an increase in neuronal activity and DA synthesis (Carlsson and Linqvist, 1963; Andén et al., 1970; Nybäch and Sedvall, 1970). Conversely, dopamine receptor agonists reduce neuronal activity and DA synthesis (Andén et al., 1967). This action may be mediated by a long feed-back mechanism involving the post-synaptic DA receptor (Bunney and Aghajanian, 1976), by activation of DA receptors in the pre-synaptic terminals (Kehr et al., 1972; Walters and Roth, 1974), or by an effect of DA on the cell body (Groves et al., 1975). This change in neuronal activity is rapid in its effect, but relatively short in its duration. There is evidence that a more long lasting control of the DA neuron is induced by changes in the sensitivity of the post-synaptic DA receptor.

1.20.5 The Sensitivity of the DA Receptor

The sensitivity of the dopamine receptor is increased whenever there is a persistent decrease in stimulation of the receptor. Within a few days of unilateral destruction of the nigro striatal DA pathway in the rat, the animal displays an increase in contralateral circling behaviour after administration of DA receptor agonists (Ungerstedt, 1971). This is interpreted as being the result of an increase in the sensitivity of the post-synaptic receptors on the denervated side due to a persistent reduction in DA stimulation. Increases in sensitivity to exogenous DA agonists can be induced by long term administration of DA antagonists (Hyttel, 1977; Gianutsos et al., 1974), which reduce effective receptor stimulation.

The molecular events which result in an increase in receptor sensitivity are not fully understood. After unilateral lesions in the nigro striatal system, homogenates of rat striatum from the denervated side shown an increased ability to generate cyclic AMP

after stimulation by DA (Mishra et al., 1974). This correlates well with in vivo behavioural studies showing increased sensitivity of post-synaptic receptors. This suggests that DA receptor sensitivity may be due to

(i) an increase in absolute DA receptor number;

(ii) an increase in the binding affinity of the receptor for DA, or

(iii) an increase in some post-synaptic mechanism involved in the generation of cyclic AMP.

A recent study has indicated that an increased binding of DA occurs at the supersensitive DA receptor (Burt et al., 1977). However, it did not indicate whether this was because of increased receptor number or affinity of binding. If a valid comparison can be made with the supersensitive insulin receptor (Goldfine, 1975), both mechanisms may apply.

The issue of the sensitivity of DA receptors and its relationship to the level of endogenous DA activity has been studied mainly in the nigro-striatal pathway, but recently, the same principle has been applied to the study of dopamine receptors present on the pituitary (Brown et al., 1976). The sensitivity of these receptors has been assessed by the degree of prolactin suppression by DA receptor agonists. While this may be considered an indirect method of assessing receptor sensitivity, Cheung and Weiner have been able to show that there is an increased response of lactotrope receptors to DA receptor agonists after pituitary stalk section, and have suggested that this is compatible with a decrease in endogenous DA activity at these receptors (Cheung and Weiner, 1976). With similar reasoning, Lal et al., (1977) concluded that pituitary lactotrope sensitivity is increased after chronic treatment with DA receptor antagonists.

1.30 The Localisation of Dopamine Containing Neural Pathways in the Brain

The description by Falck and Hillarp of a method of localising catecholamines in the brain by histochemical fluorescence (Falck et al., 1962) has enabled the neural pathways to be mapped. The method involves treatment of freeze dried tissue extracts with formaldehyde to convert monoamines to highly fluorescent compounds which can be visualised with the fluorescent microscope. This method does not identify indivudal catecholamines which requires a separate spectrofluorometric (Björklund et al., 1968) or biochemical (Butcher et al., 1970) analysis; however, serotonin can be distinguished by its yellow fluorescence from catecholamines, which show a green fluorescence.

Using this method, four major dopaminergic pathways have been defined (Ungerstedt, 1971).

1.30.1 The Nigro-Striatal DA Pathway

The A_9 group of cell bodies are found in the substantia nigra and axons from these cells pass rostrally via the crus cerebri and internal capsule to terminate in the neostriatum, particularly the caudate nucleus. Apart from histofluorescent visualisation of this pathway, evidence of its existence has also been obtained by the histological (Faull and Carman, 1968), and electron microscopic demonstration of degeneration of nerve endings in the corpus striatum (Hökfelt and Ungerstedt, 1969) after destructive lesions had been made in the substantia nigra. Moreover, electrical stimulation of the substantia nigra is associated with an increased concentration

of DA in the caudate nucleus (Poirier and Sourkes, 1965; Portig and Vogt, 1968; Connor, 1970), and radiolabelled DA can be detected in the corpus striatum following its injection into the substantia nigra (Fibiger, 1972).

1.30.2 The Meso-Limbic Pathway

The axons in this pathway arise mainly from the A_{10} cell bodies, dorsal to the interpeduncular nucleus, and ascend medial to the nigro striatal pathway to enter the medial forebrain bundle. At the level of the anterior commissure, they branch to enter the nucleus accumbens and the interstitial nucleus of the stria terminalis. Another branch is distributed to the olfactory tubercle (Ungerstedt, 1971). The functional role of the meso-limbic DA pathway is not well understood at this time. It has been suggested however, that it is involved in locomotor activity (Creese and Iversen, 1974; Pijnenberg and van Rossum, 1973), maintenance of lactational performance (Aulsebrook and Holland, 1969; Woods et al., 1969) and feedback control of prolactin secretion (Fuxe et al., 1977).

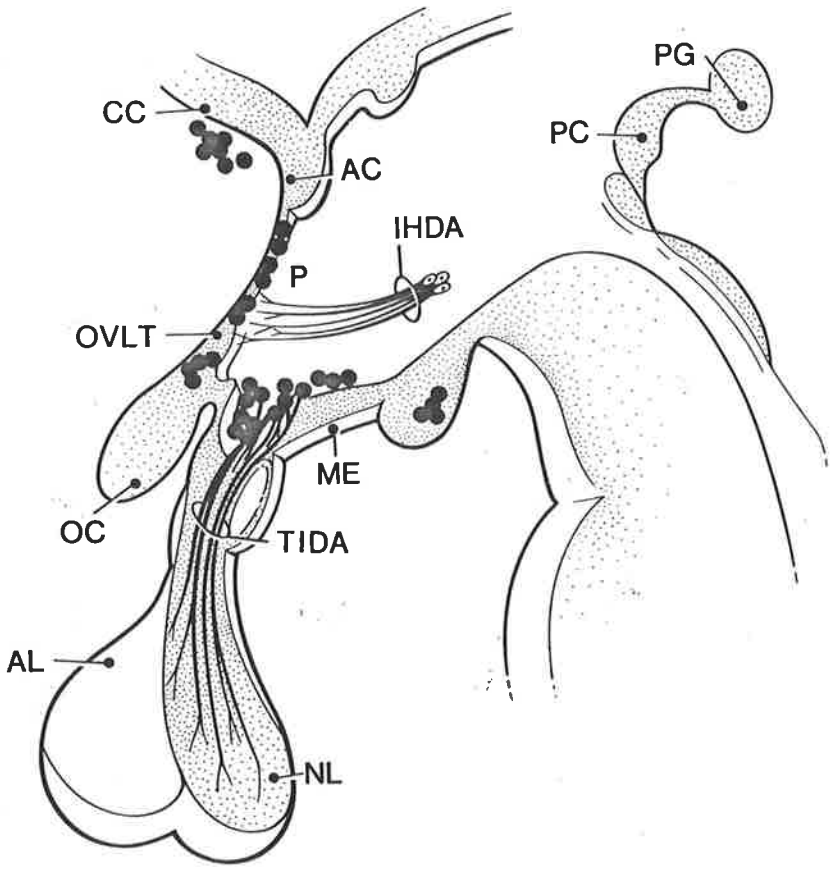
1.30.3 The Tubero-Infundibular System (Fig. 1.2)

Cell bodies of this pathway are located in the arcuate nucleus and axons are distributed to the external layer of the median eminence, particularly the lateral aspect, and also to the pars intermedia and the neural lobe (Bjorklund et al., 1973). The presence of this pathway had been previously established by histochemical fluorescence (Fuxe and Hökfelt, 1966), following Dahlstrom's earlier observation that catecholamine concentration remained high in the median eminence when widespread lesions were made in the brain stem (Dahlstrom et al., 1962).

Figure 1.2

Lateral view of the Hypothalamus and the Pituitary gland of the human showing the anatomical distribution of LRF cell bodies (●), and the tubero-infundibular DA (TIDA) and incerto-hypothalamic DA (IHDA) pathways in the brain.

CC	Corpus Callosum
AC	Anterior Commissure
P	Pre-optic Area
OVLT	Organ Vasculosum of the Lamina Terminalis
OC	Optic Chiasma
AL	Anterior Lobe of the Pituitary
NL	Neurohypophysis
ME	Median Eminence
PC	Posterior Commissure
PG	Pineal Gland



1.30.4 The Incerto-Hypothalamic Pathway (Fig. 1.2)

This pathway has been recently delineated (Bjorklund et al., 1975a) using a more sensitive fluorescent technique (glyoxylic acid histochemical fluorescence). It is composed of delicate, finely varicose neurons with low intra-neuronal amine content. The caudal part of this pathway arises from the A_{11} and A_{13} groups of cell bodies in the posterior hypothalamus and zona incerta and passes rostrally to the dorsomedial nucleus and anterior hypothalamus. The rostral part arises from the A_{14} group of cell bodies in the periventricular area and passes to the medial pre-optic area and the periventricular and suprachiasmatic nuclei.

1.40 Physiological Disturbances of Dopaminergic Neurotransmissions in the Brain.

"Enthusiasm for chemical transmission has not always been combined with critical assessment of the results being offered and there has been a tendency to lose sight of the essential quality of a transmitter substance". (Crossland, 1960).

In the last decade, however, considerable evidence has been accumulated to support the view that DA is an important neuro-transmitter in the brain and that certain clinical conditions may be associated with a disturbance in normal dopaminergic neurotransmission.

1.40.1 Dopaminergic Neurotransmission in the Nigro-Striatal Pathway

The elegant neurochemical and neuropathological studies which established that DA is present in the nerve endings of the nigro-striatal axons were given clinical relevance in the early 1960's,

by the speculation that Parkinson's disease is due to a disturbance in dopaminergic neurotransmission in this pathway. This proposal was derived from an original observation that a motor disturbance akin to Parkinson's disease could be induced in rats by reserpine and that L Dopa could reverse this effect (Carlsson et al., 1957). Although it is now recognised that both reserpine and L Dopa are non-specific in their actions, affecting both serotoninergic and catecholaminergic neurones, further support for the concept of dopamine deficiency in Parkinson's disease was added by the demonstration of a decreased urinary excretion of dopamine (Barbeau et al., 1961; Bischoff and Torres, 1962).

A more definite approach was to directly measure the concentration of dopamine in the basal ganglia of patients dying with Parkinson's disease and post-encephalitic parkinsonism (Ehringer and Hornykiewicz, 1960); very low levels of dopamine were found in the striatum. Dopamine depletion was found to be more marked in the contralateral striatum of a patient dying with hemiparkinsonism (Barolin et al., 1964), and a quantitative correlation was found between the degree of dopamine depletion and the loss of cells in the substantia nigra (Bernheimer et al., 1965). A large number of drugs which are known to block dopamine receptors in the brain induce extrapyramidal motor disorders (Hornykiewicz, 1973), while intravenous administration of L Dopa produced a short-lived improvement in patients with Parkinson's disease (Birkmayer and Hornykiewicz, 1961). The therapeutic efficacy of L Dopa is now well established (Cotzias et al., 1967; Sourkes et al., 1975), and more recently treatment with bromocriptine (Lieberman et al., 1976) and piribedil (Vakil et al., 1973) has also been found to be beneficial.

1.40.2 The Role of DA in the Physiological Regulation of Prolactin

In 1928, Riese reported the case of a woman who developed both parkinsonism and galactorrhoea, following an acute attack of epidemic encephalitis (Riese, 1928). At autopsy, this patient was shown to have considerable hypothalamic damage which was thought to be responsible for the symptom of galactorrhoea. This careful clinical pathological study is, in retrospect, the first indication that the hypothalamus normally inhibits the release of prolactin. In 1954, Everett removed the pituitary gland from rats and transplanted it beneath the renal capsule. He concluded that the secretion of "lactogenic hormone" continued even when the pituitary was separated from the hypothalamus (Everett, 1954). Hypothalamic inhibition of PRL release has been confirmed subsequently by pituitary stalk section (Dempsey and Uotila, 1940), lesioning of the median eminence (Chen et al., 1970; Welsch et al., 1971), and *in vitro* culture of the pituitary gland (Meites et al., 1961).

Extracts of the hypothalamus were prepared and found to inhibit the *in vitro* release of PRL from the pituitary gland (Talwalker et al., 1963; Schally et al., 1965; Kragt and Meites, 1967). Initially it was believed that this prolactin inhibiting factor (PIF), present in the hypothalamus, would prove to be a peptide hormone like LRF. However, it soon became apparent that DA itself, was a potent inhibitor of PRL *in vitro* (MacLeod et al., 1970). L Dopa was found to inhibit *in vivo* PRL release in the rat (Lu and Meites, 1972), the human (Malarkey et al., 1971), and the stalk sectioned monkey (Diefenbach et al., 1976). The PIF activity of partially purified extracts of porcine hypothalamus was found to be

proportional to the content of catecholamine (Takahara et al., 1974) and pre-incubation with monamine oxidase or aluminium oxide, which removes catecholamines, reduced the PIF activity (Shaar and Clemens, 1974).

The concept developed that DA is released from the tubero-infundibular nerve terminals in the median eminence, enters the portal vessels and directly affects DA receptors on the pituitary lactotrope to inhibit PRL secretion. This has found support from recent studies demonstrating the presence of DA in the portal blood (Ben-Jonathon et al., 1977), and the presence of DA receptors in the pituitary (Brown et al., 1976). Furthermore, the DA receptor agonists apomorphine (Lal et al., 1974; Smalstig et al., 1974), ergocornine (Shelesnyak, 1958), bromoergocryptine (Fluckiger and Wagner, 1969) and piribedil (Fuxe et al., 1976) inhibit PRL secretion, while drugs which reduce the concentration of DA at the post-synaptic receptor increase PRL secretion. Hence, serum PRL increases after inhibition of catecholamine synthesis by α methyl tyrosine (Donoso et al., 1971), after depletion of catecholamine stores with reserpine (Lu et al., 1970) and after blockade of DA receptors with chlorpromazine (Clemens et al., 1974) and sulpiride (L'Hermite et al., 1975).

While it is clear that DA is a potent inhibitor of PRL secretion, it cannot yet be assumed to be the only physiological inhibitor of PRL release. It has been established, for example, that hypothalamic extracts inhibit PRL secretion when injected intravenously (Amenomori and Meites, 1970; Watson et al., 1971),

and pulse injections of exogenous catecholamines have a short half-life in the circulation. A purified PIF material isolated from the hypothalamus was found to be inactivated by the proteolytic enzyme pronase, which would not be expected to affect catecholamines (Greibrokk et al., 1974; Greibrokk et al., 1975). Quijada showed that DA, when incubated with median eminence extract, released a substance which caused inhibition of PRL release and which could not be blocked by DA antagonists (Quijada, 1973). Dupont and Redding also reported the isolation of a PIF fraction which had different physiochemical characteristics from the catecholamines and which was not inhibited by perphenazine (Dupont and Redding, 1975). Subsequently, the same group suggested that this PIF was GABA (Schally et al., 1977).

A recent report has suggested that there may be two prolactin inhibiting factors obtainable from extracts of the medio-basal hypothalamus (Engalbert et al., 1977). After high speed centrifugation of an acidic extract of the hypothalamus, the supernatant contained PIF activity, which was inactivated by aluminium oxide (which reduced the DA content from 6900 pg to less than 30 pg), and was also blocked by haloperidol. The synaptosomal pellet which was recovered also contained PIF activity, but this was not affected by catecholamine adsorbing or blocking agents. This study suggests that the supernatant PIF may well be DA while the synaptosomal fraction may contain a peptide substance with PIF activity. It is well established that DA can have a direct action on the pituitary, but it remains to be determined whether it also influences the release of other PIF's from the median eminence.

In the human, persistent elevation of serum prolactin is recognised as a frequent cause of morbidity (Franks et al., 1976). In some instances, a defect in normal dopaminergic neurotransmission is clearly present, for example, when hyperprolactinaemia complicates treatment with neuroleptic drugs, which are DA receptor antagonists. In other cases, when there is damage or infiltration of the hypothalamus or when the hypothalamus is physically separated from the pituitary gland, it might be inferred that hyperprolactinaemia is a result of a decreased transport in the portal blood of DA or a DA-stimulated PIF. However, it is now appreciated that a prolactin secreting microadenoma of the pituitary gland is commonly found in many patients with hyperprolactinaemia. A question with important clinical relevance, which cannot yet be resolved, is whether a microadenoma arises de novo in the pituitary gland, or as a result of a long-standing disorder of dopaminergic neurotransmission in the tubero-infundibular pathway.

In clinical practice, surgical removal of an isolated pituitary microadenoma not infrequently results in restoration of normal serum prolactin. However, in the absence of a sensitive method of assessing dopaminergic function in the tubero-infundibular pathway, or of measurements of portal vein DA (or PIF) in these patients, it is not known whether adenectomy resolves the underlying disorder in these patients.

Another issue which makes very difficult the understanding of the basic abnormality underlying human hyperprolactinaemia, is that in the experimental animal, hyperprolactinaemia itself may increase dopaminergic activity in the tubero-infundibular neurons

(Chen et al., 1967; Clemens and Meites, 1968; Hokfelt and Fuxe, 1972a). It is by no means certain, therefore, that any of the models of hyperprolactinaemia in animals are appropriate for the study of human hyperprolactinaemia. Perhaps the best model, albeit rarely used to date, is the spontaneous development of prolactin secreting tumours in aged, constant oestrous, rats. In these animals, the dopamine concentration in the median eminence is decreased compared to young cycling rats and, consistent with this, the sensitivity of the lactotrope receptor to DA agonists is increased (Wilkes et al., 1977).

1.50 Dopamine as a Neurotransmitter Controlling Gonadotropin Secretion

Although there is general agreement that DA is an important inhibitory transmitter in the extrapyramidal system and in the control of prolactin secretion, its role in the regulation of LH and FSH has been more controversial. A major circumstantial argument which suggests that DA may influence LRF is the close anatomical relationship between LRF neurons and the tubero-infundibular and incerto-hypothalamic DA pathways. In addition, there is increasing evidence of a regulatory action of DA from various pharmacological and physiological studies in the experimental animal and the human.

1.50.1 The Luteinising Hormone Releasing Hormone (LRF) Neuron

Acidic extracts of the median eminence were found to cause release of LH from the pituitary gland (McCann et al., 1960; Harris, 1961), and subsequently the active component was isolated, purified and chemically identified as a decapeptide (Matsuo et al., 1971; Burgus et al., 1972).

Synthetic LRF (p Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) has the same primary structure as ovine and procine LRF and, when injected intravenously into humans, it causes a prompt release of LH and FSH.

LRF is released from neuron terminals in the median eminence, which end close to portal blood vessels; it is carried in the portal blood to a surface membrane receptor on the gonadotropes of the pituitary, where it activates adenyl cyclase to produce a time and dose related increase in cyclic AMP (Borgeat et al., 1972). Cyclic AMP activates protein kinase which dephosphorylates membrane proteins, causing an increase in membrane permeability. Exocytosis of secretory granules containing stored luteinising hormone then takes place by a mechanism which requires calcium (McCann, 1977).

1.50.2 Localisation of LRF in the Brain (Fig. 1.2)

Initial attempts to localise LRF in the brain used bioassay methods (McCann, 1962; Watanabe and McCann, 1968; Crighton et al., 1970). Subsequently, with the development of radioimmunoassays, LRF was measured in pooled brain slices (King et al., 1975) and pooled hypothalamic nuclei (Palkovits, 1974). All these methods suggested that LRF was present in the median eminence, the arcuate nucleus and the medial pre-optic area. In 1973, a more discriminating and precise method, using immunohisto-fluorescence, was described (Barry et al., 1973). This method enabled cell bodies and axons to be mapped in a variety of experimental animals and, more recently, also in the human (Baker et al., 1974; Setalo et al., 1975; King et al., 1974; Fuxe et al.,1976a; Barry, 1976; Barry and Dubois, 1975).

In the human, LRF cell bodies are widely scattered in the hypothalamus, but particularly in the retrochiasmatic area, the infundibular nucleus and the premamillary region (Bird et al., 1976; Barry, 1976a). (Fig. 1.2).

Nerve axons can be traced from these cell bodies and follow two main pathways. One extends down from the hypothalamus into the external layer of the median eminence, particularly the lateral aspect, and ends close to portal capillaries. The other extends from the pre-optic area and terminates on non-fenestrated capillaries in the organ vasculosum of the lamina terminalis (OVLT), a highly vascular area located at the extreme rostral end of the third ventricle.

It is assumed that LRF is synthesised in cell bodies and is transported by axoplasmic flow to the nerve terminals, where it is released. It has not, however, been excluded that LRF synthesis may also take place in the nerve terminals themselves. Although neurosecretion of LRF is well established, an alternative route of conveyance of LRF to the median eminence has been suggested on the basis of the histological description of specialised ependymal cells (tanycytes), which line the third ventricle and which extend long cytoplasmic processes through the median eminence to the portal blood vessels (Kobayashi et al., 1970; Fleischhauer, 1975). It has been proposed that LRF might enter the CSF from the OVLT and be taken up from the third ventricle by the tanycytes and thereby transported to the portal blood vessels. This rather tortuous route for LRF has received some support by the report that synthetic LRF injected into the CSF can be transported to the portal blood (Ben-Jonathon et al., 1974), and by the discovery of immunoreactive LRF in the tanycytes of the mouse (Zimmerman et al., 1974).

This latter finding however, has not been confirmed in other species (Naik, 1975).

In a recent study, it was found that the increase in serum LH and FSH in sheep, which follows intraventricular infusion of synthetic LRF, is delayed and decreased in magnitude compared to intravenous administration (Carson et al., 1976). Furthermore, the LH and FSH responses to intraventricular LRF were proportional to the level of LRF in the serum, suggesting that the response to intraventricular LRF may result from absorption into the circulation. The exact role of the tanycyte in transport or storage of LRF in the human remains to be defined.

1.50.3 The Anatomical Relationship of LRF and DA Neurons (Fig. 1.2)

Histofluorescent studies shown an accumulation of LRF and DA nerve endings in the lateral pallisade zone of the external layer of the median eminence (Löfström et al., 1976). Electron microscopic examination of this area shows numerous granules, some which are undoubtedly catecholamines and others which are probably releasing factors (Kobayashi et al., 1970). These granules are closely related but do not co-exist in the same nerve axons, since destruction of DA neurons does not change the local concentration of LRF (Kizer et al., 1975). Nerve terminals containing DA and LRF end close to capillaries of the portal plexus, which are highly fenestrated and of the specialised nature found in endocrine tissue elsewhere in the body (Kobayashi et al., 1970).

Numerous 'synaptoid' contacts have been described between nerve axons containing DA and cytoplasmic processes of the tanycytes (Guldner and Wolff, 1973).

However, it has not been possible to detect any similar connection between DA nerve terminals and LRF axons or dendrites (Ajika and Hökfelt, 1973). This need not negate the possibility that DA exerts an effect on the LRF neuron, since very few synapses can be identified in the striatum (Hökfelt, 1967), and in the neural lobe dopaminergic terminals show close contact with neurosecretory axons without actual synaptic connections being made (Baumgarten et al., 1972). It does suggest, however, that if DA does influence LRF release in the median eminence, it does so by a mechanism which is not typical of synaptic communication elsewhere in the body. A number of other mechanisms have been postulated whereby DA could influence LRF release (Ajika and Hökfelt, 1973; Kobayashi, 1970).

(i) Local release of DA from nerve terminals may alter the release of LRF from adjacent neurons by altering some aspect of membrane function or the exocytotic secretory process.

(ii) If tanocytes are involved in the transport or storage of LRF, then DA could influence this by the synaptoid contacts the neurons make with the tanycyte processes.

(iii) Local release of DA may alter portal blood flow or change the permeability of portal vessels to LRF.

(iv) DA may inhibit the action of LRF at the level of the gonadotrope receptor.

(v) Although there are no LRF cell bodies in the median eminence, a direct synaptic contact may exist between the incerto-hypothalamic tract and LRF cell bodies in the suprachiasmatic-medial pre-optic area.

At the present time, it is not possible to exclude any of these possibilities.

1.50.4 The Physiological Role of LRF in the Human Female

Secretion of gonadotropins

Gonadotropin secretion by the pituitary in normal women is composed of two components : a tonic low level secretion upon which is superimposed episodic pulsations (Yen et al., 1972). The periodicity and amplitude of these pulses vary at different times during the menstrual cycle (Midgley and Jaffe, 1971). The evidence suggests that episodic secretion of LH and FSH is a reflection of periodic secretion of LRF. Hence, LRF concentration in the portal blood of rhesus monkeys varies in a pulsatile manner (Carmel et al., 1975), antiserum to LRF abolishes pulsatile release of LH (McCormack and Knobil, 1975), and *in vitro* perfusion of the pituitary only results in episodic LH release if pulses of LRF are delivered (Osland et al., 1975).

Negative feed-back of oestrogen

In ovariectomised women, the pituitary secretion of LH and FSH is greatly increased. This appears to be mediated by an increased secretion of LRF induced by the lack of oestrogen. Increased concentrations of LRF have been found in the peripheral blood of ovariectomised women (Seyler and Reichlin, 1973), as well as in rats (Ben-Jonathon et al., 1973) and rhesus monkeys (McCormack and Knobil, 1975).

Positive feed-back effect of oestrogen at mid-cycle

During the normal menstrual cycle of women, ovulation occurs following a variable period of ovarian follicular maturation, during which time serum oestrogens are increasing. In the pre-ovulatory period, there is a more rapid increase in serum oestradiol (E_2), which is followed by an abrupt surge in LH, and to a lesser extent FSH, which normally results in ovulation (Yen et al., 1970a), LRF appears to mediate this mid-cycle surge in LH and FSH. Although there is some doubt about the validity of serum assays of LRF (Nett and Adams, 1977), increased levels of LRF have been described in the peripheral blood of women at mid-cycle (Malacara et al., 1972; Arimura et al., 1974; Mortimer et al., 1976). Using an anaesthetic which does not suppress ovulation in the rat, it has also been shown that there is an increase in LRF in portal blood during proestrus (Sarkar et al., 1976). Furthermore, administration of LRF antiserum to rats during proestrus is found to block ovulation (Arimura et al., 1974; Koch et al., 1973).

In rat experiments, LRF has also been shown to be important in the initiation of puberty and in gestation and female sexual behaviour (Arimura, 1977). However, there is no direct evidence at this time to indicate if the same applies to the human.

The apparent disparity in FSH and LH responses to LRF

Although the bulk of evidence supports the view that LRF controls both the secretion of LH and FSH (Arimura, 1977; Arimura and Schally,1974a), in different physiological and experimental states the ratio of FSH to LH secretion varies considerably. During the normal menstrual cycle, for example, there is an increase in LH : FSH at mid-cycle (Naftolin et al., 1973), and during early puberty

sleep-entrained release of LH is not associated with a similar effect on FSH (Parker et al., 1975). During early puberty, the FSH response to LRF is initially greater than LH, although as puberty proceeds, the LH response exceeds the FSH (Mortimer et al., 1975).

These disparities are most likely due to a relative insensitivity of FSH to LRF (Arimura et al., 1972), associated with a possible preferential inhibitory effect of oestradiol (Yen et al., 1975) and inhibin (Bramble et al., 1975) on FSH response to LRF. In rats however, a stimulation of the medial pre-optic area is associated with a selective rise in LH, while a more caudal stimulation releases both hormones (Kalra et al., 1971). Conversely, a suprachiasmatic lesion in rats selectively blocks the release of LH but not FSH in response to progesterone (Kalra et al., 1971). It is not possible therefore, to exclude a separate FSH stimulating hormone.

1.50.5 Catecholaminergic Involvement in the Control of Gonadotropin Secretion

The interest in catecholaminergic neurotransmission and LH secretion began in 1948, with the demonstration that local application of adrenaline to the pituitary gland stimulated ovulation in the rabbit (Markee et al., 1948), whereas dibenamine, an α adrenergic blocker, inhibited reflex ovulation in both the rabbit (Sawyer et al., 1947) and the rat (Everett, 1964).

The effect of general depletion of catecholamines

These studies led to a number of attempts to study the effect of inhibitors of catecholamine synthesis on LH release from the pituitary gland. In the absence of sensitive assays for serum LH, indirect measurements were used. Hence, α methyl tyrosine (a tyrosine hydroxylase inhibitor) was found to block spontaneous ovulation in the mouse (Bhargava and Gupta, 1966), the hamster (Lipmann, 1968) and the immature mouse (Brown, 1967), and also to prevent contralateral ovarian hypertrophy after hemicastration of female rats (Donoso and Santolaya, 1969). Reserpine and other depletors of brain catecholamines blocked ovulation in the rat (Barraclough and Sawyer, 1957; Coppola et al., 1966; Meyerson and Sawyer, 1968), although drugs which specifically depleted only peripheral amines were without effect (Coppola, 1968).

There are several difficulties in interpreting these kinds of data. The general depletion of catecholamines induced by these agents does not allow a distinction to be made between the effects of noradrenaline (NA) and DA, and failure of ovulation and ovarian hypertrophy are complex events which may not only reflect a deficiency in LH secretion.

The effect of selective depletion of Noradrenaline

Attempts have been made to improve the specificity of these type of studies by selectively decreasing NA with diethyldithiocarbamate (DDC), which blocks the conversion of DA to NA. This drug was found to reduce the LH surge induced by either progesterone treatment of the oestrogen-primed castrate rat (Kalra et al., 1972), or by

electrical stimulation of the pre-optic nucleus on the morning of proestrus (Kalra and McCann, 1973). In the castrated male rat, DDC selectively inhibits LH but not FSH secretion (Donoso et al., 1971). It seems likely that this action of NA is mediated through α receptors, since phenoxybenzamine but not propranolol, blocks the progesterone-induced surge of LH in the oestrogen primed castrated female rat (Kalra et al., 1972).

Measurement of local concentration of DA and NA.

To fully understand the nature of catecholamine control of gonadotropin secretion, it would, ideally, be necessary to correlate circulating levels of LH and FSH with the level of neural impulse flow in functionally homogenous populations of neurons. Such a correlation would best be sought in an unstressed animal during a time when physiological changes in LH were occurring. This type of analysis is not technically feasible at the present time, and in the absence of this, less direct methods have had to be used. Measurement of individual catecholamine concentrations in the brain and contemporaneous measurement of serum gonadotropins is one of these methods.

Castration was found to increase NA concentration in the anterior hypothalamus (Donoso et al., 1967) and replacement of oestradiol and progesterone reduced NA and increased DA (Donoso and Stefano, 1967). Studies of cycling female rats showed varying results; NA concentrations were reported to be elevated in the anterior and middle hypothalamus on the day of proestrus by some (Stefano and Donoso, 1967; Löfström, 1977), but not others (Kurachi and Hirota, 1969; Lichtensteiger, 1969; Kueng et al., 1976).

The difficulty in establishing a quantitative relationship between catecholamine concentration and circulating gonadotropins is largely due to the lack of an assay method sufficiently sensitive to measure catecholamines in discrete areas of the hypothalamus. Recently, however, a sensitive radioenzymatic assay has been developed and used to measure NA and DA in pooled discrete hypothalamic nuclei of female rats at 1000 hours and 1700 hours on the day of proestrus (Selmanoff et al., 1976). This study showed a significant increase in NA concentration in the suprachiasmatic nucleus, but no change in regional DA concentration. Although this study was a major technical improvement over previous methods, the limitation of sampling to only two time points may not have given a true indication of rapid changes in catecholamines. With more frequent sampling, a fall in DA concentration has been detected in the median eminence preceding the increase in serum LH (Wilkes, M.M., personal communication).

Measurement of catecholamine turn-over

This method which has been used mainly by Fuxe's group, attempts to assess neuronal activity by measuring the rate of disappearance of catecholamines, in localised areas of the hypothalamus, after synthesis of catecholamines has been blocked. This method assumes that the rate of disappearance of catecholamine is equal to the rate of new catecholamine synthesis and that these are strictly proportional to rate of impulse flow. This situation may not apply in all situations; for example, synthetic rate exceeds disappearance rate after castration (Anton-Tay and Wurtman, 1970).

The validity of Fuxe's method has been discussed at length (Löfström, 1977) with the conclusions that the method of measurement of catecholamine turn-over in the median eminence correlates well with other methods, and that the rate of disappearance of catecholamines is a valid indication of neural impulse flow (Andén et al., 1969; Arbuthnott et al., 1970).

Using this method, Fuxe's group has found that there is an inverse correlation between the rate of DA turn-over in the lateral pallisade zone of the external layer of the median eminence and the serum level of LH. DA turn-over was increased when LH levels are low during pregnancy and lactation (Fuxe et al., 1969), and was decreased in castrated rats (Fuxe et al., 1969a). Oestrogen treatment of castrated rats caused an increase in DA turn-over which correlated with the fall in serum LH (Löfström et al., 1977). A sharp fall in DA turn-over was associated with the pre-ovulatory surge of serum LH on the afternoon of proestrus in the normal cycling rat (Ahren et al., 1971), or after treatment of the immature rat with pregnant mare serum (PMS) (Fuxe et al., 1972). A decrease in portal vein DA concentration has also been described on the afternoon of proestrus (Ben-Jonathon, 1977). Other groups using catecholamine turn-over methods have shown that NA turn-over is increased on the afternoon of proestrus in normal rats (Donoso and de G. Moyans, 1970) and by PMS induced ovulation (Coppola, 1969).

Despite the difficulties intrinsic to each of these methods used to assess the effects of catecholamines on LH, the general consensus of these studies is that activation of NA pathways is associated with an increase in serum LH, while activation of DA

pathways may be associated with a decrease in serum LH (Fernstrom and Wurtman, 1977; Wilson, 1974; Löfström, 1977).

1.50.6 The Effect of Administration of DA and DA Agonists on the Secretion of LH – Animal Studies.

The initial in vitro experiments of McCann and his colleagues demonstrated that DA added to incubated pituitary glands, did not affect the secretion of LH. However, when the pituitaries were co-incubated with hypothalamic tissue, stimulation of LH and FSH occurred (Schneider and McCann, 1969; Kamberi and McCann, 1969). A subsequent report from the same group was unable to confirm these findings (Quijada et al., 1973), and in a similar experiment, it was found that DA inhibited LH secretion from the pituitary left attached to the median eminence (Miyachi et al., 1973).

Injection of both DA and NA into the third ventricle caused stimulation of LH and FSH in cycling female and normal male rats (Schneider and McCann, 1970; Kamberi et al., 1971). Furthermore, DA caused an increased release of bioassayable LRF from the brain (Schneider and McCann, 1970; Kamberi et al., 1971; Kamberi et al., 1970). In contrast however, comparatively large doses of DA were without effect on LH secretion in another study, although NA caused an ovulatory surge (Sawyer et al., 1974). This apparent paradox may be explained by a rapid uptake and conversion of DA to NA after intraventricular injection (Fuxe et al., 1970), or perhaps to oxidation and inactivation of DA (Takahara et al., 1974). A further technical factor raised as a possible explanation for McCann's findings is the observation that gonadotropin release can be induced by mechanical stretching of the ventricles by injection of fluid (Porter et al., 1972).

Local injection of DA into the arcuate nucleus during the critical period of normal cycling female rats inhibited ovulation (Craven and McDonald, 1973). Implantation of DA into the median eminence, but not the pituitary, also inhibited LH release (Uemura and Kobayashi, 1971).

A careful study by Drouva and Gallo (1976) studied the effect of various agents on the episodic release of LH in the long term ovariectomised rat. The animals were unrestrained and were bled continuously through an indwelling right atrial cannula. Intraperitoneal or subcutaneous injection of apomorphine (1.5 mg/kg) caused a marked decrease in episodic LH release in 10/12 rats, which lasted for 50 - 60 minutes. In a subsequent report, piribedil was also found to decrease episodic LH secretion for up to four hours (Drouva and Gallo, 1977). These effects of apomorphine and piribedil were blocked by a D butaclamol, but not by the inactive form, L butaclamol. Injection of saline or the dopamine antagonist pimozide had no effect on episodic secretion of LH, which led the authors to conclude that endogenous DA did not exert a tonic inhibitory effect on LH secretion in the castrate rat. Similar results were obtained by Gnodde and Schuilling (1976), also studying episodic LH secretion in the ovariectomised rat, and by Mueller et al. (1976), and Wuttke et al. (1971).

1.50.7 The Effect of Administration of DA and DA Receptor Agonists on the Secretion of LH — Human Studies.

Several studies have failed to show any effect of L Dopa on LH secretion (Hayek and Crawford, 1972; Zarate et al., 1973).

In one report, serum levels of luteinising hormone-releasing hormone (LRF) were measured and no alteration was detected (Polansky et al., 1976). In contrast, Boden et al. (1972) detected a significant decrease in serum LH after L Dopa. Lachelin et al. (1977) found that oral administration of 500 mg of L Dopa produced a small but significant decrease lasting six hours in women in the early follicular phase. This decrease was followed by a rebound increase in LH. In this latter study, samples were taken at 15 minute intervals over a period of ten hours, and the results were analysed in terms of percentage change from baseline level. It seems likely that the difference in methodology and analysis accounts for the inability of the earlier studies to demonstrate this effect.

Administration of apomorphine to normal men failed to produce any significant alteration in serum LH (Lal et al., 1973; Martin et al., 1974). Bromocriptine also was reported to have no effect on serum LH (Tolis et al., 1973). However, Lachelin et al. were also able to detect a long-standing decrease in serum LH and FSH, following acute administration of bromocriptine to women with hyperprolactinaemia.

There is only one previous report of the effects of prolonged intravenous infusion of DA on serum LH in the human (Leblanc et al., 1976). These authors found a persistent decrease in serum LH levels during the infusion of DA and a small rebound increase at the end of the infusion. The infusion of DA in the dose used (4 µg/kg/min) did not induce any change in blood pressure or pulse rate, and was free of any major complication.

1.60 Summary and Aims of Study

Since 1957, when DA was established as a neurotransmitter in brain tissue, a number of studies have sought to define its role in human disease. It is firmly established that DA is a major neurotransmitter in the extrapyramidal system and deficiency of effective dopaminergic neurotransmission in the nigro striatal pathway results in parkinsonian-like motor disturbances. DA is also a major regulator of prolactin secretion from the pituitary gland, and the possibility has been explored that hyperprolactinaemia in the human may be a result of a defect in dopaminergic neurotransmission in the tubero-infundibular pathway.

Precise neurochemical studies have determined that LRF producing cells and LRF neurons are positioned in close anatomical relationship to DA neurons of the incerto-hypothalamic and tubero-infundibular neurons. Experimental studies in animals have suggested that there may be some physiological significance to this anatomical arrangement. Although technical difficulties have tended to limit the validity of many of these studies, there is evidence that DA may inhibit the release of LH from the pituitary. This action seems to be mediated indirectly by decreasing the release of hypothalamic LRF.

Technical difficulties have, in the past, made it difficult to study the role of DA in the control of LH secretion in the human. However, the recent demonstration that DA, given by intravenous infusion, causes a decrease in serum LH, has offered a new means of investigating this problem.

The hypothesis that this dissertation examines is that DA is a major regulator of the release of gonadotropin as well as prolactin secretion in the human female. It also examines the possible modulating effect of gonadal steroids on the LH and PRL responses to DA in normal women and the possibility that a disturbance in dopaminergic neurotransmission is associated with the syndrome of hyperprolactinaemic chronic anovulation.

CHAPTER 2

MATERIALS AND METHODS : HUMAN STUDIES.

2.10 Normal Subjects and Patients

Most of the studies to be described were performed on normal women either at the Garvan Institute of Medical Research, Sydney, or, in San Diego, at the Clinical Research Center or the Ambulatory Research unit at University Hospital. The protocol for these latter studies were approved by the Human Subjects Committee of the University of California, San Diego. The nature and purpose of the investigations were explained and informed written consent was obtained from all the volunteers who took part in them.

Clinical details of the normal women are summarised in Table 3.1. These women had normal, regular, ovulatory cycles and most were studied on several occasions. Some of the women were experienced in recognising symptoms of lower abdominal discomfort or increased vaginal discharge in the periovulatory part of their cycle, which helped in the planning of studies during the late follicular phase. Careful attention was paid to the stage of the menstrual cycle at which these women were studied and details of this are given in appropriate sections of the text. Other investigations were performed on normal men, agonadal women, or hyperprolactinaemic women as described. None of the normal subjects or patients had taken any medication for six months, unless specifically stated otherwise.

2.20 Experimental Studies

During these studies, which commenced at 0800 hours, the subjects remained supine in a comfortable state, but were not permitted to smoke, sleep or drink coffee.

2.20.1 Dopamine Infusions

Dopamine HCL ("Inotropin"; Arnar-Stone Lab., Inc., Mt. Prospect, Ill.) was given in a standard dose of 4 µg/kg/min. This was delivered over a period of four hours through a scalp vein needle into a forearm vein by means of a constant infusion pump (Model 2681; Harvard Apparatus, Millis, Mass.).

An intravenous plastic catheter ("Medicut #19", Sherwood Med. Industries, St. Louis, Miss.) was inserted into a large antecubital vein at the beginning of the study and its patency was maintained by a slow infusion of normal saline (15 mls/hour). Every 15 minutes, the catheter was cleared of saline by removing 2 ml of blood which was discarded and then a 6 ml sample was collected. DA infusions commenced after blood samples had been collected for one hour.

Diuresis was a common association with the DA infusion and nausea developed in one hyperprolactinaemic woman, but not to the extent that the study had to be terminated. No change in blood pressure or pulse rate was detected in any subject.

2.20.2 Metoclopramide (MCP) Studies

The same batch of metoclopramide (Batch #37, Beecham Research Lab., Moorabbin, Victoria) was used in the studies performed at the Garvan Institute. In the studies performed at U.C.S.D., metoclopramide, Lot #2303, obtained from Robins and Co., Richmond, Virginia, was used. The drug was given by pulse intravenous injection of 2.5 mg or 0.5 mg after a 1 or 3 hour basal collection

of blood. Blood samples were drawn through an indwelling plastic catheter at 15 minute intervals over the duration of the study. Apart from occasional drowsiness, no side effects were observed. In particular, no extrapyramidal reactions and no change in blood pressure occurred.

2.30 Pituitary Hormone Measurements

Blood samples were allowed to clot and then centrifuged at $4^{o}C$ and the serum separated. Serum was stored at $-20^{o}C$ until assayed. All the samples were assayed within one month of collection. None of the samples were thawed and refrozen more than twice. Serum was assayed for prolactin (PRL), LH, FSH, 17β-oestradiol (E_2), oestrone (E_1), and progesterone (P) by radioimmunoassay.

2.30.1 The Human Prolactin Assay

Serum PRL was measured using the same basic radioimmunoassay procedure that has previously been described (Ehara et al., 1973). However, the purified human PRL used in the standard curve and for radio-iodination was the VLS #3 preparation distributed by NIAMDD, Bethesda. Rabbit anti-human PRL antiserum (NIAMDD) was used in a final tube dilution of 1:200,000. Labelled I^{125} PRL was repurified for each assay using a Sephadex G100 column and only the monomer fraction was used.

A nine dose standard curve was constructed from triplicate determinations. This is shown, with details of the maximum binding, non-specific binding, range and sensitivity of the assay, in Figure 2.1. A control serum run in 30 consecutive assays and binding at 75.4% of maximum gave a coefficient of variation of 19.2%.

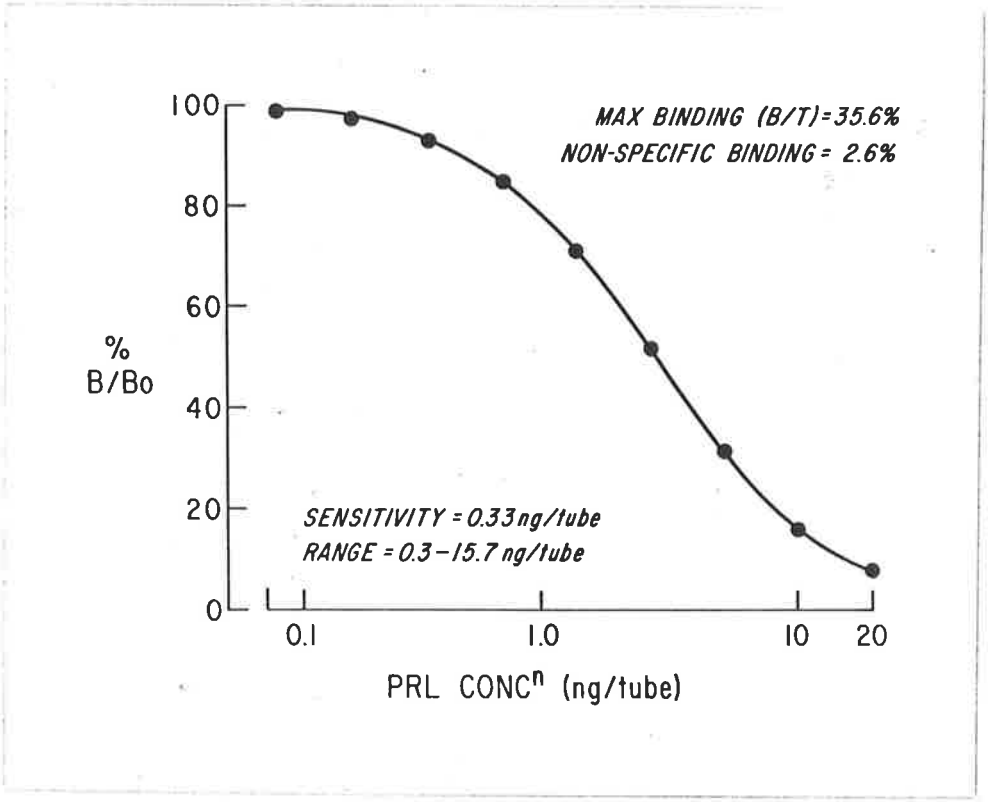
Figure 2.1

A typical standard curve for the human PRL assay showing maximum binding, non-specific binding, sensitivity and range.

Bo = Maximum binding with no unlabelled PRL minus non-specific binding.

B = Binding with added unlabelled PRL minus non-specific binding.

Maximum binding is calculated from a mean of nine samples.



2.30.2 The Human LH Assay

Serum LH was measured using a previously described radioimmunoassay procedure (Yen et al., 1968). However, the LER 907 pituitary gonadotropin preparation (NIAMDD, Bethesda) was used in the standard curve and the LER 960 material for radio-iodination. Nine serial dilutions of HMG 2nd IRP were also run in triplicate in each assay. Results are expressed in terms of this HMG 2nd IRP preparation by multiplying the nanograms of LER 907 by a factor which relates these two preparations at 50% of maximum binding. Using this factor, 1 μg of LER 907 is equivalent to 210 mIU of the HMG 2nd IRP material. Interassay variation over 30 consecutive assays was 12.9% at 20% binding. A typical standard curve, maximum binding, non-specific binding, sensitivity and range are shown in Figure 2.2.

2.30.3 The Human FSH Assay

FSH was measured using some modifications of the previously described assay (Yen et al., 1970). The NIAMDD preparation LER 1801 #3 was used to prepare I^{125} labelled FSH tracer and LER 907 was used in the standard curve. Rabbit anti-human FSH antiserum was purchased from Dr. A. Parlow (Professional Staff Association, U.C.L.A.) and used in a final dilution of 1:160,000.

I^{125} labelled FSH was repurified before each assay by chromatography on a Sephadex G100 column (55 x 0.9 cms), using Dextran blue which yielded a highly immunoreactive labelled FSH, eluting with the void volume (Bell et al., 1975). A typical standard curve, sensitivity and range are shown in Figure 2.3.

Figure 2.2

A typical standard curve for the human LH assay with maximum and non-specific bindings, range and sensitivity of the assay.

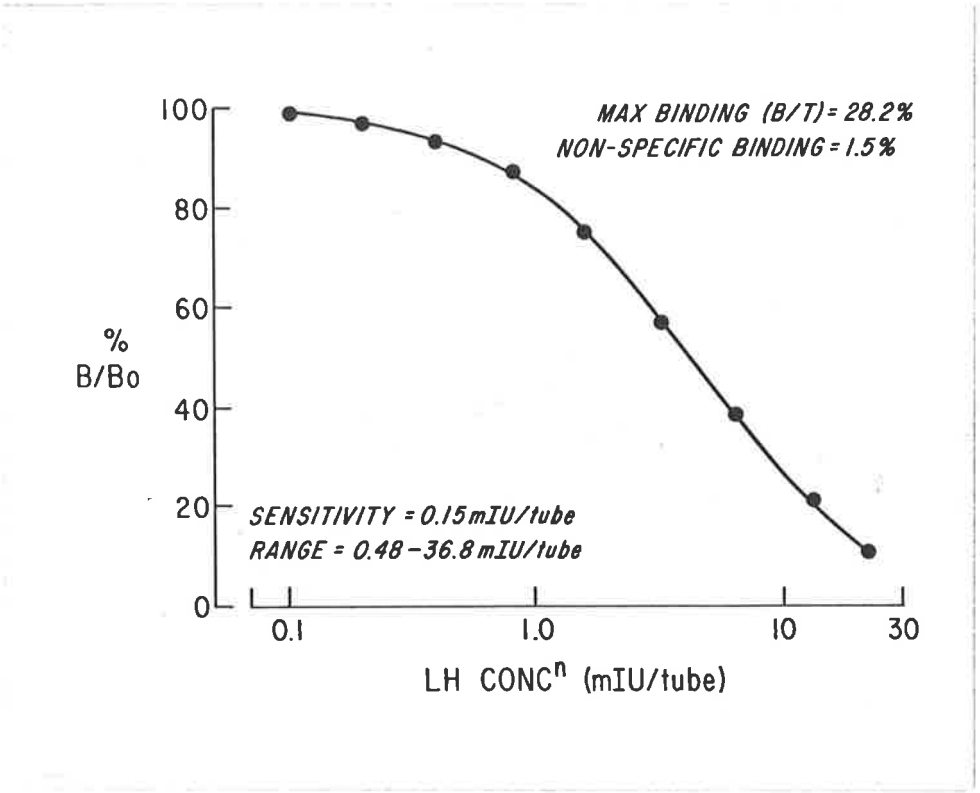
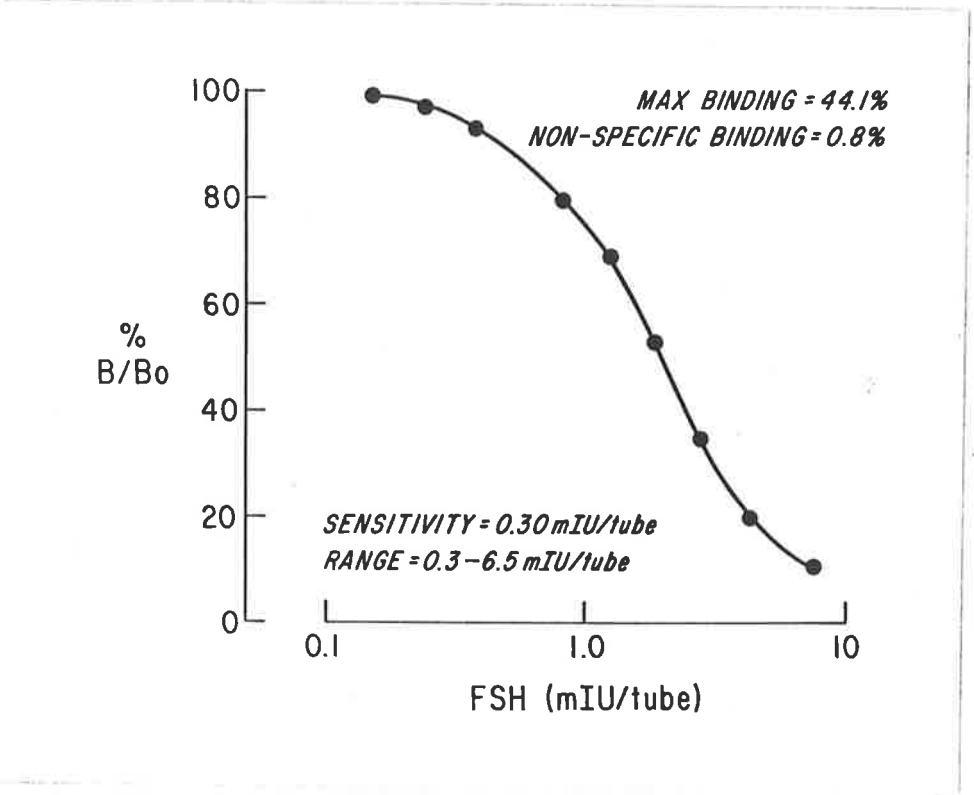


Figure 2.3

A typical standard curve for the human FSH assay also showing maximum binding, non-specific binding, sensitivity and range of the assay.



Results are expressed in terms of HMG 2nd IRP standard; 1 µg of LER 907 was equivalent to 38.5 mIU of the HMG 2nd IRP in this assay. Interassay variation of two control samples run in 25 consecutive assays were 13.8% (74% of maximal binding) and 16.0% (52% of maximal binding).

2.31 Steroid Assays

Oestradiol 17 β (E_2), Oestrone (E_1) and Progesterone (P) were extracted from serum and measured by radioimmunoassay, using modifications of previously published methods (DeVane et al., 1975).

2.31.1 Extraction and Separation Procedure

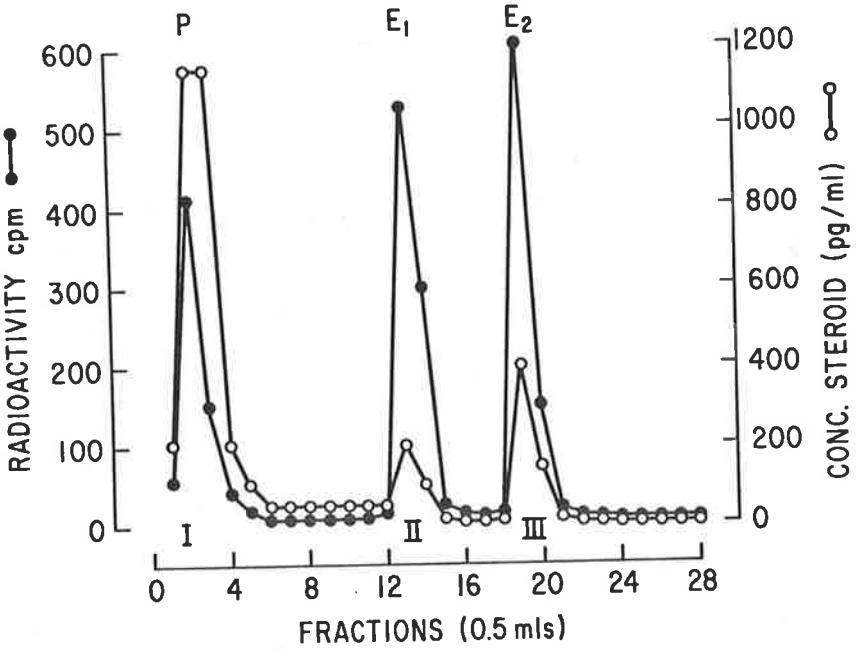
E_2, E_1 and P are extracted from serum with ether and the extract is applied to a microcelite column with ethylene glycol as the stationary phase. Progesterone is extracted by elution with iso-octane (Fraction 1, Figure 2.5), E_1 is eluted with 15% ethyl acetate in iso-octane (Fraction II, Figure 2.5) and E_2 is eluted with 40% ethyl acetate in iso-actane (Fraction III, Figure 2.4). The fractions are dried and then reconstituted with 0.1 M Phosphate buffer. A fraction of this is counted to determine the internal recovery of a known amount of labelled hormone and 0.5 ml is used for assaying.

2.31.2 Radioimmunoassay of E_2, E_1 and P

The same antiserum, prepared in rabbits by injection of oestriol 3, 16, 17 trihemisuccinate (Abraham S5310 #5), was purchased from Dr. G.E. Abraham and used for the assay of both E_2 and E_1. The antiserum is used in a final dilution of 1:70,000

Figure 2.4

A comparison between the elution patterns of Progesterone (P), Oestrone (E_1), and Oestradiol (E_2) on celite columns as determined by simultaneous radioimmunoassay of various fractions and measurement of radioactivity when a trace dose of purified labelled hormone is added to normal female serum.



for the E_1 assay and 1:110,000 for the E_2 assay. The Progesterone antiserum (S7 #4) was prepared in rabbits by injection of 11 ∝ - hydroxyprogesterone and used in a final dilution of 1:14,000.

2,4,6,7-H^3 oestradiol or 1,2,6,7,-H^3 Progesterone (Amersham - Searle) were repurified on celite columns and added in a final concentration of 5,000 cpm per tube in the various assays. Bound and free fractions were separated with dextran charcoal.

Representative standard curves with sensitivity and useful range are shown for each of the assays in Figures 2.5, 2.6 and 2.7. Water blanks run in each assay were less than assay sensitivity. The specificity of each assay has been determined by measuring the immunoreactivity of fractions collected from a celite column to which purified labelled E_2, E_1 and P, in human female plasma, had been added. The radio-active and immunoreactive peaks coincide (Figure 2.4) and are well separated by the column. No immuno-reactivity was detected in other fractions of the serum sample which indicates that no cross-reactivity exists with other closely related steroids present in human female plasma.

Interassay variation of a control serum binding at 72% of maximum binding in 12 successive E_2 assays was 15% and for a control serum binding at 80% of maximum binding in 12 successive E_1, assays was 20%. Interassay variation for a control serum binding at 76% of maximum binding over 12 successive Progesterone assays was 8.2%.

Figure 2.5

A typical standard curve for the 17 β Oestradiol (E_2) assay showing sensitivity, range and recovery rates of added tracer.

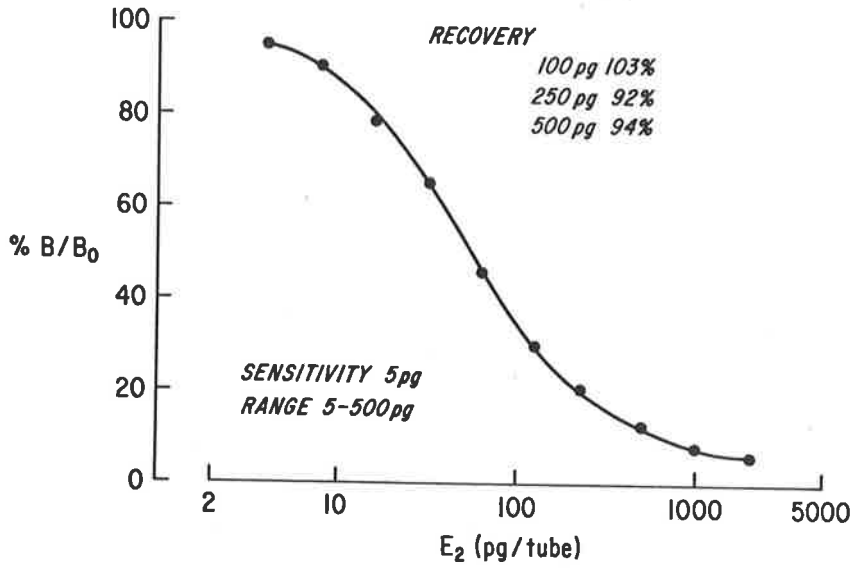


Figure 2.6

A typical standard curve for the Oestrone (E_1) assay including sensitivity and range of the assay and internal recovery rates of added tracer.

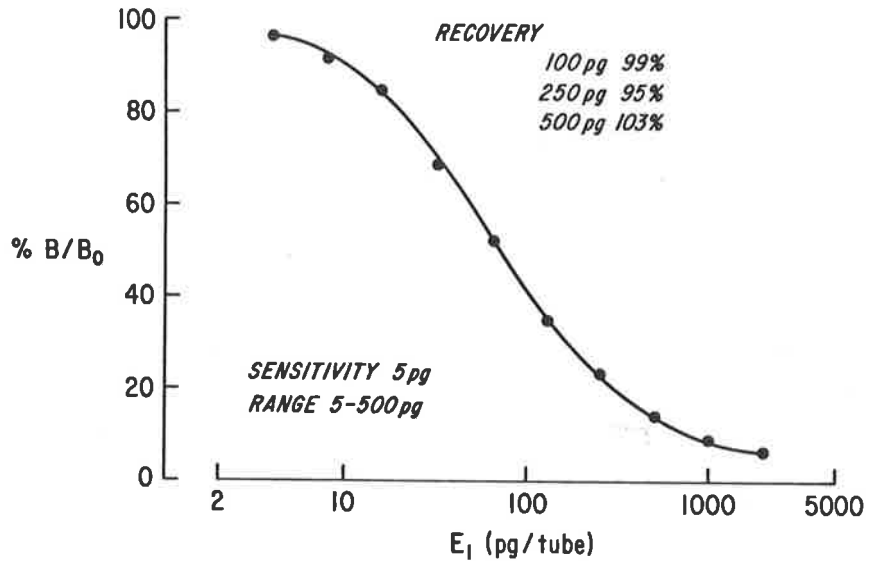
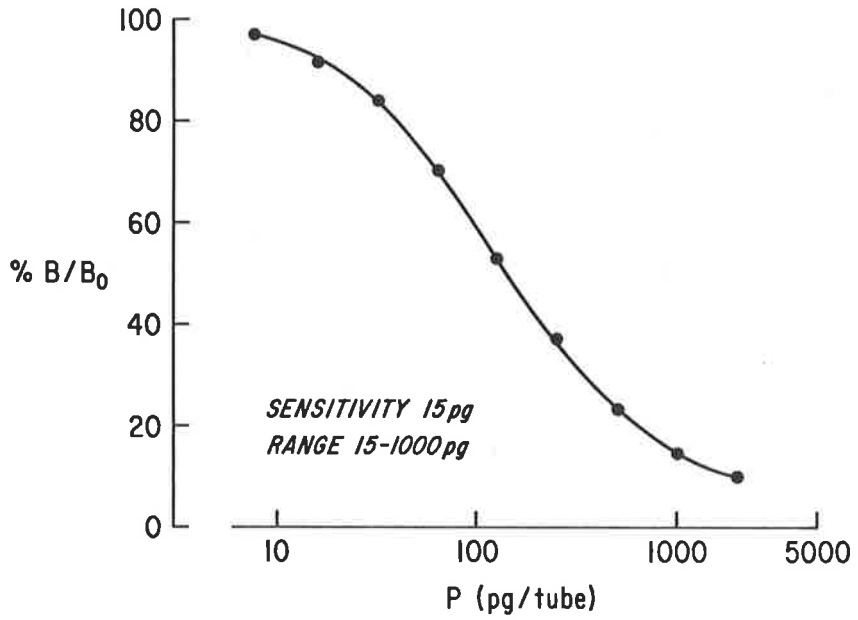


Figure 2.7

A typical standard curve for the Progesterone (P) assay including sensitivity and range.



2.40 Statistical Methods

The effect of DA on serum levels of LH, FSH and PRL was assessed in each subject by calculating the net change of these hormones from a baseline level derived from the mean of five samples at 15 minute intervals taken before the start of DA. To allow comparison between groups, mean net changes (± SEM) for each hormone were calculated at 15 minute intervals over the duration of the study, using a programme devised for use on the Hewlett Packard Desktop calculator (Model 9830A Hewlett Packard, Loveland, Col.) by Mr. G. Brink (Dept. of Reproductive Medicine, U.C.S.D.). For statistical purposes, the net change of a particular hormone during the last two hours of the DA infusion were compared in different groups using two way analysis of variance. An example of the format used for these analysis is shown in Table 2.2. The programme used for these calculations was designed for the Hewlett Packard Company by the Statistical Analysis Unit of Colorado State University. This programme allows unbalanced numbers of observations to be statistically analysed using either the method of fitting constants or the method of square means.

Hormone responses to metoclopramide (MCP) were analysed by determining the mean levels of LH, FSH, and PRL from the 13 samples taken before MCP and comparing these values to the mean level from 20 samples taken after MCP by paired "t" test.

Group "t" tests were used to compare baseline levels of hormones between individual groups.

Table 2.1.

The format for a two way analysis of variance (in this case balanced) comparing the change of serum PRL during DA infusion of six normal women on Day 2 of their menstrual cycle and six normal women on Day 12 of their menstrual cycle.

	Column 1 (Day 2)	Column 2 (Day 12)
Row 1 Sample #14	Observations (n = 6) (1 for each subject)	Observations (n = 6) (1 for each subject)
Row 2 Sample #15	"	"
Row 3 Sample #16	"	"
Row 4 Sample #17	"	"
Row 5 Sample #18	"	"
Row 6 Sample #19	"	"
Row 7 Sample #20	"	"
Row 8 Sample #21	"	"

CHAPTER 3

THE INHIBITION OF SERUM GONADOTROPINS AND PROLACTIN BY INFUSIONS OF DOPAMINE IN NORMAL WOMEN : THE MODE OF ACTION OF DA AND THE EFFECT OF OESTROGEN.

3.10 Introduction

It has been pointed out that the nerve terminals of the tubero-infundibular DA pathway and the LRF neurons are closely associated in the external layer of the median eminence. This area of the brain is generally considered to lie outside the "blood-brain barrier" and hence is freely influenced by substances in the systemic circulation (Wislocki and Leduc, 1952; Wilson et al., 1962). Although the hypothalamus takes up only small quantities of labelled biogenic amines (Olendorf 1971; Weil-Maherbe et al., 1959), intra-peritoneal administration of large doses of DA to the rat (500 mg/kg) is associated with an increase in histofluorescence of the parenchyma of the median eminence (Bertler et al., 1963).

These observations suggested that if a sufficient dose of dopamine was given to exceed its rate of metabolism by MAO, then the tubero-infundibular and LRF neurons in the median eminence might be directly influenced. This possibility was tested by Leblanc et al. (Leblanc et al., 1976), who found that a constant infusion of 4 μg/kg/min of DA was associated with a consistent decrease in serum PRL and LH.

Two other important observations were made in this latter study. Firstly, it was found that DA exerted a greater inhibitory effect in women than men which suggested that oestrogen may modulate the action of DA. Secondly, on cessation of DA, there was found to be a rebound increase in serum prolactin and to a lesser extent LH.

In this chapter are reported a number of studies which are designed to further investigate these effects of DA. In particular, these studies attempted to define the mode of action of DA in suppressing serum LH and to assess the modulating effect of oestrogen on the inhibition of LH, FSH and PRL by DA.

3.20 Subjects and Methods

Twenty adult women participated in these investigations and many were studied on more than one occasion. Clinical details of each patient, including age, age of onset of menarche, % average body weight and reproductive history are shown in Table 3.1.

The women had regular cycles ranging in length from 26 - 33 days with a mean of 30.2 days. Since the aim was to study women at different, but comparable, stages of follicular maturation and endogenous oestrogen secretion, the day of study varied slightly according to individual cycle lengths. The three specific times during which the women were investigated were defined as follows:

1. The Day 2 group included women studied on Day 2 or 3 of their cycle;
2. The Day 12 group was studied 3 - 5 days before the day of peak LH secretion;
3. The Day 14 group was studied the day immediately before the peak secretion of LH (see Figure 3.1).

These times were roughly predicted in individual subjects by the careful recording of previous menstrual cycle lengths aided in some women by the recognition of pre-ovulatory changes in vaginal secretion or mittelschmerz. Serial daily blood samples were taken after the studies and assayed for LH, FSH, E_2, E_1 and P to confirm

Table 3.1

Clinical details of 22 normal women who took part in the various studies described in this dissertation.

* % average body weight derived from the tables of the Society of Actuaries Build and Blood Pressure Study, Vol. I, p. 16, Chicago, 1969.

** Indicates that this subject was included in the group designated 'Day 2', 'Day 12' or 'Day 14' for study with DA infusion.

*** Metoclopramide studies.

EF = Early Follicular phase studies using 2.5 mg MCP.

ef = Early Follicular phase studies using 0.5 mg MCP.

LF = Late Follicular phase studies using 2.5 mg MCP.

lf = Late Follicular phase studies using 0.5 mg MCP.

Table 3.1

Subject	Age	Menarche	% Av. Body wt.*	Gravid/ Para/ab	Mean Cycle Length (±ISD)	Studies		
						DA **	MCP***	Others
1. J.C.	30	12	104	4/4/0	31.6 ± 2.9	D_2, D_{12}, D_{14}	EF LFx2 ef lf	DA + LRF NE DA+MCP
2. M.K.	27	13	118	0/0/0	31.2 ± 1.3	D_2, D_{12}		Saline
3. R.F.	26	12	94	0/0/0	32.0 ± 1.0	D_2, D_{12}		Saline
4. J.T.	32	12	140	5/5/0	28.7 ± 2.3	D_2, D_{12}	EFx2 LF ef lf	DA + MCP
5. M.D.	28	13	83	3/1/2	32.3 ± 1.5	D_2, D_{14}	LF	DA + LRF
6. L.M.	19	13	109	0/0/0	27.3 ± 1.5	D_{12}, D_{14}		6 hr DA
7. S.D.	35	12	125	5/4/1	30.0 ± 1.3	D_{14}		
8. B.H.	25	13	96	2/1/1	28.8 ± 2.7	D_{14}	EFx2	DA + LRF
9. S.F.	31	13	100	4/4/0	32.1 ± 2.1		EFx2 ef lf	
10. J.J.	34	11	99	3/3/0	28.0 ± 1.0		LF	

Subject	Age	Menarche	% Av. Body wt.*	Gravid/ Para/ab	Mean Cycle Length (± ISD)	Studies		
						DA**	MCP***	Others
11. R.N.	22	13	106	0/0/0	28.6 ± 1.1	D_2		8 hr DA NE Saline
12. V.L.	25	13	121	0/0/0	27.2 ± 1.8	D_{12}	EFx3 ef lf	
13. P.W.	28	13	90	0/0/0	32.6 ± 0.6		EF	Saline
14. S.L.	30	12	129	0/0/0	31.5 ± 1.0			DA + LRF NE
15. E.B.	30	12	113	4/4/0	30.5 ± 2.0			NE
16. W.K.	30	13	133	4/4/0	32.3 ± 1.2			DA + LRF
17. J.K.	27	11	89	0/0/0	32.8 ± 2.1			DA + LRF
18. K.D.	26	11	84	0/0/0	27.7 ± 2.1			DA + LRF
19. C.A.	32	14	119	1/1/0	29.3 ± 3.1			DA + LRF
20 N.W.	29	13	90	1/1/0	29.6 ± 2.3			DA + LRF
21. B.N.	27	11	103	4/4/0	29.8 ± 1.6	D_{14}	EF LF ef lf	

Table 3.1

Subject	Age	Menarche	% Av. Body wt.*	Gravid/ Para/Ab	Mean Cycle Length (±ISD)	Studies		
						DA**	MCP***	Other
22. K.M.	28	12	101	0/0/0	30.1 ± 0.3		ef 1f	
Mean ± SEM	28.2 ± 0.8	12.4 ± 0.2	103 ± 3.6		30.2 ± 0.4			

Figure 3.1

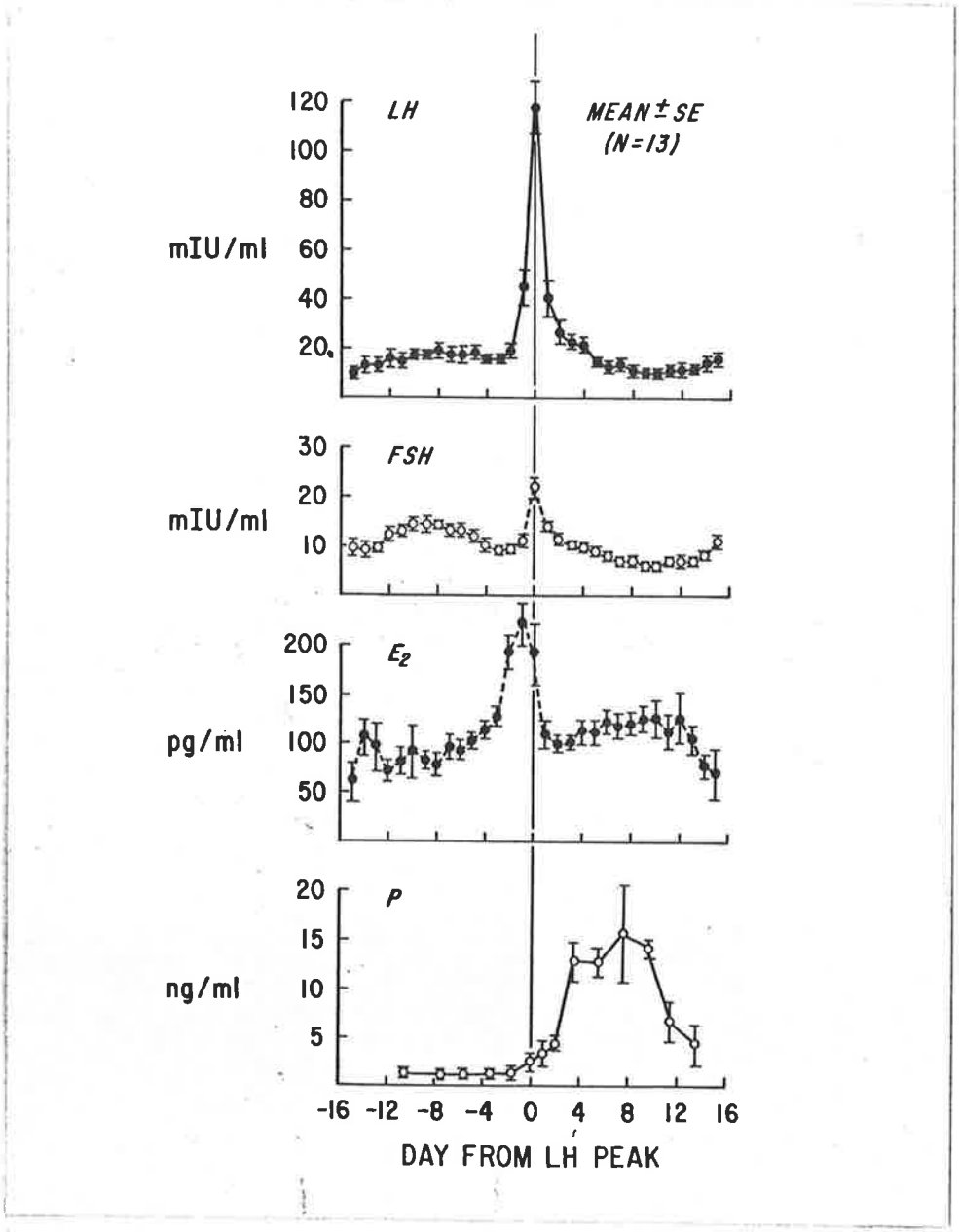
Normal alterations of LH, FSH, E_2 and P during the menstrual cycle. These values were obtained from a different group of women then described in the text (Yen, S.S.C., unpublished).

The three follicular groups of women were studied at the times indicated:

Day 2 (Day of cycle)

Day 12 (3 - 5 days before LH peak)

Day 14 (1 day before LH peak).



these times in retrospect. If these subsequent results did not confirm these times, the study was excluded altogether or reassigned to the appropriate group. Ovulation was confirmed in each cycle investigated by a rise of serum progesterone in excess of 5 ng/ml following the occurrence of the LH surge.

The technique of the DA infusion, collection of blood samples, and the assay of hormones is as described (2.20.1). In order to compare the hormonal response to DA in the various groups, the results are expressed in terms of the net change (Δ) in serum hormone concentration from a baseline level, calculated from the mean of five samples taken at 15 minute intervals before the start of the infusion. The results are analysed by Group t tests or analysis of variance where appropriate.

3.30 Results

3.31 The Response of LH, FSH and PRL to Infusion of DA During the Follicular Phase of the Normal Menstrual Cycle.

Eleven normal women received a standard infusion of DA on 1 - 3 occasions (Table 3.1) so that there were six women in each of the three follicular phase groups described above. The mean ($\pm$ SEM) basal concentrations of LH, FSH, PRL, E_2 and E_1 for each of the three groups of women is shown in Table 3.2. The uniformity of serum E_2 and E_1, within the three groups and the progressive increase at different times during the follicular phase is consistent with the experimental aim. The basal levels of LH in the Day 2 and Day 12 groups are the same, but there is a four-fold increase in serum LH in the Day 14 group which indicates that this group was studied after the initiation of the mid-cycle increase in serum LH; however, in subsequent daily samples, it was determined that the

maximum secretion of LH did not occur until the day after the study.

3.31.1 LH and FSH Responses to DA Infusion

In each of the three groups, there is a significant decrease in serum LH during the course of the DA infusion (Figure 3.2 and 3.2a). Despite an oestradiol concentration which is four times higher in the Day 12 group, the LH response to DA (Max Δ = -4.0 $\pm$ 0.7 mIU/ml) is not significantly different from the Day 2 group (Max Δ = -4.5 $\pm$ 0.7 mIU/ml). In marked contrast in the Day 14 group, there is a substantially greater decrement in LH (Max Δ = -30.2 $\pm$ 15.8 mIU/ml), which, over the four hours of DA infusion, is significantly greater than the other two groups ($P < 0.0001$, by analysis of variance).

Individually, there is a significant correlation between this response to DA and the basal level of LH($r = 0.979$, $P < 0.0001$) but not with circulating E_2 ($r = 0.103$, $P > 0.01$). A rebound increase in serum LH, above baseline levels, after discontinuation of DA is only consistently seen in the Day 14 group (Figure 3.2a). This episodic increased pulsation of LH occurred in each of these women, although not concurrently.

Serum FSH remains little affected by DA in the Day 2 and Day 12 groups, but is significantly decreased in the Day 14 group (Figure 3.3). The maximum Δ is however small (-3.0 $\pm$ 0.7 mIU/ml). There is significant correlation between the maximum FSH response and the basal serum level of LH ($r = 0.788$, $P < 0.001$) in individual women.

Table 3.2

Basal concentrations (mean ± SEM) of circulating oestrogen, gonadotropins and prolactin in normal women receiving DA infusions at different stages of the follicular phase of the menstrual cycle.

Subjects	E_2 pg/ml	E_1 mIU/ml	LH mIU/ml	FSH mIU/ml	PRL ng/ml
Day 2 (n = 6)	25.2 ± 3.0	27.2 ± 4.9	11.7 ± 1.1	8.7 ± 0.5	17.6 ± 2.7
Day 12 (n = 6)	103 ± 7.3	57.0 ± 3.5	11.3 ± 1.4	8.0 ± 0.3	22.4 ± 1.7
Day 14 (n = 6)	209 ± 41	137 ± 28	49.6 ± 27	10.2 ± 1.4	20.1 ± 5.5

Figure 3.2

The effect of Dopamine infusion on serum LH (mlU/ml) in three groups of six normal women at different times during the follicular phase of the cycle.

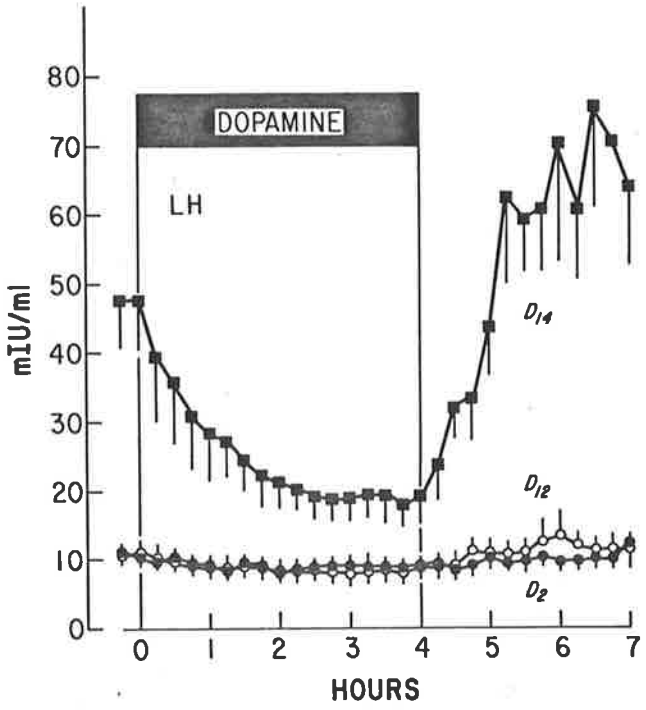


Figure 3.2 (a)

The effect of Dopamine infusion on the net change (Δ) of serum LH in three groups of six normal women at different times during the follicular phase. The P values refer to the level of significance between the response in the Day 14 group compared to the Day 2 group (by analysis of variance).

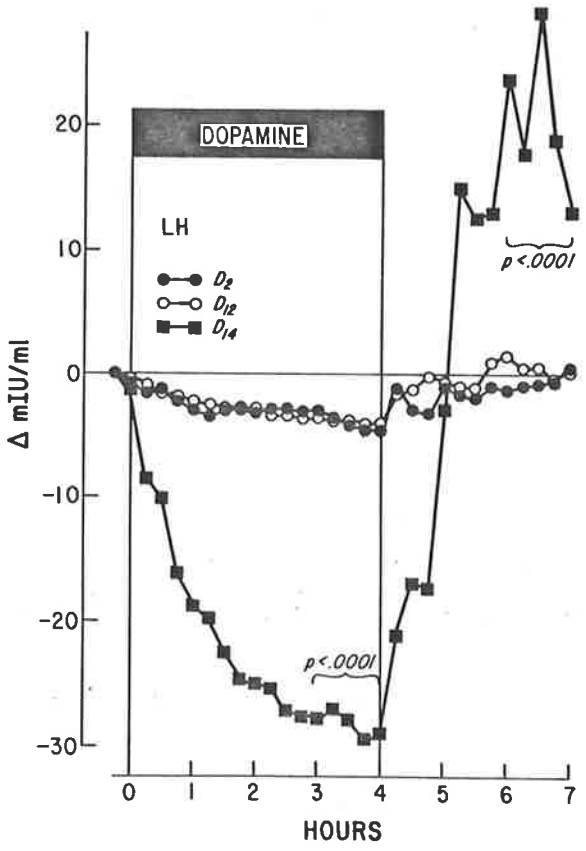


Figure 3.3

The effect of Dopamine infusion on the net change (Δ) of serum FSH in three groups of six normal women at different times during the follicular phase. The P value refers to the level of significance between the responses in the Day 14 and the Day 2 groups.

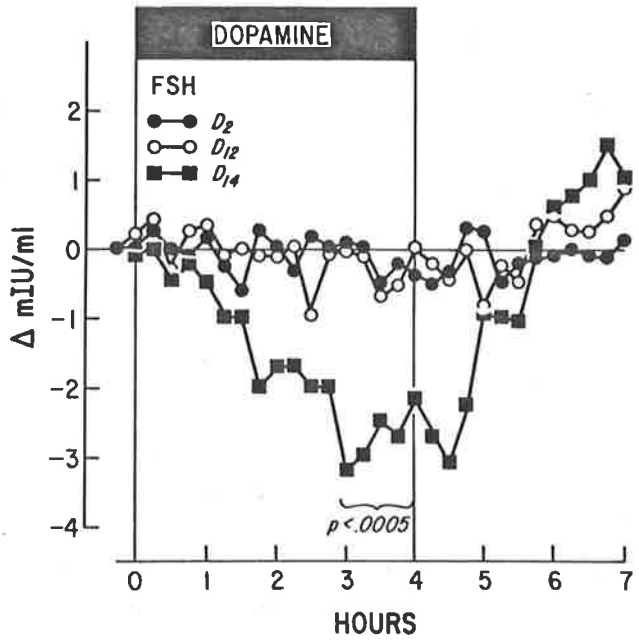
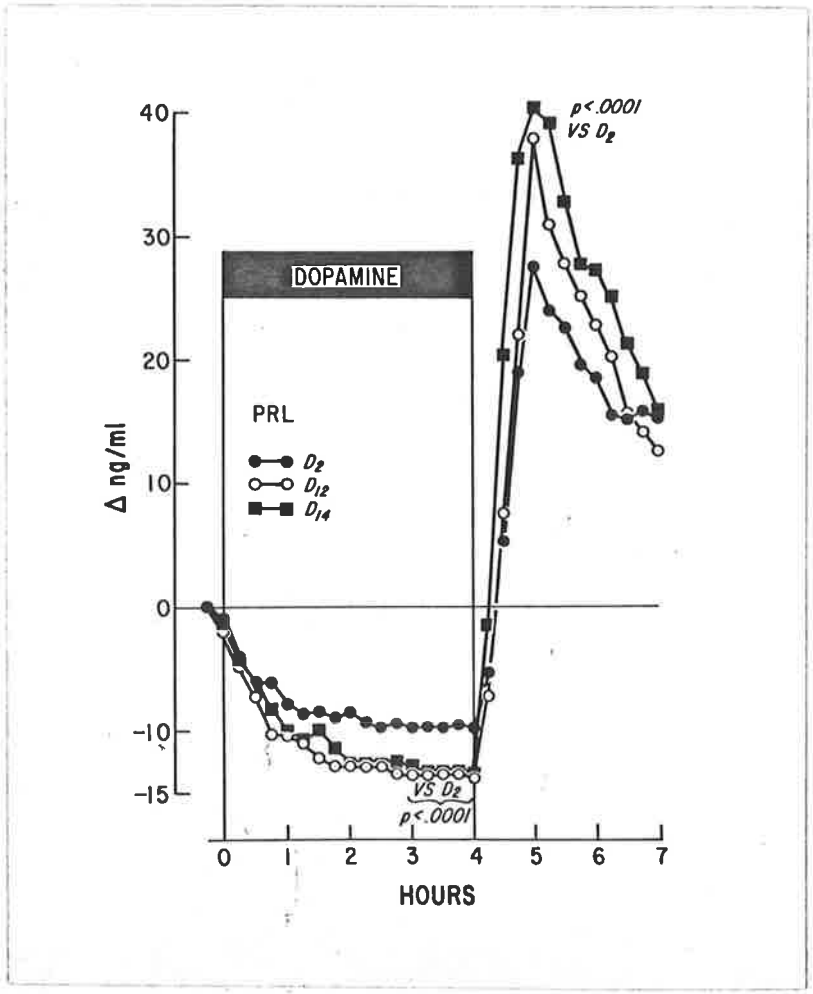


Figure 3.4

The effect of Dopamine infusion on the net change (Δ) of serum PRL in three groups of six normal women at different times during the follicular phase of the menstrual cycle. The P values indicate the level of significance between responses in the Day 14 and the Day 2 groups.



3.31.2 The PRL Response to DA Infusion

Basal levels of serum PRL are not significantly different in the three groups (Table 3.2), but there is a consistently greater degree of suppression by DA in the Day 12 (Max Δ = -14.0 $\pm$ 0.4 ng/ml) and Day 14 groups (Max Δ = -13.5 $\pm$ 1.3 ng/ml), compared to the Day 2 group (Max Δ = -9.7 $\pm$ 0.5 ng/ml) (Figure 3.4). This decrement in both the Day 12 and Day 14 groups, over the four hours of DA infusion, is significant ($P < 0.0001$, by analysis of variance) compared to the Day 2 group. The maximum net decrease in PRL is significantly correlated with both the basal level of PRL ($r = 0.878$, $P < 0.001$) and the circulating E_2 level ($r = 0.685$, $P < 0.001$). In all three groups, there is a significant rebound increase in serum PRL above baseline levels ($P < 0.00001$) although this is greatest in the Day 14 group.

A comparison was made of the increment of serum PRL after DA with the decrement during DA infusion by measuring the areas under and above the mean curves in each of the three groups (Table 3.3). In each case, the post-infusion increment exceeded the intra-infusion decrement by two to three-fold.

3.32 The Effect of Infusion of Normal Saline on LH, FSH and PRL in Normal Women

Four normal women on Day 2 of their cycles were studied to determine the effect of the infusion procedure, per se, on serum levels of LH, FSH and PRL. The procedure of these investigations was the same as for the women receiving DA except that normal saline was infused at a rate of 0.02 ml/min instead of DA.

Table 3.3

Comparison of the magnitude of suppression of PRL during DA infusion and the rebound release following cessation of DA.

Group	Suppression during DA	Release After DA	Ratio increment / decrement
	Area above curve ng-hr/ml	Area under curve ng-hr/ml	
Day 2	48	105	2.2
Day 12	81	155	1.9
Day 14	84	266	3.2

The basal levels of serum LH, FSH, PRL, E_2 and E_1 were not significantly different in this group from the previous Day 2 group (Table 3.4). Infusion of saline was not associated with any significant change in LH or FSH (Figure 3.5). However, there was a small decrease in serum PRL (Max Δ = -4.2 $\pm$ 2.8 ng/ml) which was significant when compared to baseline levels ($P < 0.005$ by analysis of variance). Despite this effect on PRL, the decrement of PRL in the Day 2 group during DA infusion remains significant when compared to this saline infusion group ($P < 0.00001$).

3.33 The Effect of Prolonged Infusion of DA on Serum LH, FSH and PRL in Normal Women

Two normal women on Day 3 or Day 12 of their cycle received extended infusions of DA over a period of eight hours and six hours respectively, to test the stability and biological activity of DA over a longer period of time. Blood samples were collected as before over a period of ten hours. The responses of LH, FSH and PRL, shown in Figure 3.6, are the same as those seen with the four hour infusions. In particular, there is no loss of inhibitory effect of DA on serum LH and PRL.

3.34 The Effect of Infusion of Noradrenaline on the Secretion of LH, FSH and PRL in Normal Women

Four normal women in the Day 12 group received infusions of noradrenaline (NA) to determine if there was any similarity in the effects of NA and DA on serum hormone concentrations and to determine if an increase in NA could trigger a premature LH surge in women in the late follicular phase of the cycle.

Table 3.4

Basal concentrations (mean ± SEM) of circulating oestrogen, gonadotropins and prolactin in four normal women receiving saline infusions on Day 2 of the menstrual cycle.

	E_2 (pg/ml)	E_1 (pg/ml)	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)
Day 2	21.3 ± 2.0	38.2 ± 3.6	10.9 ± 0.8	9.4 ± 1.4	19.2 ± 2.8

Figure 3.5

The effect of Saline infusion on serum levels of LH, FSH and PRL in four normal women on Day 2 of the menstrual cycle.

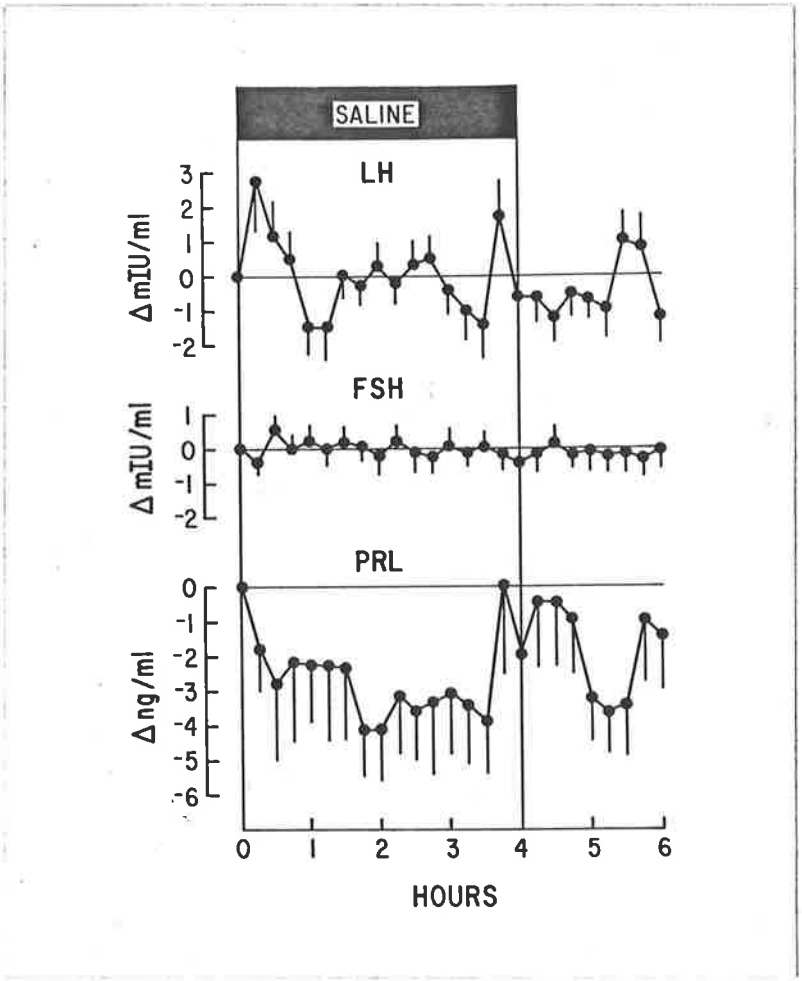
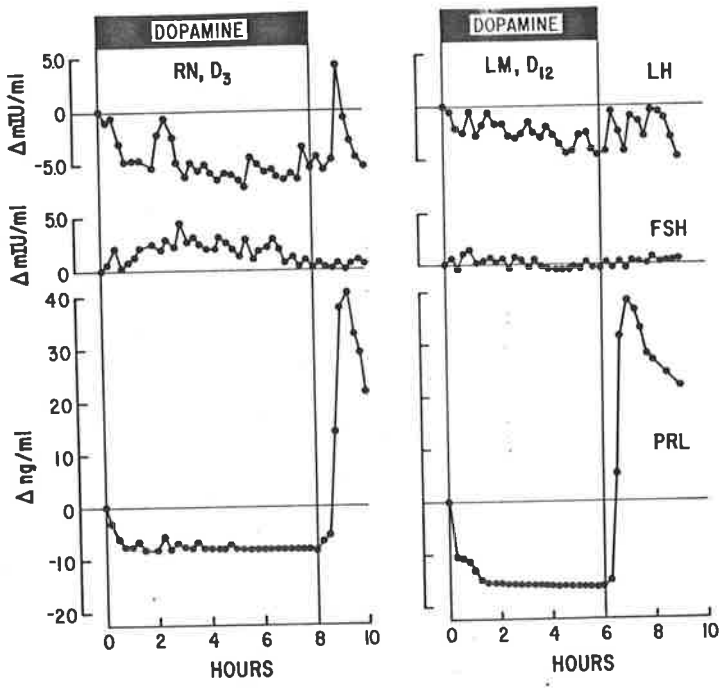


Figure 3.6

The effect of infusion of DA for six hours (LM) or eight hours (RN) on the net change (Δ) of serum LH, FSH and PRL in two normal women.

D_3 = Day 3 of the menstrual cycle

D_{12} = Day 12 of the menstrual cycle.



Noradrenaline ("Levophed" Winthrop Labs.) was infused at a rate of 4 µg/min for four hours into a large antecubital vein. A preliminary study in a normal male, who received varying rates of infusions from 0.2 - 6.0 µg/min, while his blood pressure and pulse rate were monitored by intra-arterial measurements, had determined that NA, given at a rate of 4 µg/min, did not cause an excessive elevation of blood pressure. Frequent blood pressure and pulse rates were measured during the infusions in the normal women and serial blood samples were collected as previously described.

There was a moderate increase in blood pressure from a mean of 118/80 to 145/104 associated with a slight decrease in pulse rate, but the procedure otherwise was not associated with any noticeable side effects.

Basal circulating levels of LH, FSH, PRL, E_2 and E_1 are shown in Table 3.5; they are not significantly different from basal levels of the Day 12 group receiving DA. The episodic secretion of LH was not affected by infusion of this dose of NA, however, a small decrease occurs in both serum FSH and PRL (Figure 3.7). Only the change in FSH was significantly different from the saline control study; DA has a significantly greater effect on both LH and PRL than NA (Table 3.6). Hence, it is apparent that while this dose of NA, which causes a significant effect on the cardiovascular system, has a small effect on serum FSH and PRL, these responses are qualitatively and quantitatively different from those seen with DA.

3.35 The Effect of DA Infusion in Normal Women on the LH and FSH Response to Luteinizing Hormone-Releasing Hormone (LRF)

Nine women participated in this study which was designed to determine if DA exerts its effect on LH and FSH by a direct action

Table 3.5

Basal concentrations (mean ± SEM) of circulating oestrogens, gonadotropins and prolactin in four normal women receiving infusions of norepinephrine on Day 12 of the menstrual cycle.

	E_2 (pg/ml)	E_1 (pg/ml	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml
Day 12	98.3 ± 10.2	96.8 ± 3.7	14.7 ± 1.6	8.3 ± 0.4	15.0 ± 1.0

Figure 3.7

The effect of noradrenaline (norepinephrine) on net change (Δ) of LH, FSH and PRL in four normal women on Day 12 of the menstrual cycle.

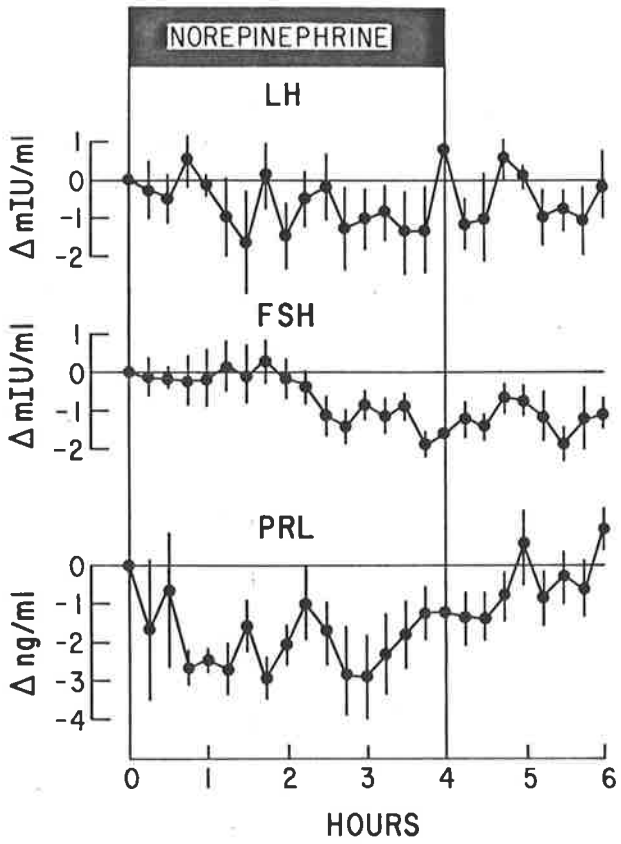


Table 3.6

Comparison of the maximum net change (Δ) in LH, FSH and PRL during the infusions of DA, NA or saline.

Groups	Max Δ LH (mIU/ml)	Max Δ FSH (mIU/ml)	Max Δ PRL (ng/ml)
DA infusion n = 6	-4.5 $\pm$ 0.7 (a)	-0.9 $\pm$ 0.4 (N.S.)	-14.0 $\pm$ 0.4 (b)
NA infusion n = 4	-0.9 $\pm$ 0.6	-1.9 $\pm$ 0.2	-3.0 $\pm$ 0.5
Saline infusion n = 4	-0.7 $\pm$ 0.4 (NS)	-0.1 $\pm$ 0.3 (a)	-4.2 $\pm$ 2.8 (N.S.)

(a) $P < 0.01$ compared to NA

(b) $P < 0.00001$ compared to NA

N.S. Not significant compared to NA

at the level of the pituitary gland. Clinical details of these women is included in Table 3.1. Each of the women received two pulse injections of 10 μg of LRF, intravenously, five hours apart. Previous studies (Wang et al., 1976) had shown that the LH response was not altered by successive doses of 10 μg of LRF during this part of the menstrual cycle. In this study, the first dose of LRF was given after one hour of basal blood sample collection and two hours before the start of a standard DA infusion. The second dose of LRF was given three hours after the commencement of DA (Figure 3.8). Samples of blood were collected, as previously described, through an indwelling venous catheter over the nine hours of the study. Baseline levels of LH were calculated from the mean of five samples taken in the hour proceeding the injection of LRF. The mean ± SEM baseline level of LH before the first injection was 13.9 ± 0.7 mIU/ml, whereas the mean ± SEM of the baseline level before the second pulse, collected during the DA infusion, was 10.7 ± 1.2 mIU/ml (Table 3.7). The individual maximum net changes were calculated for each individual from the corresponding baseline level. The mean maximum net change after the first dose of LRF was 20.0 ± 2.1 mIU/ml and after the second dose was 13.5 ± 1.6. This difference was significant ($P < 0.02$). The FSH response was too small to allow statistical analysis (Figure 3.8).

3.36 The Effect of Exogenous Oestrogen on the LH, FSH and PRL Responses to the Infusion of DA.

In order to more fully define the effect of oestrogen on the LH, FSH and PRL responses to DA, three ovariectomised women, whose clinical details are summarised in Table 3.8, received standard

Table 3.7

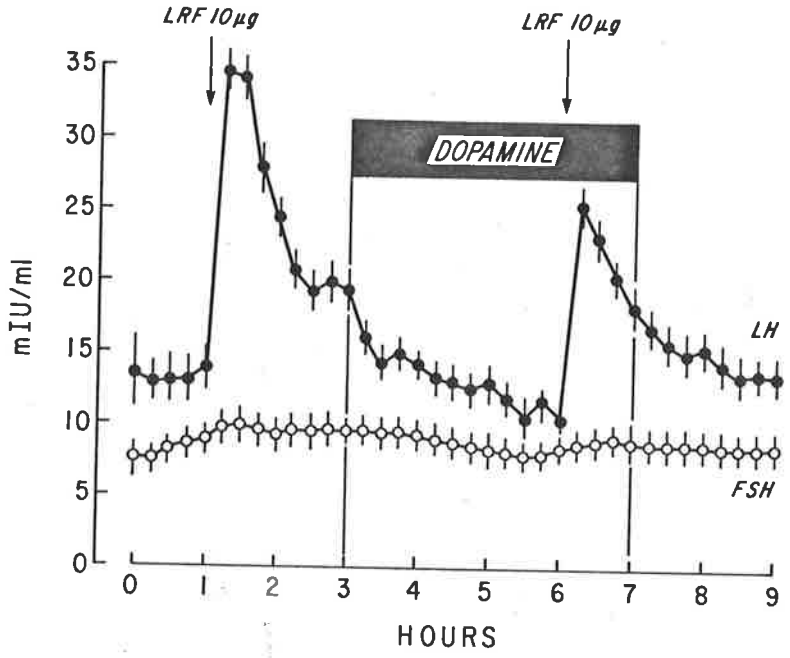
The mean ± SEM baseline levels of LH and maximum responses to LRF before and during DA infusion.

	Baseline LH * (mIU/ml)	Peak response LH	Max Δ LH
Before DA	13.9 ± 0.7	33.9 ± 1.8	20.0 ± 2.1
During DA	10.7 ± 1.2	24.8 ± 1.3	13.5 ± 1.6 $P < 0.02$

* Mean of five samples collected before DA or before second pulse of LRF.

Figure 3.8

The LH and FSH responses to intravenous pulses of 10 μg of synthetic LRF given before and during an infusion of Dopamine in nine normal women on Day 2 of the menstrual cycle.



infusions of DA before and after treatment for seven days with ethinyl oestradiol (50 μg b.d. orally). The last dose of ethinyl oestradiol (EE) was taken two hours before the commencement of DA.

Basal serum levels of LH (94.1 ± 7.8 mIU/ml) and FSH (74.0 ± 5.2 mIU/ml) were significantly reduced by treatment with ethinyl oestradiol to levels of 35.1 ± 3.1 mIU/ml (LH) and 33.1 ± 1.9 mIU/ml (FSH) respectively (both $P < 0.0001$). Conversely, serum PRL increased from 12.3 ± 1.0 to 24.5 ± 0.6 ng/ml ($P < 0.0001$) with EE treatment.

After EE treatment, contemporaneous with the decrease in basal levels of serum LH and FSH, there was a significant attenuation in these responses to DA (Figure 3.9). The mean decrement of LH over the four hours of the infusion, before EE, was 15.6 ± 2.8 mIU/ml; after EE, the mean decrement was 4.4 ± 1.2 mIU/ml ($P < 0.000001$). The mean decrement in FSH during DA infusion before EE was 3.9 ± 1.7 mIU/ml, which was also significantly greater than the post EE response (2.3 ± 1.3 mIU/ml; $P < 0.005$). Conversely, EE augmented the inhibition of PRL by DA from a mean decrement of 1.9 ± 0.6 ng/ml to 6.2 ± 1.1 ng/ml ($P < 0.00001$). There was also a significant augmentation of the rebound in serum PRL after the end of DA infusion in the EE treated group.

3.40 Discussion

Infusion of saline into normal women is clearly associated with a fall in serum PRL from the baseline level, although serum LH and FSH levels were unaffected (Figure 3.5). This is likely due to the effect of stress during the early part of the procedure

Table 3.8

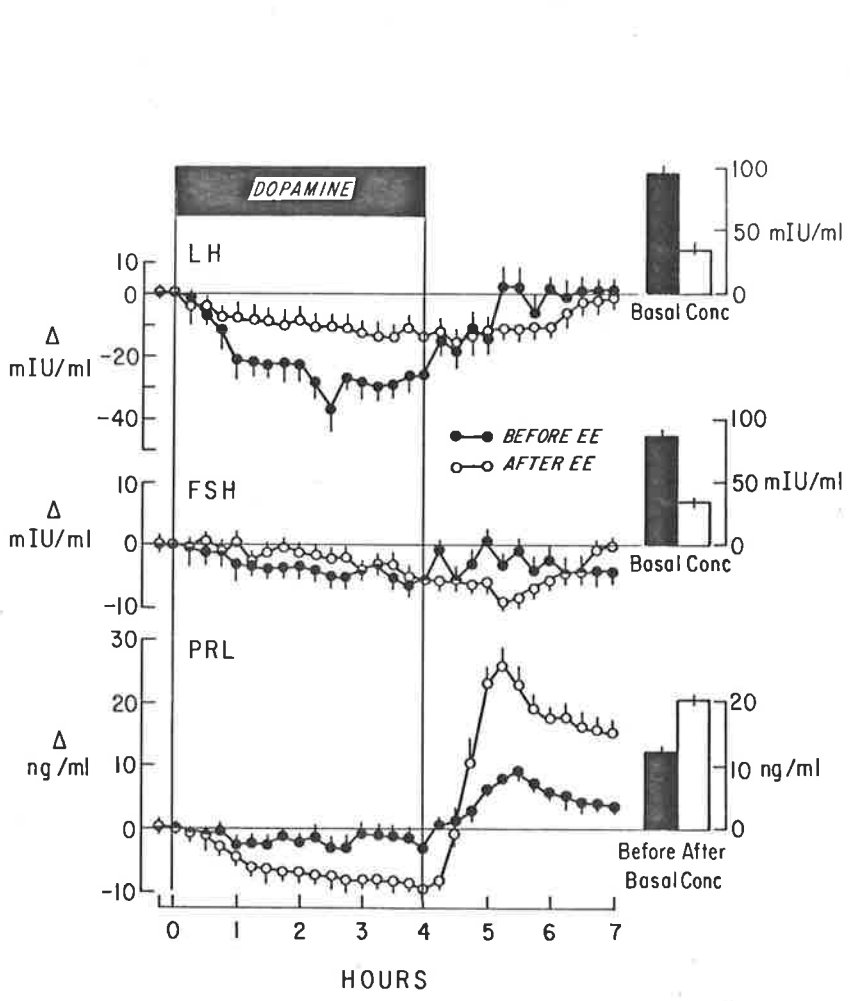
Clinical features of three agonadal women receiving DA infusions before and after ethinyl oestradiol.

Subject	Age	Menarche	Gravid/Para/Ab	Years since Ovariectomy	Reason for Ovariectomy
1. N.G.	37	14	0/0/0	3	Endometriosis
2. E.M.	58	13	2/2/0	8	Chronic P.I.D.
3. E.T.	40	11	5/1/4	12	Menometrorrhagia

Figure 3.9

Dopamine infusion in three agonadal women before and after the administration of ethinyl oestradiol (EE) 50 μg b.d. for seven days.

Mean values and standard error are shown for the basal levels of serum LH, FSH and PRL and for the responses to DA.



(Noel et al., 1972), which would tend to elevate the PRL level in the initial blood samples from which the baseline level is calculated. In addition, there is a normal diurnal variation of PRL (Ehara et al., 1973) and it is possible that part of this decrease during saline infusion is related to a carry-over of a physiological decline occurring in the early morning. This study emphasised the need for a baseline collection of at least an hour to allow for adjustment to these changes.

It seems likely that the effects noted during the infusion of DA were not a result of metabolic conversion of DA to NA, which could theoretically occur after uptake of DA by NA neurons. The infusion of NA was associated with quite different effects from DA; NA infusion caused an increase in mean blood pressure and no significant effect on LH and PRL (Figure 3.7). Prolonged infusion of DA was not associated with any loss of effect on serum LH and PRL (Figure 3.6), which might be expected if conversion to NA was occurring. Very recently, plasma concentrations of DA, NA and A have been measured by radio-enzymatic assay during the course of DA infusion, and although there is a 2 - 3 fold increase in NA and A, serum DA increases several hundred-fold (Wilkes, M.M., personal communication).

3.40.1 The Effect of DA Infusion on Serum Concentrations of LH and FSH

In each of the studies described, infusion of DA caused a decrease in serum levels of LH and PRL and, in some studies, also FSH. These findings support the previous observations of Leblanc et al. (1976) and, in the absence of a similar effect of saline and NA infusion, suggests that this may be specific to DA. The previous

reports are also extended by the demonstration that there is a variation in the net change of LH, FSH and PRL at different times during the normal follicular phase of the menstrual cycle.

Both LH and FSH show a remarkable change in their pattern of response to DA on the day before the maximum secretion of LH. Two major changes are known to occur at this stage of the menstrual cycle; firstly, there is a rapid increase in serum oestrogens contemporaneous with maturation of the ovarian follicle, and secondly, endogenous LRF secretion is increased (Malacara et al., 1972; Arimura et al., 1974). The augmentation of the gonadotropin response to DA at this particular time of the cycle could be related, in theory, to either of these factors.

It is unlikely, however, that oestrogen directly influences the effect of DA on gonadotropin secretion. No correlation has been found relating serum oestradiol concentration and the response of LH or FSH to DA; a four-fold increase in serum oestradiol concentration in the Day 12 group, compared to the Day 2 group, did not alter the gonadotropin response to DA (Figure 3.2). A further increase in oestrogen concentration in the Day 14 group was associated with an augmented gonadotropin response to DA, but EE treatment of agonadal women reduced the response to DA (Figure 3.9).

In contrast, an excellent correlation could be seen between the basal level of LH and the subsequent responses of both LH and FSH to DA. As previously discussed (1.50.4), there is evidence that the increase in basal level of LH at mid-cycle and in agonadal women reflects an increased secretion of endogenous LRF.

Augmented inhibition of both LH and FSH by exogenous infusion of DA in the Day 14 and untreated agonadal women could therefore be seen as circumstantial evidence that DA inhibits either the release or the effect of endogenous LRF. Consistent with this interpretation is the finding that DA infusion consistently suppressed episodic secretion of LH and FSH, which is also thought to depend on LRF secretion (Carmel et al., 1975; McCormack and Knobil, 1975; Osland et al., 1975). If this interpretation is correct, then the rebound increase in serum LH and, to a lesser extent FSH, may be the result of release of accumulated LRF, combined with an oestrogen-induced increase in sensitivity of the pituitary gland; the reduced response of FSH to LRF at mid-cycle compared to LH is well recognised in normal women (Naftolin et al., 1973).

Uptake of exogenous DA by tubero-infundibular neurons with subsequent inhibition of LRF release from adjacent nerve endings in the median eminence, is one possible mechanism to explain the effects of DA infusion on the gonadotropins. This can only be verified by measurement of portal blood or gonadotrope receptor levels of LRF which is presently not technically possible.

An attempt was made to exclude another possible explanation of the effect of DA, viz. that DA inhibits the action of LRF at gonadotrope receptors (3.34). However, in this study a small but consistent impairment of LH release by exogenous LRF was found during DA infusion (Figure 3.10). An alternative explanation for this finding is that DA, by inhibiting endogenous LRF secretion, inhibits the accumulation of acute releasable LH, and hence the

response to the second pulse of LRF. However, it is not possible to exclude a direct action of DA, inhibiting LRF action, at the gonadotrope receptor level; in vitro studies considered in a later chapter (6.32) and elsewhere (Schneider and McCann, 1969) however, argue against this.

The possibility that DA may affect the permeability of portal blood vessels to LRF or the rate of blood flow in portal vessels has also been considered. Neither of these possibilities can be excluded; however, it has been noted that Growth Hormone (GH) secretion is unaffected by DA infusion (Leblanc et al., 1976) which suggests that if a change in permeability occurs, passage of the putative GH releasing factor is unaffected. Extensive studies in the effect of DA infusion on cerebral blood flow in the dog have also been reported (Van Essen, 1974). In low doses (2 µg/kg/min) cerebral blood vessels initially constrict, an action which is opposed by phentolamine but not by pimozide, suggesting that this is mediated by α adrenergic receptors. Since NA, an α agonist, did not produce the same change in LH, it seems unlikely that a decreased portal blood flow could account for these effects. In higher doses, dopamine causes a specific increase in cerebral blood flow by an action which is blocked by pimozide (Van Essen, 1974).

3.40.2 The Effect of DA Infusion on Serum PRL Concentration

Infusion of DA causes a rapid and consistent decrease in serum PRL. This action may be mediated through uptake and release of DA by tubero-infundibular neurons or by a direct action on DA receptors on the lactotrope.

There appears to be a more direct correlation between the circulating level of oestradiol and the PRL response to DA; the steady increase in oestrogens during the early part of the follicular phase and administration of oestrogen to agonadal women is associated with an increase in the inhibition of PRL by DA.

The means by which oestrogen increases the PRL response to DA is not clear. In theory, oestrogen might increase the number of DA receptors on the lactotrope and hence augment the action of DA. Another possibility is that oestrogen acts indirectly to decrease dopaminergic activity in the tubero-infundibular neurons affecting PRL section, and hence cause an increased sensitivity of DA receptors on the lactotrope. However, a recent study which measured DA turn-over by assessing rate of decline of median eminence DA levels, found that DA turn-over was unchanged in hypophysectomised oestrogen-treated male rats (Eikenburg et al., 1977).

The further increase in oestradiol in the Day 14 group was not associated with a further augmentation of DA inhibition of PRL (Figure 3.4). This need not, however, negate the conclusion that oestrogen modulates the action of DA since the duration of exposure to these higher mid-cycle levels of oestrogen is relatively short and if, for example, oestrogen is producing its effects by increasing the number of lactotrope receptors, a longer duration of exposure may well be necessary.

The mechanism of the rebound increase in serum PRL can only be speculated upon. If it is assumed that before infusion of DA, the serum PRL is held in a steady state by a balance in the rate of elimination from the circulation, then DA may act either at the

pituitary or in the periphery to bring about a decrease in serum PRL. It is likely that the major effect of DA is to reduce the rate of secretion of PRL from the pituitary (MacLeod et al., 1970). However, the increase in PRL which follows withdrawal of DA exceeds that which would be expected from the secretion of unreleased PRL and the return to the same steady state. It is possible that the rebound increase in PRL secretion is a reflection of a continued or increased PRL synthesis during DA infusion or of a mobilisation of previously inactive stores within the lactotrope. Alternatively, it is conceivable that the increase in PRL secretion results from an inadequate secretion of endogenous DA, a consequence of a feed-back decrease in neuronal firing rate (Bunney et al., 1973), or that the exposure to exogenous DA induces a state of hyposensitivity in the putative DA receptors on the lactotrope so that renewed endogenous DA secretion is functionally insufficient to maintain the previous level of suppression of PRL secretion. The relatively short duration of exposure to DA need not negate this speculation, since hyposensitivity has been described within two hours of administration of the DA agonist, apomorphine (Costentin et al., 1975; Baudry et al., 1977).

A further additional factor may be that DA decreases the half life of PRL in the peripheral circulation. This possibility is made less likely by the demonstration of the rebound phenomenon in studies of the isolated pituitary gland (6.34 and Thorner et al., 1977), which suggests that a local mechanism involving the pituitary is operative.

Whatever the mechanism, it is apparent that the PRL response of the pituitary following discontinuation of DA is rapid, which suggests either that the disappearance of exogenous DA from the lactotrope receptor is rapid or that the receptor promptly responds to a relative decline in local DA concentration. This observation is consistent with the rapid PRL response seen after administration of dopamine receptor antagonists (4.30).

CHAPTER 4

THE EFFECT OF METOCLOPRAMIDE ON SERUM LEVELS OF LH, FSH AND PRL IN NORMAL WOMEN : ITS MODE OF ACTION AND INTER-RELATIONSHIP WITH OESTROGEN.

4.10 Introduction

Administration of DA receptor antagonists is associated with an increase in serum PRL levels (Lu et al., 1970; Clemens et al., 1974; L'Hermite et al., 1975), which is compatible with the suggestion that DA exerts a tonic inhibitory action on normal PRL secretion by a mechanism which involves specific DA receptors. However, several previous studies have demonstrated that the DA antagonists pimozide and sulpiride, either have no effect, or decrease serum LH. (Collu et al., 1975; Beattie et al., 1976; Ojeda et al., 1974; Mancini et al., 1975). A difficulty in interpreting these studies is that both these drugs are major tranquillisers and the general suppression of neuronal activity induced by drugs of this kind may itself reduce the secretion of LRF. Furthermore, in several of these studies pimozide was given at a dose level which has been shown to interfere with noradrenaline receptors, as well as DA (Anden et al., 1970).

In order to investigate the effect of acute DA receptor blockade on serum LH and FSH, it was thought preferable to use a specific, non-neuroleptic, DA antagonist which could be given safely by intravenous injection to human subjects. There were several reasons to believe that metoclopramide (N-diethylaminoethyl-2-methoxy-4-amino-5 chlorobenzamide), a procainamide derivative which is used widely as an antiemetic, might be appropriate for this purpose. A preliminary report had drawn attention to its ability to increase PRL (McNeilly et al., 1974) and the early literature, investigating its antiemetic effect, had determined that it blocked emesis induced by the potent dopamine receptor agonist, apomorphine (Malméjuc

et al., 1964; Klein et al., 1968). Furthermore, analysis of its structure showed a similarity to other known DA antagonists (Smythe, G., personal communication).

The studies described in this chapter have confirmed the usefulness of metoclopramide (MCP) as a stimulator of PRL secretion and have investigated its interaction with DA and DA receptor agonists. The acute effect of intravenous administration of this drug on serum levels of LH, FSH and PRL has also been assessed in normal women at two different times during the follicular phase of the normal menstrual cycle.

4.20 Subjects and Methods

Initial studies to determine an appropriate dosage of MCP and to examine its interaction with DA and DA receptor agonists, were performed in eight normal adult men aged 24 to 33 years. These men were all in good physical health, were non-smokers and had not taken any medication for at least three months before the investigations commenced. Subsequently, ten normal adult women, whose clinical details are outlined in Table 3.1, received bolus injections of either 0.5 mg or 2.5 mg of MCP. These investigations were performed in either the early follicular phase (EFP) (Day 2-4) or the late follicular phase (LFP) (Day 13-15), as assessed by menstrual history and serum levels of E_2, E_1 and P. Most of the women were studied on more than one occasion but none received MCP more than twice in any one cycle and always at an interval of at least one week.

Blood samples were taken, as previously described (2.20.1), at 15 minute intervals for a baseline period of three hours and

for five hours after administration of MCP, unless otherwise stated. All the samples in each study were run in one assay for LH, FSH or PRL.

Results are expressed either as the net change (Δ) from a baseline level determined from the first five samples collected or else in terms of the mean of all samples taken before or after MCP. These results were analysed by two-way unbalanced analysis of variance, paired "t" test or group "t" test where appropriate.

4.30 Results

4.31 Dose Response Studies With MCP

In this initial study, two normal men received intravenous injections of MCP, on three occasions each, at intervals of at least one week apart. The first subject (SJ) was given doses of 0.1 mg, 0.5 mg or 2.5 mg in a random order. The second subject (GM) received doses of 0.5 mg, 2.5 mg and 10 mg and, on a fourth occasion, normal saline. Baseline blood samples were taken for one hour before injection of MCP and for either three hours (SJ) or five hours (GM) thereafter.

Basal levels of LH, FSH, PRL and sex steroids are shown for each subject in Table 4.1. Serum LH and FSH were assayed in each study and no significant alteration from baseline levels was detected after any dose of MCP. In addition, Growth Hormone, Thyroid Stimulating Hormone (TSH) and Triiodothyronine (T3) were assayed in both subjects before and after study with the 2.5 mg dose of MCP. There was no significant change in serum T3; serum levels of

Table 4.1

Basal serum concentrations (± SEM) of LH, FSH and PRL (mean of 20 samples) and E_2, E_1. Androstenedione (A) and Testosterone (T) (mean of four samples) in two normal male subjects receiving various doses of MCP.

	Age	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)	E_2 (pg/ml)	E_1 (pg/ml)	A (pg/ml)	T (pg/ml)
S.J.	33	14.3 ± 1.3	8.2 ± 0.2	8.5 ± 0.3	20.0 ± 2.0	30.0 ± 3.0	749 ± 41.0	2225 ± 130
G.M.	32	8.3 ± 0.3	5.3 ± 0.4	23.9 ± 2.1	19.0 ± 3.2	31.0 ± 1.5	736 ± 40.0	3626 ± 166

Figure 4.1

The effect of varying doses of metoclopramide (MCP) on the net change (Δ) in serum PRL in two normal men.

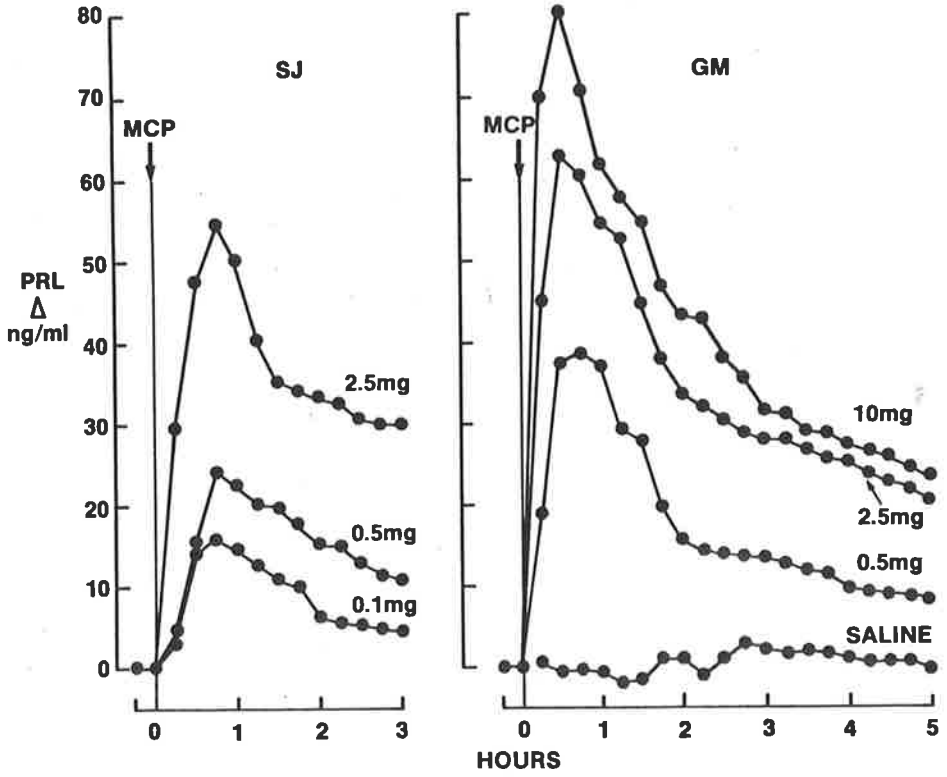


Figure 4.2

The dose-response relationship between dose of MCP and the maximum PRL response (from data derived from studies of two male subjects).

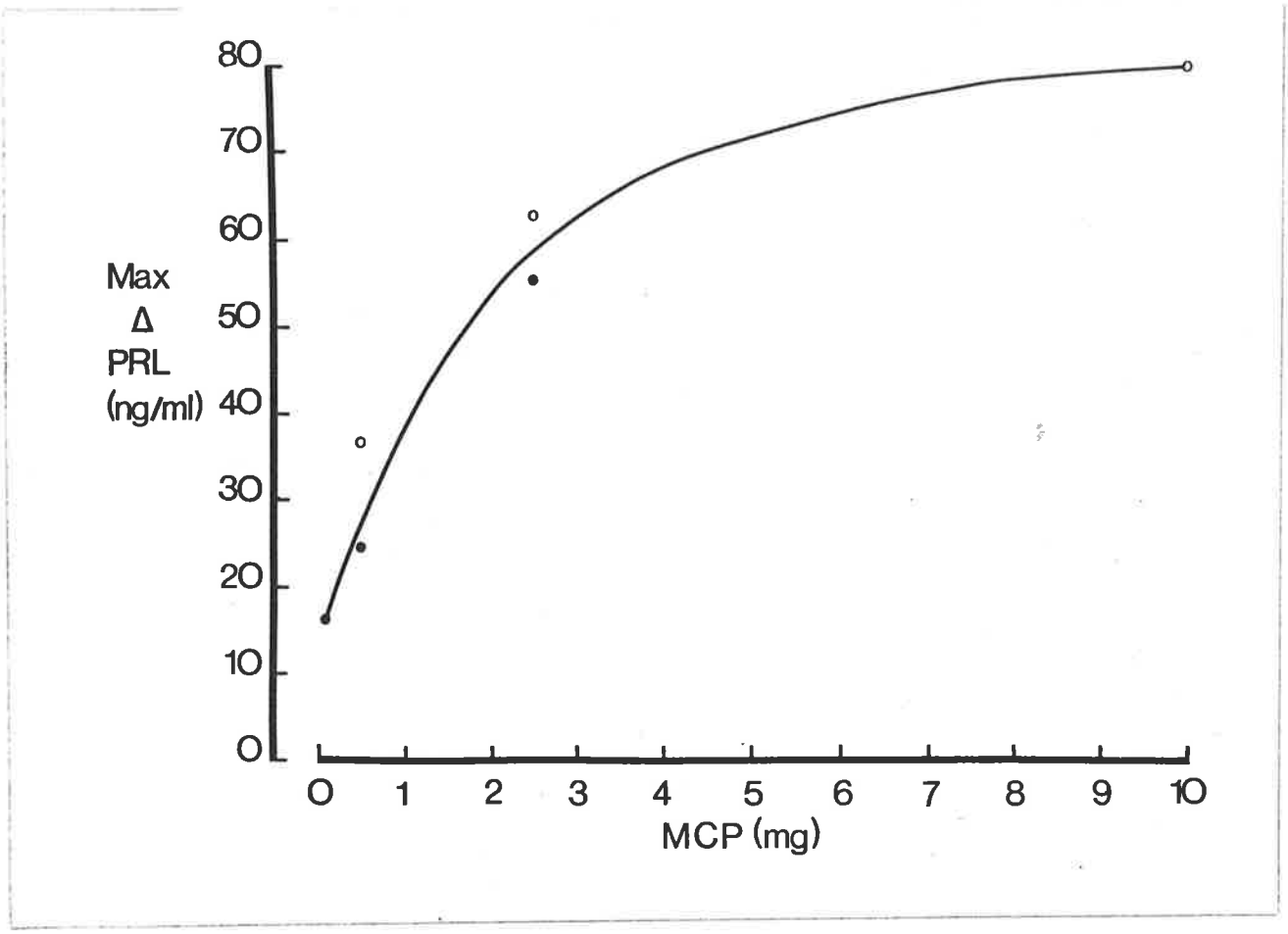
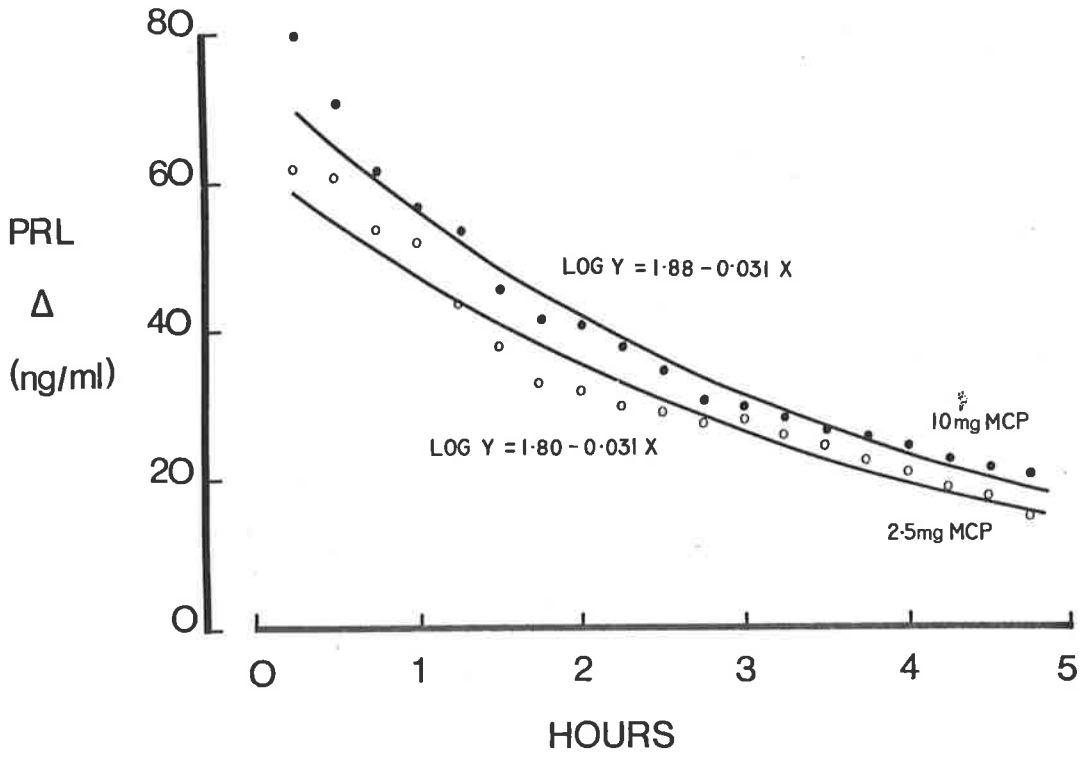


Figure 4.3

The slopes of decline in serum PRL, as analysed by logarithmic regression in a normal male (GM) after 10 mg and 2.5 mg of MCP.



GH and TSH were both less than the assay sensitivity ($<$1.2 ng/ml, $<$ 1.0 μU/ml respectively) and did not increase after MCP.

A significant increase in serum PRL occurred at each dose level of MCP (Figure 4.1) and a significant degree of correlation was found between the serum PRL response and the dose level of MCP, ($r = 0.865$, $P < 0.05$). By combining the results of these two subjects, a dose response curve was obtained; the response to 2.5 mg of MCP is on the upper part of the linear portion of this curve (Figure 4.2).

After maximum PRL stimulation, the rate of disappearance of PRL from the circulation is curvilinear (Figure 4.3). The disappearance curves are similar after administration of 10 mg and 2.5 mg of MCP with a half-life of 2.40 and 2.43 hours respectively.

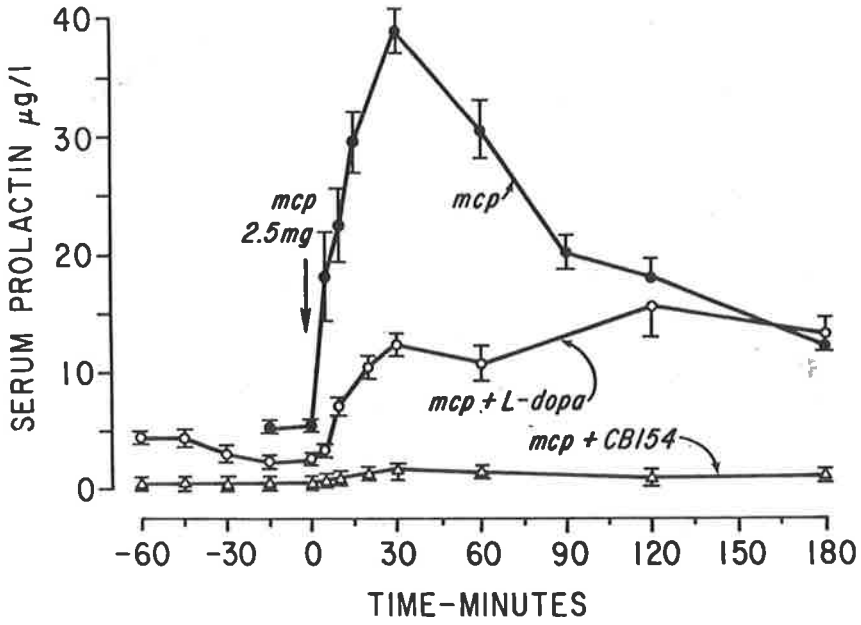
4.32 The Effect of Pretreatment with L Dopa and 2 ∝ Bromoergocryptine on the PRL Response to MCP.

In this investigation, the same six male subjects were studied on three separate occasions. Initially, 2.5 mg of MCP was given as a bolus intravenous injection and the PRL response was followed over a period of three hours. Subsequently, on two separate occasions, an oral dose of either 500 mg L Dopa (Laradopa Roche) or 2.5 mg of 2 brom ∝ ergocryptine (CB 154, Sandoz) were given 12 hours and one hour before a repeat study with MCP.

Basal levels of PRL (measured in the Garvan assay) were 5.2 $\pm$ 1.3 ng/ml in the control study before MCP. Basal serum levels of PRL were decreased compared to this level, 12 hours after bromoergocryptine, (1.7 $\pm$ 0.3 ng/ml), $P < 0.05$), but not after L Dopa

Figure 4.4

The effect of pretreatment with L Dopa (500 mg orally) and bromoergocryptine (CB 154, 2.5 mg orally) on the PRL response to MCP in six normal men.



(4.0 ± 1.0 ng/ml). However, during the first hour after administration of the second dose of L Dopa or bromoergocryptine, both groups showed a significant decrease in baseline levels, compared to the control group (Figure 4.4).

Following MCP, serum PRL increased to 38.2 ± 3.7 ng/ml in the control study; this level was significantly greater than the maximum response in either the L Dopa treated (16.3 ± 4.3 ng/ml, $P < 0.005$) or the bromocriptine treated groups (3.1 ± 0.6 ng/ml, $P < 0.00001$). Serum PRL remained suppressed throughout the duration of the study in the group pretreated with bromocriptine (Figure 4.4), but in the L Dopa treated group there was a gradual loss of antagonism to MCP, so that three hours after L Dopa serum PRL levels were not significantly different from the control group.

4.33 The Effect of MCP on the Suppression of LH and PRL by DA

Two normal women (JC and JT, Table 3.1) were studied on two occasions each. Firstly, each received a bolus injection of either 2.5 mg MCP (JC) or 10 mg MCP (JT), three hours after the commencement of a five hour infusion of DA. Subsequently, in a control study, each woman received a second dose of MCP without DA. Both women were studied in the late follicular phase of the cycle and basal serum hormone concentrations are shown in Table 4.2.

The period of special interest in these initial investigations is the two hour period immediately after the injection of MCP during which time the DA infusion is continuing. The mean LH, FSH and PRL levels in the eight samples collected during this time are compared to the mean level over an equivalent two hour period immediately before MCP and again after the DA infusion was discontinued (Table 4.3).

Table 4.2

Basal serum concentrations of LH, FSH and PRL (mean ± SEM of five samples each) and E_2, E_1 and Progesterone (P), in two women receiving MCP with or without DA.

	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)	E_2 (pg/ml)	E_1 (pg/ml)	P (ng/ml)
JC						
MCP + DA	24.4 ± 0.6	9.1 ± 0.2	15.9 ± 0.5	122	58	0.54
MCP (2.5 mg)	19.6 ± 0.7	6.5 ± 0.3	14.3 ± 0.2	181	92	0.36
JT						
MCP + DA	10.6 ± 0.3	10.8 ± 0.4	36.3 ± 0.6	126	85	0.49
MCP (10 mg)	9.5 ± 0.3	4.8 ± 0.3	26.3 ± 1.0	207	112	0.17

Table 4.3

Mean net change of LH, FSH and PRL in two hour periods, during a DA infusion, before and after MCP and on discontinuation of DA. (Mean ± SEM of nine samples per time interval).

(a) Indicates $P < 0.05$

(b) Indicates $P < 0.005$

(c) Indicates $P < 0.0005$

N.S. Indicates Not significant.

} Compared to level after MCP but during DA infusion.

Table 4.3

	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)		LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)
JC Before MCP During DA	- 8.5 ± 0.4 (b)	- 0.7 ± 0,2 NS	- 6,9 ± 0,6 NS	JT Before MCP During DA	- 0,9 ± 0.4 (a)	+ 0.3 ± 0.8 NS	- 18.4 ± 1.0 (c)
After MCP During DA	- 5.9 ± 0.6	- 0.5 ± 0.3	- 7.3 ± 0.7	After MCP During DA	+ 0.4 ± 0.3	+ 0.3 ± 0.4	+ 57.4 ± 12.3
After DA	- 0.9 ± 1.2 (b)	- 0.7 ± 0.2 NS	+ 78.3 ± 7.4 (c)	After DA	+ 1.6 ± 0.6 NS	+ 0.5 ± 0.5 NS	+ 76.3 + 12.0 NS

Figure 4.5

The effect of 2.5 mg of MCP on the LH, FSH and PRL responses to DA infusion (4 mg/kg/min.).

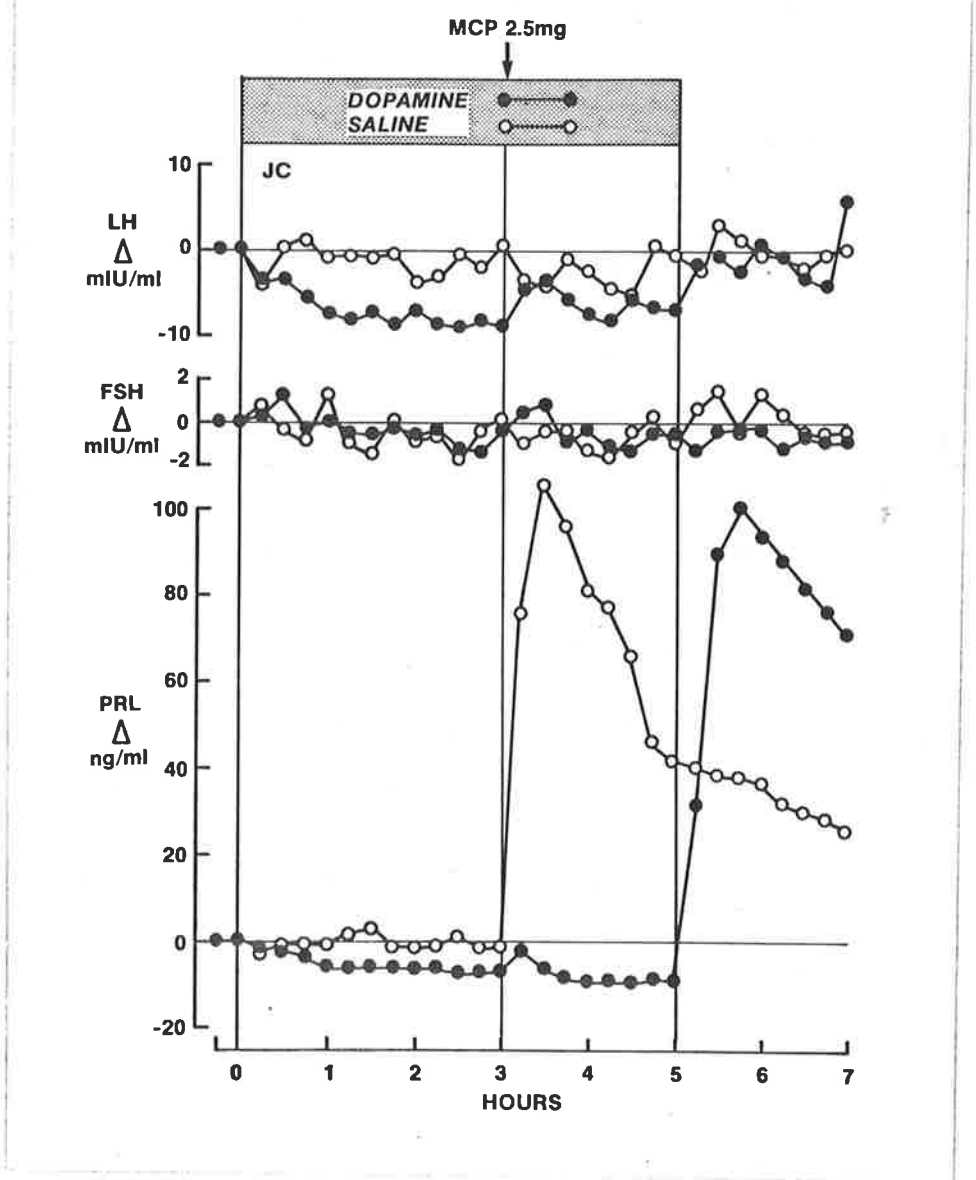
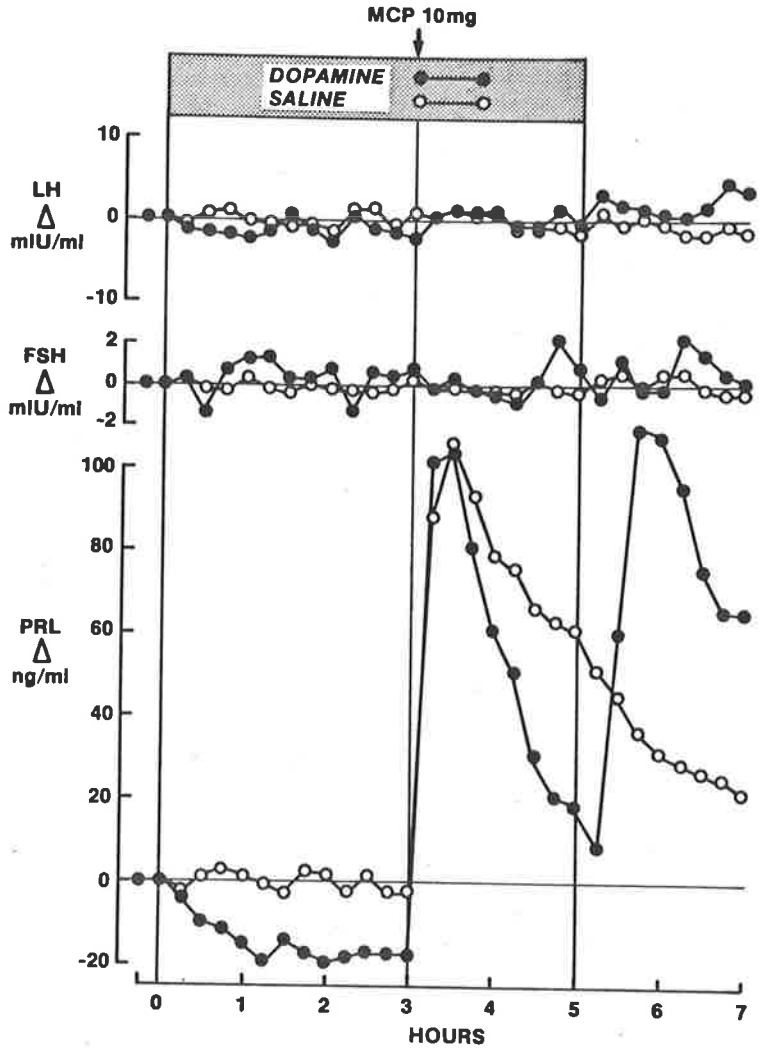


Figure 4.6

The effect of 10 mg MCP on the LH, FSH and PRL responses to DA (4 mg/kg/min) in normal subject, JT.



These results are analysed by Students"t" test.

During the first three hours of DA infusion, there was a decrease in both LH and PRL, but not FSH in the first subject (JC). When 2.5 mg of MCP was given after three hours of DA infusion, there was an insignificant increase in serum PRL, in contrast to the control study which shows a clear response to this dose of MCP (Figure 4.5). At the end of the DA infusion, there is a rapid increase in PRL to an equivalent peak response (100 ng/ml) as that seen in the control study (106.7 ng/ml). Despite the absence of effect of this dose of MCP on serum PRL during DA, there is a small but significant increase in the mean level of serum LH after MCP (Table 4.3). At the end of DA infusion, there is a further increase in the mean level of LH.

In subject JT, there is a marked decrease in serum PRL during DA, but an insignificant decrease in serum LH and FSH when compared to the saline control study (Figure 4.6). The reason for the absence of a significant LH response in this particular study is not known, since on previous occasions clear LH responses to DA had been obtained in this woman. Administration of 10 mg of MCP during DA produced a marked increase in serum PRL which was not significantly different from that seen when MCP was given without DA (Figure 4.5). However, there was a faster rate of decline in serum PRL from peak levels during the DA infusion ($t_{\frac{1}{2}}$ = 1.07 hours) compared to the saline control ($t_{\frac{1}{2}}$ = 2.87 hours). When DA was discontinued there was again an increase in serum PRL (peak response 110 ng/ml) which was similar in magnitude to the initial response to MCP (104 ng/ml). Although the change in serum LH after DA was not significantly different from the control study with MCP alone, there

was a small increase in the mean level of LH after MCP when compared to the immediate preceding two hour period (Table 4.3).

4.34 The Effect of Metoclopramide on Serum Levels of LH, FSH and PRL During the Early and Late Follicular Phases of the Menstrual Cycle.

A total of 28 studies were performed on ten normal women (Table 3.1) in either the early follicular phase (EFP) or the late follicular phase (LFP) of the cycle. 17 of these studies were performed during the EFP (Day 2-4) using either 2.5 mg of MCP (n = 12) or a smaller dose of 0.5 mg MCP (n = 5). The remaining 11 studies were performed in the LFP, six with the 2.5 mg dose and five with the 0.5 mg dose. In the LFP groups, serial daily blood sampling determined that four studies were performed within two days of peak LH secretion (three with the 2.5 mg dose, JC, JC and MD, and one with the 0.5 mg dose, JC; Table 4.4).

4.34.1 The Response to 2.5 mg of MCP

The mean basal serum levels of LH, FSH and PRL (13 samples for each hormone) before the administration of 2.5 mg of MCP are compared to the mean levels after injection (22 samples for each hormone) in Table 4.4. Following MCP, there is a rapid increase in serum PRL which reaches a peak level by 30 minutes, (Figure 4.7). By analysis of variance, the Δ PRL in the LFP over the five hour period after MCP is significantly greater than in the EFP group ($P < 0.005$). The rate of disappearance of PRL from the circulation after the maximum response follows a curvilinear slope in both groups of subjects and fits a logarithmic regression better than a linear

Table 4.4

Mean serum levels (± SEM) of LH, FSH and PRL before and after administration of 2.5 mg of MCP. The basal levels are calculated from the mean of 13 samples for each patient before MCP and the response after MCP from the mean of 20 samples for each patient. Serum E_2 and E_1 are also shown.

Twelve women were studied in the early follicular phase (EFP) and six in the late follicular phase (LFP).

The responses to MCP are compared by paired t test.

(a) Indicates $P < 0.00001$

(b) Indicates $P < 0.005$

N.S. Indicates Not Significant

} Compared to mean basal level of the corresponding hormone before MCP.

Table 4.4

Early Follicular	BASAL SERUM CONCENTRATION (± SEM)			MEAN LEVEL AFTER MCP (± SEM)			
	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)	$\frac{E_2}{E_1}$
1. SF	14.6 ± 0.2	8.7 ± 0.3	9.7 ± 0.6	14.4 ± 0.4	9.9 ± 0.3	81.1 ± 7.7	29/49
2. SF	9.1 ± 0.3	7.0 ± 0.3	9.4 ± 0.5	10.5 ± 0.9	6.6 ± 0.2	87.8 ± 9.2	39/15
3. JC	13.9 ± 0.4	8.1 ± 0.2	11.3 ± 0.3	13.7 ± 0.3	9.3 ± 0.2	65.5 ± 5.3	39/42
4. JT	13.1 ± 0.4	12.6 ± 0.8	27.5 ± 2.0	12.5 ± 0.3	10.5 ± 0.2	79.1 ± 10.4	35/45
5. JT	9.6 ± 0.3	6.8 ± 0.2	29.9 ± 0.5	11.8 ± 0.3	9.4 ± 0.9	57.2 ± 4.1	51/56
6. VL	10.9 ± 0.5	6.5 ± 0.2	25.6 ± 1.5	9.7 ± 0.2	6.6 ± 0.2	63.6 ± 3.8	45/17
7. VL	16.2 ± 0.5	9.0 ± 0.3	11.8 ± 0.6	13.9 ± 0.5	8.0 ± 0.2	67.2 ± 8.2	89/50
8. VL	13.4 ± 0.8	12.0 ± 0.3	13.0 ± 0.6	11.8 ± 0.5	10.8 ± 0.1	58.5 ± 6.4	86/53
9. PW	11.1 ± 0.5	7.4 ± 0.2	26.1 ± 0.5	11.5 ± 0.3	8.6 ± 0.2	73.6 ± 3.2	45/44
10. BH	7.5 ± 0.3	10.1 ± 0.3	22.7 ± 0.8	7.4 ± 0.3	9.7 ± 0.3	65.2 ± 3.3	43/35
11. BH	9.4 ± 0.4	8.9 ± 0.2	18.0 ± 0.8	12.4 ± 0.6	9.1 ± 0.2	77.2 ± 3.7	43/32
12. BN	10.7 ± 0.5	6.5 ± 0.4	6.4 ± 0.4	10.5 ± 0.2	6.0 ± 0.1	64.1 ± 3.3	53/53
Mean ± SEM	11.6 ± 0.8	8.6 ± 0.6	17.6 ± 2.4	11.7 ± 0.6 NS	8.7 ± 0.5 NS	70.0 ± 2.8 (a)	$\frac{49.8 \pm 5.4}{40.9 \pm 3.9}$

Table 4.4 (Contd.)

Late Follicular	BASAL SERUM CONCENTRATION (± SEM)			MEAN LEVEL AFTER MCP (± SEM)			
	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)	$\frac{E_2}{E_1}$
1. JC	28.3 ± 0.8	6.9 ± 0.3	25.7 ± 0.6	25.9 ± 0.7	7.2 ± 0.5	56.1 ± 4.4	181/92
2. JC	19.6 ± 0.7	6.3 ± 0.2	14.0 ± 0.4	18.6 ± 0.5	6.3 ± 0.2	68.0 ± 6.6	200/118
3. JT	9.5 ± 0.3	4.7 ± 0.2	25.2 ± 0.8	9.3 ± 0.4	4.5 ± 0.1	71.5 ± 6.5	224/112
4. JJ	12.4 ± 0.5	5.6 ± 0.1	9.7 ± 0.4	11.4 ± 0.3	5.6 ± 0.1	102.0 ± 11.3	310/86
5. BN	12.3 ± 0.4	11.4 ± 0.7	18.8 ± 0.6	12.3 ± 0.4	11.7 ± 0.4	80.4 ± 4.7	423/180
6. MD	20.1 ± 0.7	8.0 ± 0.2	12.2 ± 0.4	22.8 ± 0.9	7.6 ± 0.2	112.4 ± 7.3	190/124
Mean ± SEM	17.0 ± 2.8	7.2 ± 1.0	17.7 ± 2.8	16.7 ± 2.8 NS	7.2 ± 1.0 NS	81.7 ± 8.7 (b)	$\frac{255.0 \pm 39.0}{119.0 \pm 13.7}$

Figure 4.7

The effect of 2.5 mg of MCP on the net change (± SEM) in serum levels of LH, FSH and PRL in 12 normal women in the early follicular phase (EFP) and 6 women in the late follicular phase (LFP).

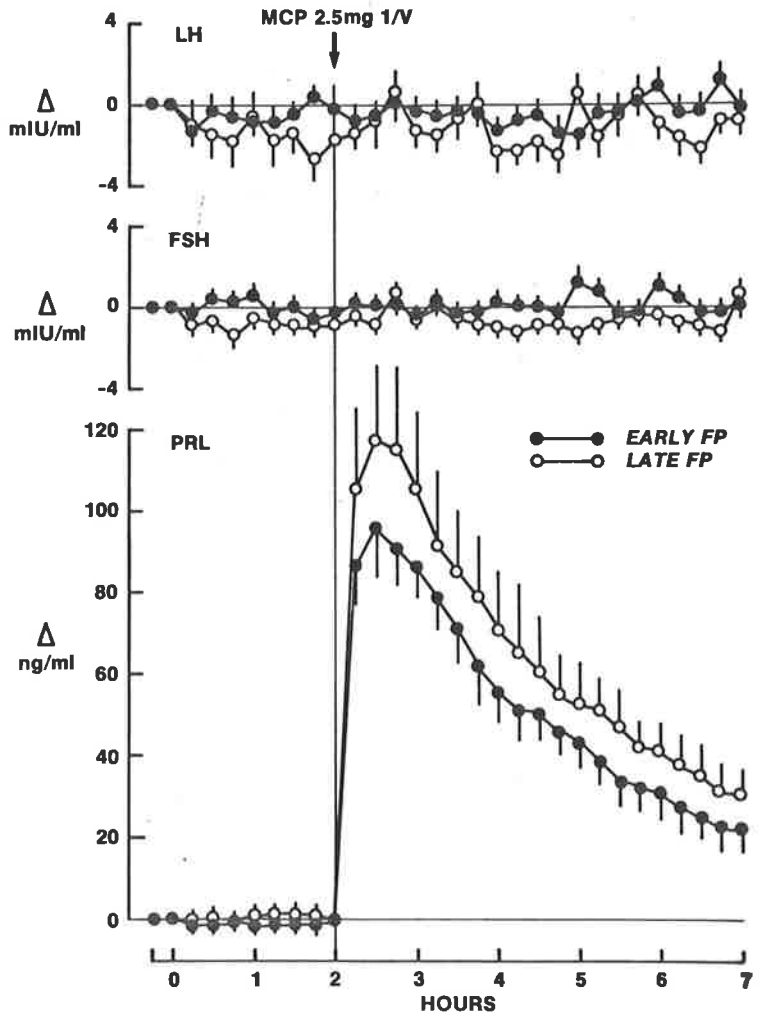
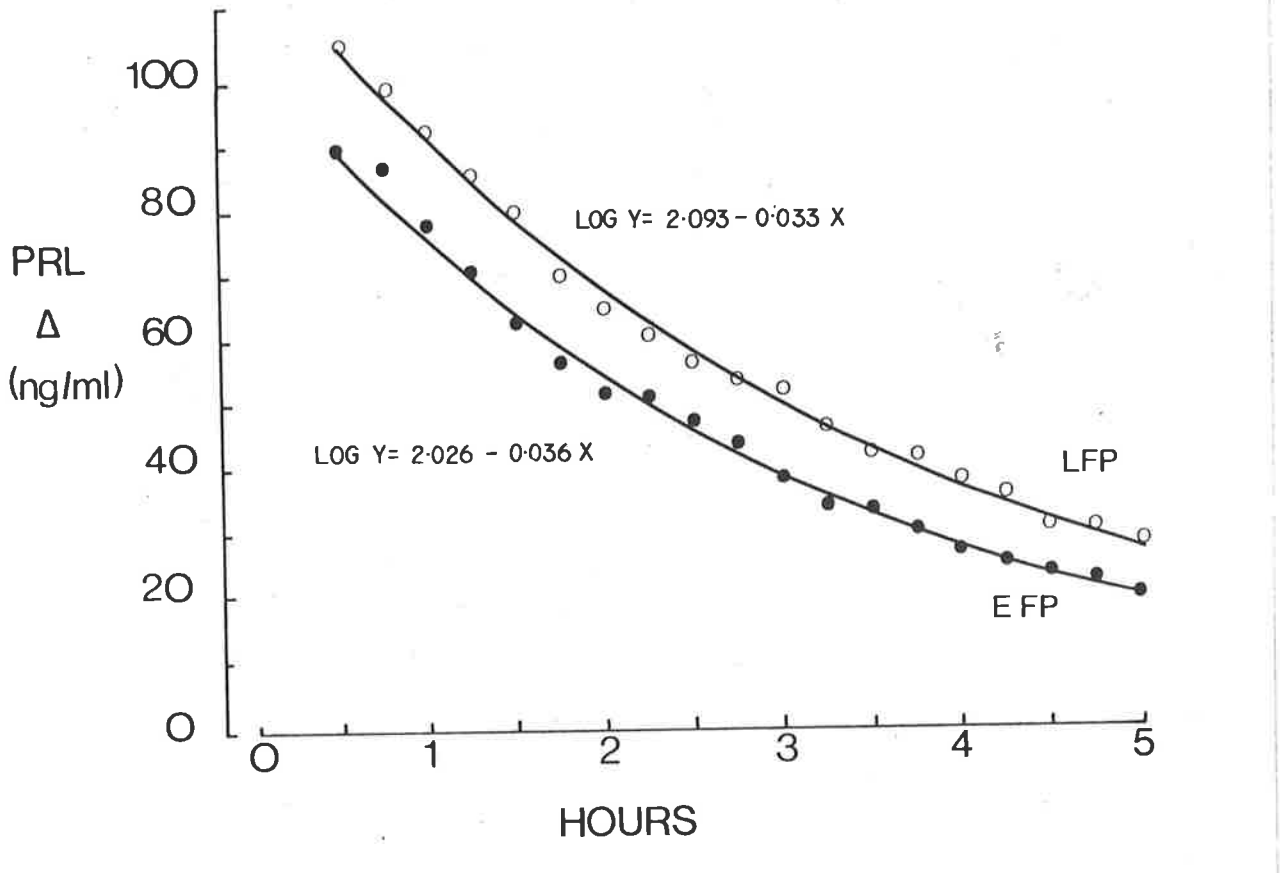


Figure 4.8

The slope of decline in serum PRL after administration of 2.5 mg of MCP, as analysed by logarithmic regression, in 12 normal early follicular phase women (EFP) and 6 women in the late follicular phase (LFP).



regression. The slopes of these curves are very similar (Figure 4.8) and they resemble the slope and half-life of disappearance of PRL after MCP in men (Figure 4.3).

There were a highly significant negative correlation between the individual baseline levels of PRL and the mean levels in the five hours after MCP ($r = -0.631$; $P < 0.01$). There was a positive, though insignificant, individual correlation between circulating oestradiol and the mean response to MCP ($r = 0.316$; $P > 0.05$).

The effect of MCP on serum LH and FSH was assessed by analysis of variance of the net change from baseline (Figure 4.8) as well as by paired t test of the mean levels in individual subjects before and after MCP (Table 4.4). No significant alteration was found in serum levels of either LH or FSH.

4.34.2 The Response to 0.5 mg of MCP

The LH, FSH and PRL responses to this dose of MCP paralleled the responses with the 2.5 mg dose. Serum PRL again increased within five minutes of injection of MCP and reached a peak level within 30 minutes (Figure 4.9). The maximum net response in both the EFP group (49.5 ± 7.6 ng/ml) and the LFP group (62.7 ± 9.5 ng/ml) is less than the corresponding responses with the 2.5 mg dose of MCP (95.9 ± 10.5 and 117.8 ± 19.5 ng/ml, respectively). These differences are both significant ($P < 0.05$).

No significant change was detected in the levels of serum LH or FSH after the administration of MCP. (Table 4.5).

Figure 4.9

The effect of 0.5 mg of MCP on the net change (± SEM) in serum levels of LH, FSH and PRL in five normal women in the early follicular phase (EFP), and five women in the late follicular phase (LFP).

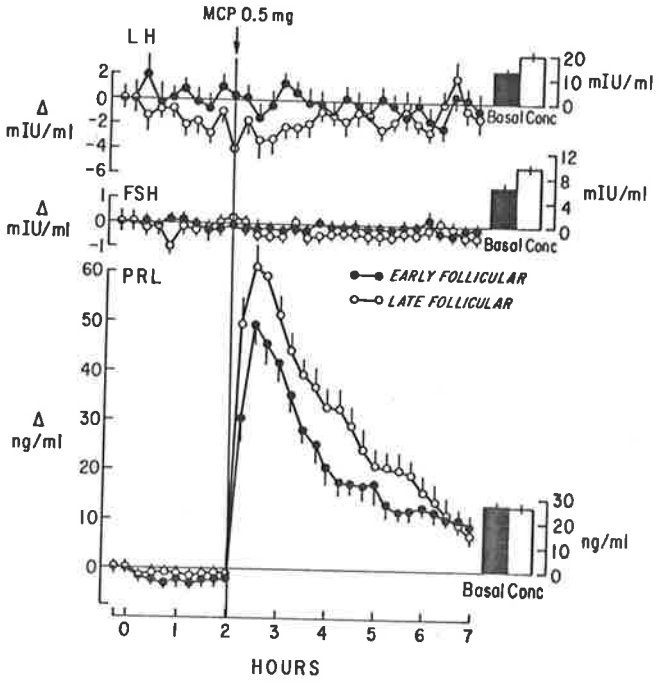


Table 4.5

Individual basal serum levels of LH, FSH, PRL (mean ± SEM, 13 samples each hormone) and E_2 and E_1, compared to the mean levels (± SEM, 20 samples each hormone) after administration of 0.5 mg MCP in a total of 10 studies: 5 in the early follicular phase (EFP) and 5 in the late follicular phase (LFP).

(a) Indicates $P < 0.01$ compared to basal serum PRL before MCP.

N.S. Indicates Not significant compared to corresponding basal hormone concentration.

Table 4.5

	BASAL SERUM CONCENTRATION (± SEM)			MEAN LEVEL AFTER MCP (± SEM)			
Early Follicular	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)	PRL (ng/ml)	$\frac{E_2}{E_1}$
1. SF	13.0 ± 0.4	6.8 ± 0.1	17.6 ± 0.4	14.6 ± 0.4	6.6 ± 0.1	50.0 ± 1.5	59/53
2. SF	6.1 ± 0.4	4.2 ± 0.1	20.4 ± 0.4	5.2 ± 0.3	4.2 ± 0.1	61.4 ± 2.3	38/29
3. JC	21.7 ± 0.7	10.7 ± 0.1	21.1 ± 0.4	20.2 ± 0.9	10.3 ± 1.0	44.4 ± 2.4	43/26
4. JT	4.6 ± 0.2	5.5 ± 0.2	38.8 ± 1.2	5.6 ± 0.2	5.1 ± 0.1	34.4 ± 2.0	32/46
5. KC	9.9 ± 0.8	4.5 ± 0.1	22.3 ± 3.2	6.9 ± 0.4	4.4 ± 0.1	59.8 ± 4.1	30/31
Mean ± SEM	11.1 ± 3.0	6.3 ± 1.2	24.0 ± 3.8	10.5 ± 3.0 NS	6.1 ± 1.1 NS	50.0 ± 5.0 (a)	$\frac{40.4 \pm 5.2}{37.0 \pm 5.3}$
Late Follicular							
1. JC	15.0 ± 0.4	4.4 ± 0.1	34.4 ± 0.6	17.4 ± 0.7	4.2 ± 0.1	63.0 ± 4.0	155/79
2. JC	34.8 ± 1.1	6.4 ± 0.2	31.8 ± 0.7	33.8 ± 1.6	4.8 ± 0.1	65.2 ± 4.7	205/94
3. VF	16.2 ± 0.5	9.4 ± 0.1	23.7 ± 1.6	15.6 ± 0.6	9.5 ± 0.3	45.4 ± 4.2	106/56
4. KC	17.4 ± 0.5	4.2 ± 0.1	18.6 ± 1.0	16.5 ± 0.4	4.2 ± 0.1	42.3 ± 3.4	96/44
5. KC	18.1 ± 1.2	4.9 ± 0.1	20.6 ± 0.7	16.4 ± 0.7	4.7 ± 0.1	67.3 ± 4.4	134/33
Mean ± SEM	20.3 ± 3.7	5.9 ± 1.0	25.8 ± 3.1	19.9 ± 3.5 NS	5.5 ± 1.0 NS	56.2 ± 5.5 (b)	$\frac{139.0 \pm 19.4}{61.2 \pm 11.2}$

4.40 Discussion

It is clear from these studies that metoclopramide (MCP) is a potent and rapid stimulus of PRL secretion. Administration of MCP is not associated with any major side effect; lassitude was noticed by a number of subjects but there were no problems with sedation, postural hypotension or extrapyramidal reactions.

The response to MCP extends over a wide dose range from 0.1 mg to 10 mg. Further dose levels will need to be tested to determine the true dose-response curve, but it appears that it may be linear up to a dose 2.5 mg (Figure 4.2).

4.40.1 The Mode of Action of MCP

Metoclopramide is widely used as an antiemetic and was first synthesised in 1953 as part of a systematic investigation of the diverse biological activities of the para-aminobenzoic acid derivatives (Justin-Besçanon et al., 1964). The pharmacodynamics of the drug have not been well investigated in the human, largely because assay methods are insensitive and lack specificity. It has been reported that intramuscular injection of 40 mg of MCP produces serum levels of between 20 - 200 ng/ml after 2 - 3 hours in man (Hucker et al., 1966). However, the assay system used in that study also measures the major metabolites of MCP. Also, consistent results have been obtained in the rat, dog and rabbit, using a more specific assay system; large intravenous injections (10 mg/kg) produce serum concentrations in all three species of around 8 µg/ml when extrapolated to zero time, and the rate of disappearance follows first order kinetics (Bakke and Segura, 1976). In their study, a high volume of distribution (in excess of 1 litre/kg) and a short half-life (20 - 30 minutes) was also described.

The major mode of metabolism of MCP is by N de-ethylation (Arita et al., 1970) and several of these metabolites are excreted in the urine with the glucuronide and sulphurate conjugates together with free MCP (Beckett and Huizing, 1975). Oral administration of MCP produces variable serum levels in different species; in the rabbit and the dog only 10% reaches the systemic circulation unchanged, the rest is apparently metabolised in its first passage through the liver (Bakke and Segura, 1976).

In the studies described in this chapter, variability of absorption and delivery of MCP to the systemic circulation were avoided by intravenous administration of the drug. Although the pharmacological half-life of the drug has not been determined in the human, the biological half-life, as determined indirectly by the rate of fall of serum prolactin from its peak level after 2.5 mg MCP, is the same in women and men (around 2.2 hours) and was unaltered at the two different stages of the menstrual cycle studied.

Metoclopramide appears to induce PRL secretion from the pituitary by virtue of its Dopamine receptor blocking ability.

The ability of MCP to block DA receptors concerned in the regulation of PRL secretion has been assessed by examining the interaction between MCP and DA agonists on serum PRL concentration. Both L Dopa and bromocriptine inhibit the PRL secreting effect of MCP (Figure 4.3). Oral administration of 2.5 mg of bromocriptine has a longer duration of action in suppressing PRL than 500 mg of L Dopa; this is consistent with the observed clinical efficacy of these drugs at these dose levels in controlling pathological hyperprolactinaemia.

Competition between MCP and DA has also been demonstrated. In the two women studied, 2.5 mg of MCP was found to be insufficient to antagonise PRL suppression by DA (Figure 4.5), although 10 mg of MCP appeared to completely block DA action (Figure 4.6). Competition was also suggested by the more rapid decline in MCP induced PRL elevation when the DA infusion continued (Figure 4.6).

Although this type of mutual competition is suggestive of an action at a common receptor, it is not conclusive. Hence, PRL elevation induced by TRH (a non-dopaminergic lactotrope stimulator) is also antagonised by DA (Besses et al., 1975). A more definitive study of the ability of MCP to block DA receptors would be the demonstration that MCP displaces labelled DA from a purified preparation of lactotrope receptors, in a dose-related manner. This has not yet been performed.

There are several other indications that MCP is a DA receptor antagonist. MCP has been found to cause a specific increase in DA turn-over and homovanillic acid formation, without a change in turn-over of noradrenaline or serotonin (Peringer at al., 1975). This biochemical effect is typical of other DA antagonists and is related to the feedback increase in neuronal firing rate induced by receptor blockade (Carlsson and Lindqvist, 1963; Bunney et al., 1973). Other properties of DA antagonists shared by MCP are the inhibition of apomorphine and amphetamine-induced stereotypic behaviour (Janssen et al., 1967) and circling behaviour induced by unilateral destruction of the nigro-striatal pathway (Dolphin et al., 1975). However, not all the actions of MCP are typical of a DA antagonist; for example, there is an absence of anti-psychotic activity (Nakra et al., 1975), an absence of antagonism

of the therapeutic action of L Dopa in Parkinson's disease (Tarsy et al., 1975) and the inability to block DA stimulation of cyclic AMP in the rat striatal homogenate (Peringer et al., 1976). In relation to this, it has been observed that bromocriptine does not stimulate cAMP accumulation from the rat striatal homogenate model (Kebabian and Calne, 1978. Personal communication) and it is possible that the nature of these dopamine receptors differs from those on the pituitary. Despite these atypical effects on the mesolimbic and nigro-striatal pathways, the most likely mode of action of MCP on PRL secretion is as an antagonist of endogenous DA activity at the level of the pituitary lactotrope.

4.40.2 Modulation of the Effect of MCP by Oestrogen

There is a significantly greater increase in serum PRL after both doses of MCP in the late follicular phase group, compared to the early follicular phase, which suggests that oestrogen may augment this effect of MCP. A similar augmentation of PRL response after oral perphenazine has been previously described in the late follicular phase (Buckman et al., 1976) or after exogenous administration of oestrogen (Buckman and Peake, 1973). This effect of oestrogen is also in keeping with the greater PRL response to MCP seen in women compared to men (Figure 4.3, compared to Figure 4.7), and may be best explained by postulating a direct effect of oestrogen on the pituitary gland to increase the pituitary stores of PRL. Several studies have, in fact, shown that oestrogen increases the mitotic activity and secretory ability of the pituitary lactotrope (Nicoll and Neites, 1962; Gersten and Baker, 1970; Davies et al., 1974; Haug and Gautvik, 1976) and this mechanism has been invoked

previously to explain the augmentation of PRL release by other stimuli (Rakoff et al., 1973; Carlson et al., 1973; Noel et al., 1974). This potentiation of stimulated prolactin release by oestrogen may occur in the absence of any change in basal serum level, as it was in this study.

4.40.3 The Effect of MCP on Serum Gonadotropins

It is apparent that MCP did not significantly alter serum levels of LH or FSH (Tables 4.4 and 4.5). It is unlikely that this is due to an inability of MCP to reach DA receptors in the brain, since it has been shown, in mice, that tritiated MCP passes easily into the brain (Ingrand and Boulu, 1970); intraperitoneal injection of MCP is associated with a prompt increase in DA turnover in the brain (Peringer et al., 1975), which also suggests that there is easy access of systemically injected MCP to brain receptors.

Other possibilities remain to explain the lack of effect of MCP on basal serum gonadotropins. Firstly, it is possible that specific DA receptors of the type that control PRL secretion may not control the release of LRF, the action of DA may be less specific, perhaps by influencing ion flux in nerve membranes. Another possibility is that DA receptors do control LRF secretion, but they are of a type more akin to those present in the striatal homogenate model and are not easily blocked by MCP. It may well be that the dose of MCP required to block putative receptors controlling LRF release is critical, since it is recognised that a compensatory increase in DA neuronal activity results from blockade of DA receptors (Carlsson et al., 1966) or an increase in serum PRL

(Olson et al., 1972; Hokfelt and Fuxe, 1972; Eikenburg et al., 1977). The two doses chosen for these studies may have been insufficient to block DA receptors controlling LRF release. The studies with DA infusions and MCP were both somewhat inconclusive and it will be necessary to further pursue this question using DA infusions in agonadal women to cause a substantial reduction in serum LH and FSH and then injecting MCP, while DA infusion continues, to see if this trend can be reversed.

Finally, it is conceivable that DA neurons do not exert a constant inhibitory influence on LRF release, but that the role of DA is rather to co-ordinate the release of LRF at critical times when this is required. Hence, a fall in DA in the pre-ovulatory phase may co-ordinate the LRF releasing action of other neurotransmitter substances at this time. These possibilities remain to be analysed.

CHAPTER 5

DISTURBANCES OF DOPAMINERGIC NEUROTRANSMISSION IN PATIENTS WITH HYPERPROLACTINAEMIA AND CHRONIC ANOVULATION

5.10 Introduction

If, as has been proposed previously, endogenous DA inhibits the release of LRF as well as PRL, then the possibility exists that some disorders of human ovulation result from a chronic and inappropriate elevation of dopaminergic activity in the median eminence.

Persistent hyperprolactinaemia is commonly associated with chronic anovulation in women and although this association is being recognised with increasing frequency (Franks et al., 1975), the nature of the disturbance in the hypothalamic-pituitary-ovarian axis is poorly understood. However, an attenuation of pulsatile release of gonadotropins (Boyar et al., 1974a; Bohnet et al., 1976) and a lack of positive feedback secretion of gonadotropins after exogenous oestrogen (Glass et al., 1975) are in keeping with a hypothalamic disturbance. Most importantly, it is clear that reduction of serum PRL levels to normal reverses chronic anovulation (Hardy, 1975; Thorner et al., 1974; Bohnet et al., 1976; Malarkey and Johnson, 1976; Del Pozo et al., 1974; Lloyd et al., 1975).

In the rat, it has been demonstrated that administration of exogenous PRL is associated with an increase in DA turnover in the tubero-infundibular neurons, including those of the lateral pallisade zone (Hökfelt and Fuxe, 1972a; Gudelsky et al., 1976), and it has been previously suggested that if a similar situation existed in the human, it might explain the disturbance in gonadotropin secretion in hyperprolactinaemia (Lachelin et al., 1977).

Several previous studies of hyperprolactinaemic women have shown that although serum PRL is consistently reduced by treatment with L Dopa or bromocriptine, serum LH is not affected (Malarkey et al., 1971; Friesen et al., 1972; Zarate et al., 1973; Tolis et al., 1973). However, a careful study by Lachelin et al. (1977) demonstrated a clear suppression of both LH and FSH by bromocriptine. The PRL response to chlorpromazine in patients with hyperprolactinaemia is attenuated (Zarate et al., 1973b; Malarkey and Johnson, 1976; Malarkey, 1975).

Several factors limit the value of these previous investigations in delineating possible abnormalities in the dopaminergic control of LH and PRL in hyperprolactinaemic women. L Dopa and chlorpromazine are both non-specific in their actions, non-uniform groups of hyperprolactinaemic patients have been investigated and finally, most of the previous investigations lacked appropriate control groups of normoprolactinaemic, fertile women.

In the studies described in this chapter, a relatively uniform group of women, each with a demonstrated pituitary microadenoma, was studied with both DA and metoclopramide. Control studies were performed on a comparable group of normal cycling women in the early follicular phase.

5.20 Subjects and Methods

Ten hyperprolactinaemic women, each of whom had a surgically proven or radiologically obvious microadenoma of the pituitary gland, were selected for these studies. None of these women had any underlying condition known to be associated with hyperprolactinaemia and in particular, all were euthyroid and none had received any

tranquillising or neuroleptic drugs. Symptoms of galactorrhoea were noticed by five women in the group whilst they were taking oral contraceptives, and three others developed persistent amenorrhoea after these were stopped (Table 5.1). In all the patients studied, loss of fertility was a major problem. The only two patients in the group who had previously been pregnant had developed amenorrhoea and galactorrhoea post partum (JG) or after surgical termination of the pregnancy (HK).

Each of the women was studied on two separate occasions, at least two days apart. On the first occasion they received a standard infusion of DA and on the second a bolus intravenous injection of 2.5 mg of MCP was given. Basal serum samples were collected for the measurement of 17 β Oestradiol (E_2), Oestrone (E_1), Androstenedione (A), Testosterone (T), Progesterone (P) and Dehydroepiandrosterone (DHEA). Serial blood samples were collected through an indwelling venous catheter, at 15 minute intervals, and were assayed for LH, FSH and PRL. The hormone responses to DA and MCP in these patients were compared to a control group of six women on Day 2 of their cycle, previously studied with DA (3.31) or a control group of 12 early follicular phase women, previously studied with 2.5 mg MCP (4.34.1).

The response to both DA and MCP was determined, as previously described, by calculating the net change in serum LH, FSH and PRL from their baseline levels. The mean net exchanges of these hormone levels in hyperprolactinaemic women were then statistically compared to those in normal women by analysis of variance.

Table 5.1

Clinical details of ten hyperprolactinaemic women who were studied with DA and MCP.

* % average body weight derived from tables of the Society of Actuaries; Build and Blood Pressure Study, Vol. I, p. 16, Chicago, 1969.

BCP = Birth Control Pills
A/G = Amenorrhoea and Galactorrhoea
LNMP = Last Normal Menstrual Period
TOP = Termination of Pregnancy

Table 5.1

Subject	Age	Menarche	Gravid/ Para/Ab	% Av. Wt.*	Menstrual History	Microadenoma	
						Tomogram	Surgery
MK	25	14	0/0/0	92	Galact. on BCP Post Pill Amen. 18/12	+	No surgery
CS	27	11	0/0/0	88	BCP 8 years LNMP 8 years	+	+
JG	22	13	1/1/0	126	Galact. on BCP Post-partum A/G 5 years	+	+
HK	27	12	1/0/1	96	Galact. on BCP A/G post TOP 8 years	+	+
VP	33	13	0/0/0	100	Galact. on BCP 4 years later A/G	+	No surgery
DT	33	15	0/0/0	108	Amenorrhoea 11 years	+	+
GP	30	14	0/0/0	103	Post Pill Amenorrhoea 6 1/2 years	+	+
CN	26	12	0/0/0	130	Amenorrhoea 5 years	+	+
SF	30	14	0/0/0	124	Post Pill Amenorrhoea 5 years later A/G	+	No surgery
HN	22	14	0/0/0	97	Galact. on BCP Amenorrhoea 1 year	+	+

5.30 Results.

The mean basal serum PRL concentration in the hyperprolactinaemic women was 130 ± 19 ng/ml, which was significantly greater ($P < 0.00001$) than either of the control groups (both 17.6 ng/ml; Table 5.2). Serum E_1 concentrations was also significantly greater in hyperprolactinaemic women ($P < 0.001$). Although the serum E_2 concentration was not statistically different in the hyperprolactinaemic and the Day 2 group studied with DA infusions, the early follicular phase normal women studied with MCP had a slightly greater basal level of serum E_2 (Table 5.2). Basal serum levels of LH, FSH, A, T, P and DHEA were not statistically different.

5.31 The LH, FSH and PRL Responses to DA

Infusion of DA is associated with a marked reduction in the serum levels of PRL in the hyperprolactinaemic group of women compared to the normal group, (Figure 5.1). The difference between the two groups is significant after 30 minutes of infusion ($P < 0.001$) and by four hours the mean decrement in the hyperprolactinaemic group is 83 ± 7.4 ng/ml compared with 11.5 ± 2.8 ng/ml for the Day 2 group ($P < 0.00001$). The maximum decrement in PRL is significantly correlated with the baseline level of PRL ($r = 0.685$, $P < 0.05$). On cessation of the DA infusion, there is a rapid increase in serum PRL, so that during the first hour after DA the net change in PRL, from the suppressed level, is 100.5 ± 10.9 ng/ml in the hyperprolactinaemic women and for the Day 2 group is 38.6 ± 8.6 ng/ml ($P < 0.001$).

Table 5.2

Basal serum levels of PRL, LH, FSH, 17 β Oestradiol (E_2), Oestrone (E_1), Androstenedione (A), Testosterone (T), Progesterone (P) and Dehydroepiandrosterone (DHEA) in ten hyperprolactinaemic women and the two groups of normal women in the early follicular phase, previously described, who provided controls for these studies.

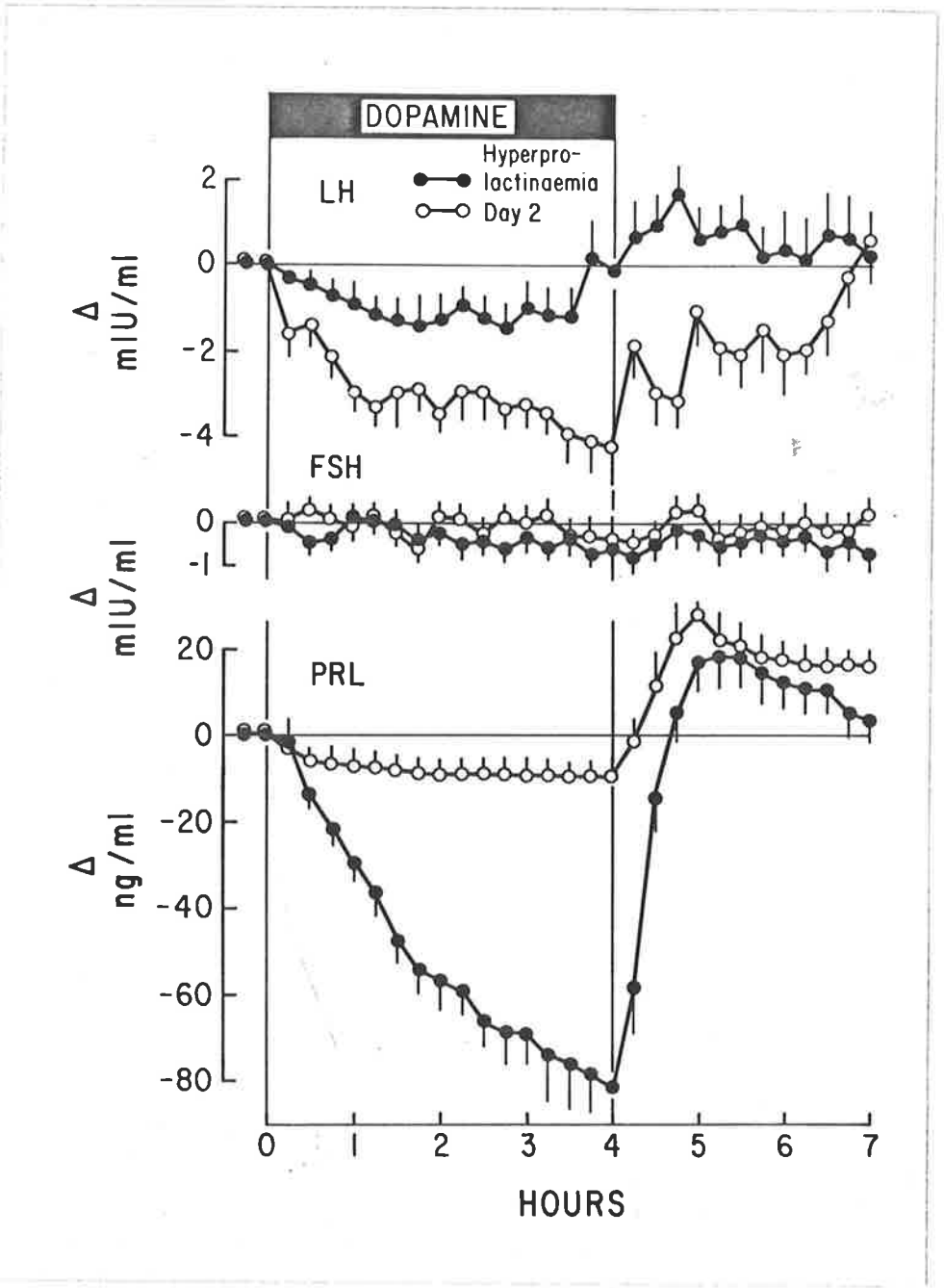
The 'Day 2 women' acted as controls for the DA studies; the 'Early Follicular phase women' acted as controls for the MCP studies.

Table 5.2

Subject	PRL ng/ml	LH mIU/ml	FSH mIU/ml	E_2 pg/ml	E_1 pg/ml	A pg/ml	T pg/ml	P ng/ml	DHEA ng/ml
MK	77.6 ± 1.0	10.4 ± 0.2	8.4 ± 0.1	37	55	1328	278	0.38	4.96
CS	82.2 ± 0.9	13.7 ± 0.3	7.1 ± 0.1	20	59	702	237	0.47	2.47
JG	86.0 ± 1.1	10.6 ± 0.4	8.4 ± 0.2	23	58	1010	211	0.43	4.31
HK	93.0 ± 1.9	4.6 ± 0.1	8.7 ± 0.2	17	50	958	353	0.67	3.18
VP	93.4 ± 3.1	10.4 ± 0.8	10.0 ± 0.4	18	35	814	309	0.28	3.47
DT	108.0 ± 0.7	19.9 ± 1.1	12.6 ± 0.2	19	59	1902	452	0.54	3.55
GP	142.0 ± 4.8	7.8 ± 0.3	7.0 ± 0.2	17	55	1089	336	0.43	4.94
CN	166.0 ± 3.6	6.6 ± 0.3	2.9 ± 0.1	21	42	955	299	0.36	2.67
SF	208.0 ± 5.3	6.1 ± 0.1	9.4 ± 0.2	23	35	1267	447	0.35	4.34
HN	247.0 ± 7.4	12.1 ± 0.6	9.2 ± 0.1	19	56	1214	482	0.61	3.86
MEAN Hyperprolactinaemic women	130.0 ± 19.0	10.2 ± 1.4	8.4 ± 0.8	21.4 ± 1.9	50.4 ± 3.0	1124 ± 106	340 ± 29	0.45 ± 0.04	3.78 ± 0.03
MEAN Normal Day 2 women	17.6 ± 2.7	11.7 ± 1.1	8.7 ± 0.5	25.2 ± 3.0	27.2 ± 4.9	1055 ± 78	267 ± 148	0.38 ± 0.2	3.90 ± 0.4
MEAN Early Follicular Phase women	17.6 ± 2.4	11.6 ± 0.8	8.6 ± 0.6	49.8 ± 5.4	40.9 ± 3.9				

Figure 5.1

The effect of DA infusion on serum levels of LH, FSH and PRL in ten hyperprolactinaemic women and six norman women on Day 2 of their menstrual cycle.



Conversely, the response of LH during the infusion of DA is significantly attenuated in hyperprolactinaemic women (Figure 5.1). The difference in the two groups becomes significant after the first hour of infusion ($P < 0.001$) and reaches a maximum decrement after two hours in the hyperprolactinaemic group (-0.9 ± 0.4 mIU/ml). However, in the Day 2 group, there is a continued decline until the end of the infusion (-4.5 ± 0.7 mIU/ml). There is a significant negative correlation between the maximum decrement in serum LH and the basal level ($r = 0.881$, $P < 0.001$). Serum concentrations of FSH are not significantly altered by the infusion of DA (Figure 5.1).

5.32 The LH, FSH and PRL Responses to MCP

There is no significant effect of MCP on serum levels of LH or FSH in either normal or hyperprolactinaemic women (Figure 5.2). However, in both groups there was a significant PRL response to MCP. In normal women, following injection of MCP, there is a rapid increase in serum PRL to a maximum increment of 108 ± 16.6 ng/ml, which is significantly greater than the increment in the hyperprolactinaemic group (10.3 ± 7.3 ng/ml, $P < 0.0001$). There is a significant negative correlation between the maximum net change in PRL after MCP and the basal level in the hyperprolactinaemic women ($r = 0.686$, $P < 0.05$). Hence the four women with the highest baseline level of PRL (142 - 247 ng/ml), had no significant response to MCP, while the other women, with baseline levels of PRL less than 110 ng/ml, had a significant increase in PRL (19.6 ± 4.0 ng/ml) (Figure 5.3).

Figure 5.2

The effect of 2.5 mg of MCP on serum levels of LH, FSH and PRL in ten hyperprolactinaemic women and twelve normal women in the early follicular phase.

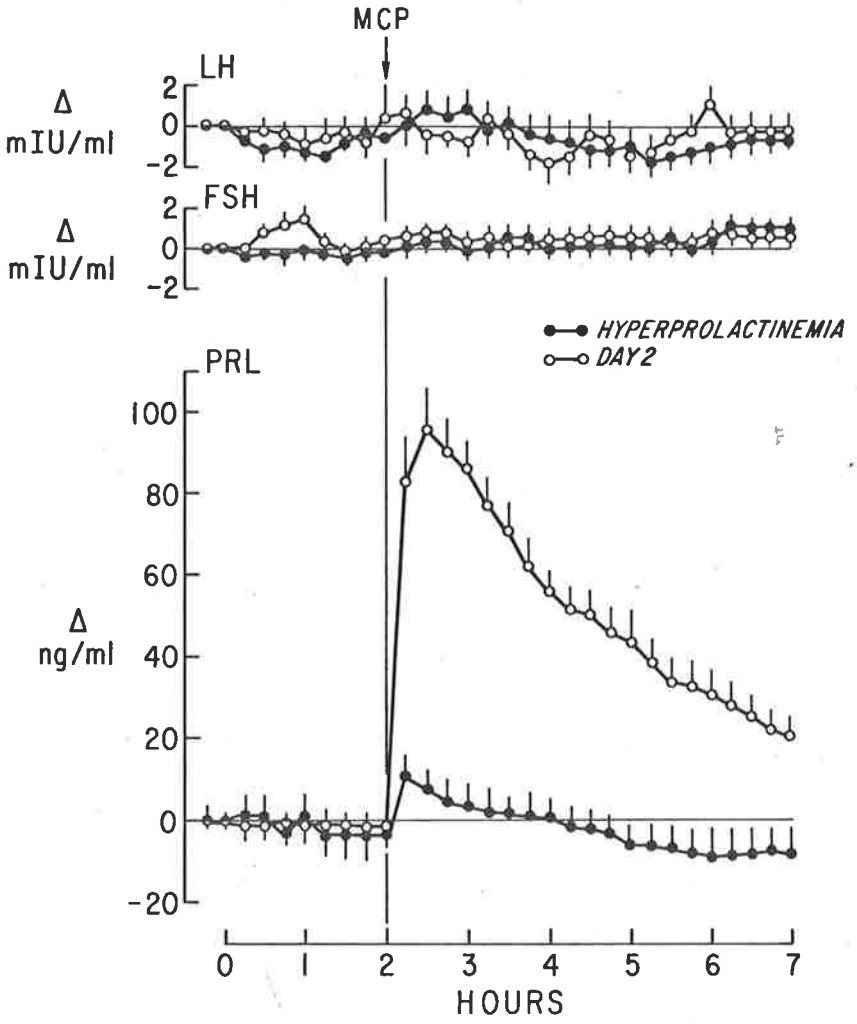
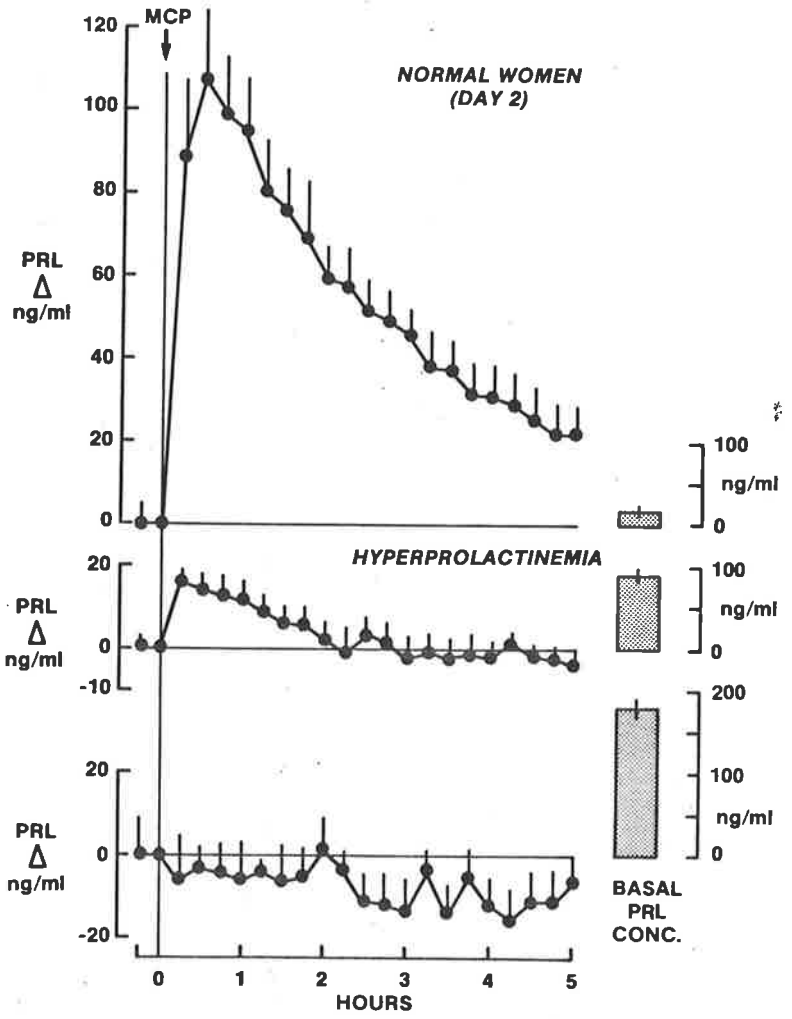


Figure 5.3

The relationship between basal PRL concentration and the PRL response to MCP in ten hyperprolactinaemic women and twelve normal women in the early follicular phase.



5.40 Discussion

The group of hyperprolactinaemic women studied all had a demonstrated microadenoma of the pituitary gland. Long term follow-up studies of patients with hyperprolactinaemia and galactorrhoea, but without obvious abnormalities of the sella turcica, have suggested that there may be a gradual progression to the stage of microadenoma formation and noticeable x-ray changes (Young et al., 1967; Lachelin et al., 1977). If this is the case, then these patients all represent a relatively late stage of the disease spectrum.

The control groups of patients were as previously described in previous chapters. They were comparable in age and mean body weight, and have basal hormone levels which are not significantly different apart from the elevation in PRL and E_1.

A relative excess of serum E_1 is a common finding in many patients with chronic anovulation, whatever the cause (Yen, 1978). In the absence of follicular development and ovarian secretion of E_2, there is a greater contribution to circulating oestrogen of E_1 which is largely derived from peripheral conversion of androstenedione secreted by the adrenal and the ovary. This contribution becomes particularly important in patients in whom there is an excess secretion of androstenedione combined with increased conversion in peripheral fat (De Vane et al., 1975).

In both normal and hyperprolactinaemic women, DA infusion is associated with a decrease in serum LH. The hyperprolactinaemic group however, shows a significant reduction in the LH response to

DA, indicating a possible resistance to the inhibitory effect of DA (Figure 5.1). If, as has been previously proposed (3.40.1), the LH response to DA is a reflection of the rate of endogenous LRF secretion, then it may be concluded that LRF is decreased in hyperprolactinaemic women. This is consistent with the previously reported impairment of episodic LH secretion (Boyar et al., 1974a), and, since endogenous LRF is an important factor determining the size of the acute releasable pool of LH (Hoff et al., 1977), it would also explain the reduced LH response to LRF in hyperprolactinaemic women (Lachelin et al., 1977). Although post-partum lactation may not be strictly analogous to pathological forms of hyperprolactinaemia, it is interesting that a decrease in LRF activity has been described in suckling rats (Minaguchi and Meites, 1967).

If there is indeed a reduced secretion of endogenous LRF in these hyperprolactinaemic women, then it might also be hypothesised that this may result from an excessive inhibition by DA neurons controlling LRF secretion. This suggestion has, in fact, been previously proposed on the basis of a demonstration of increased DA turn-over in the median eminence of hyperprolactinaemic rats (Hökfelt and Fuxe, 1972; Olson et al., 1972).

In a recent study, hyperprolactinaemic rats were found to be resistant to the LH lowering action of apomorphine (Beck and Wuttke, 1977). These findings were interpreted as being the result of desensitisation of DA receptors controlling LRF release presumably as a result of persistently increased stimulation by endogenous DA. If this was the case, then it would be expected that blockade of these putative receptors with metoclopramide would cause an increase in serum LH in hyperprolactinaemic women.

This is clearly not the case in the studies described above since MCP in doses which consistently blocked DA receptors controlling PRL release, did not affect serum levels of LH (Figure 5.2). Hence, while these studies support the view that endogenous LRF is decreased in hyperprolactinaemic women and are consistent with the hypothesis that endogenous DA activity is increased in hyperprolactinaemia, it is not possible to conclude that this decrease in endogenous LRF results from excessive stimulation of DA receptors.

In marked contrast to the effects of DA and LH secretion, the magnitude of the response of PRL to DA is significantly greater in the hyperprolactinaemic women than the normal control group (Figure 5.1). The importance of this observation is that it excludes the possibility that hyperprolactinaemia is a result of a loss of response of DA receptors of the lactotropes of the PRL secreting microadenoma. Since lactotrope receptors are able to respond to DA, it is concluded that persistent hyperprolactinaemia in these patients must result from a relative or absolute deficiency in dopaminergic activity affecting the lactotrope receptors. The reduced PRL response to MCP in these patients is certainly consistent with this proposed reduction in dopaminergic activity at the lactotrope receptors. Furthermore, if it is assumed that lactotrope receptors become supersensitive when dopaminergic activity is chronically reduced (Cheung and Weiner, 1976), then an accelerated PRL response to exogenous DA would be anticipated.

Acute administration of MCP is associated with an increase in serum PRL in both groups (Figure 5.2), but the magnitude of the response is inversely correlated to the basal level of PRL. This observation suggests that, with increasing serum levels of PRL, there is an associated decrease in DA stimulation of lactotrope receptors, and hence a decrease in the response to MCP. The possibility exists, but cannot be concluded from these studies, that a decrease in dopaminergic activity at the lactotrope receptor is the primary abnormality which gives rise to hyperprolactinaemia and that there is a gradation in the severity of this underlying abnormality, as serum levels of PRL increase.

If excessive PRL secretion is, in fact, normally controlled by a short loop feedback which produces increased DA activity in the hypothalamus, then it could be hypothesised that hyperprolactinaemia results when there is a break-down in this control system. If this abnormality existed in the efferent arm of the feedback loop, then it is conceivable that, while DA was unable to exert an effect on DA receptors controlling PRL release, excessive DA might be able to suppress LRF secretion and cause anovulation.

CHAPTER 6

IN VITRO STUDIES OF THE RESPONSE OF ISOLATED RAT PITUITARIES TO DOPAMINE AND METOCLOPRAMIDE

6.10 Introduction

The availability of synthetic hypothalamic releasing hormones for clinical use offered a means to directly stimulate the pituitary gland and, it was hoped, provide a means of discriminating between disorders of the hypothalamus and the pituitary gland. It has become apparent, however, that the hypothalamic-pituitary axis functions as a very complex and integrated unit, controlled by feedback signals not only from distant target organs, but also from within itself. This is certainly true for LH and FSH secretion, which is controlled by feedback signals (both positive and negative) from gonadal steroids operating on both the hypothalamus and the pituitary, combined with cyclical stimulation by LRF (Yen et al., 1971; Brown-Grant, 1977; Yen and Lein, 1976).

The problem of differentiating the site of action of DA and MCP has been mentioned several times during this dissertation. It has been pointed out, for example, that a reduced LH response to LRF during a DA infusion (Figure 3.8) need not necessarily imply that DA interferes with the action of LRF on the pituitary gonadotrope.

In this chapter are described a number of *in vitro* experiments using incubated, hemisected, rat pituitary glands in an attempt to better understand the potential of the pituitary to respond, in isolation from the hypothalamus, to MCP and DA.

6.20 Materials and Methods

6.21 Animals

Male rats of the Sprague Dawley strain were purchased from Holtzman Co., Madison, Wisconsin, and were housed in cages in temperature-controlled (75 ± 1°F) and artificially illuminated conditions (lights on from 0500 to 1900 hours daily). The rats were maintained on a standard diet of "purina rat chow," and were given tap water ad libitum.

6.22 Preparation of Hypothalamic Extract

Male donor rats weighing 300-350 gms were decapitated by guillotine and the brains removed within five minutes. The hypothalamic portion, including the median eminence and pituitary stalk, was removed by blunt dissection with fine pointed scissors. This portion was bounded rostrally by the optic chiasma, caudally by the mammillary bodies, laterally by the hypothalamic sulcus and dorsally by the cleft of the third ventricle. These hypothalami were placed in a centrifuge tube containing 2 - 4 mls of 0.1 N HCl kept in an ice bath. At the end of the collection, fragments were homogenised with a Brinkman Polytron cell homogeniser (Brinkman Instrument Inc., Westburg, New York) and were stored overnight at -20°C. The next morning the homogenate was thawed and then heated in a water bath at 100°C for two minutes. The extract was cooled and then centrifuged at 12,000 x G for 40 minutes at 4°C in a Sorvall RC - 2B automatic refrigerated centrifuge (Ivan Sorvall Inc., Norwalk, Connecticut). The supernatant was separated and the volume measured. The supernatant was incorporated into Krebs Ringer Buffer containing glucose and buffered to PH 7.4 with 1.0 N NaOH.

The volume of the buffer was adjusted so that the equivalent of ½ hypothalamus was added in 2 mls of incubation medium.

6.23 Preparations of Incubation Media

6.23.1 Krebs Ringer Buffer

This was prepared freshly for each experiment. Drugs and hypothalamic extract were added immediately before the start of each experiment and the PH was adjusted to 7.30 - 7.40. The following reagents were added to one litre of distilled water to prepare the Krebs Ringer Buffer (Dunn and Arditti, 1968).

NaCl	8.0 gms	Na_2HCO_3	1.0 gms
KCl	0.2 gms	$Mg\ Cl_2\ 6H_2O$	0.10 gms
$CaCl_2.2H_2O$	0.2 gms	Glucose	1.0 gms
NaH_2PO_4	0.05 gms		

Magnesium chloride was obtained from J.T. Baker Chemical Co., Phillipsburg, New Jersey. All the other reagents were obtained from Mallinckrodt Inc., St. Louis, Missouri.

6.23.2 Dopamine

Dopamine HCl (Inotropin) was obtained from Arnar-Stone Lab. Inc., Mt. Prospect, Illinois, in ampoules containing 40 mg/ml. 250 microlitres (µl) of this was added to 99.75 mls of Krebs Ringer Buffer (KRB) with glucose to give a starting concentration of 5.29×10^{-4} M. This was further diluted as necessary.

6.23.3 Metoclopramide

Metoclopramide (Beecham Lab., Moorabbin, Victoria) was obtained in ampoules containing 5 mg/ml. 600 µl of this was added to 99.4 mls of KRB and glucose to provide an initial concentration of 1×10^{-4} M and further dilutions were made from this solution.

6.23.4 1% Bovine Serum Albumin

Specimens of incubation media were collected in 1% Bovine Serum Albumin (BSA) prepared by adding 1 gm of BSA (Armour Pharmaceutical Co., Phoenix, Arizona), to 100 mls of distilled water.

6.24 In Vitro Incubation Technique

Mature male rats (300 - 350 gms body weight) were decapitated by guillotine. The pituitary gland was removed and the posterior lobe was separated. The anterior pituitary was placed in a petri dish containing filter paper moistened with KRB and was hemisected with a scalpel blade. The pituitary halves were placed in paired incubation tubes containing KRB and incubated for 30 minutes in a Dubnoff metabolic shaking incubator (Labline Inc., Chicago, Illinois), operating at 60 cycles per minute, under constant gassing with 95% O_2, 5% CO_2 at 37°C. At the end of this time, the pituitary halves were gently removed and replaced in paired tubes containing 2 mls of either control medium or experimental medium, previously warmed to 37°C and oxygenated in the incubator. At one hourly intervals for the next three hours, 100 µl of medium was removed and placed in tubes containing 900 µl of 1% BSA. 100 µl of fresh control or experimental media were then replaced into the appropriate incubation

tubes to restore the volume to 2 mls. Samples of incubation media were stored at -20°C until assayed for rat PRL or LH. All samples from the one experiment were measured in the same assay.

6.25 Rat Hormone Assays

6.25.1 Rat Prolactin

Rat PRL was measured by radioimmunoassay using the kit distributed by the NIAMDD, N.I.H., Bethesda. The NIAMDD - RAT PRL - RPI material is diluted from a starting dose of 5 ng for use in the standard curve. Unknown samples and standard solutions are added in a volume of 100 µl. 100 µl of NIAMDD anti-rat PRL antiserum #4, prepared in rabbits, is added in a dilution of 1 : 2500 in 1/200 normal rabbit serum. The mixture is incubated for 24 hours at 4°C. The next day, I^{125} rat PRL prepared from NIAMDD rat PRL-I-2 is added in a dilution of 20,000 cpm per tube and the mixture incubated for a further 24 hours at 4°C. Following this, 100 µl of 1 in 30 anti-rabbit gamma globulin (USCD #17) is added and the mixture incubated for 72 hours before centrifugation, separation of the supernatant and counting of the residue. A typical standard curve with range and sensitivity is shown in Figure 6.1.

6.25.2 Rat LH Assay

Rat LH is measured using materials provided by the rat hormone distribution programme of NIAMDD, N.I.H. The basic procedure is the same as that used to measure rat PRL described above. NIAMDD Rat LH - RPI is used in the standard curve and purified NIAMDD rat LH-I-4 is iodinated with I^{125}.

Figure 6.1

A typical standard curve for the rat PRL assay showing maximum binding, non-specific binding and sensitivity and range. Each point on the standard curve is measured in triplicate.

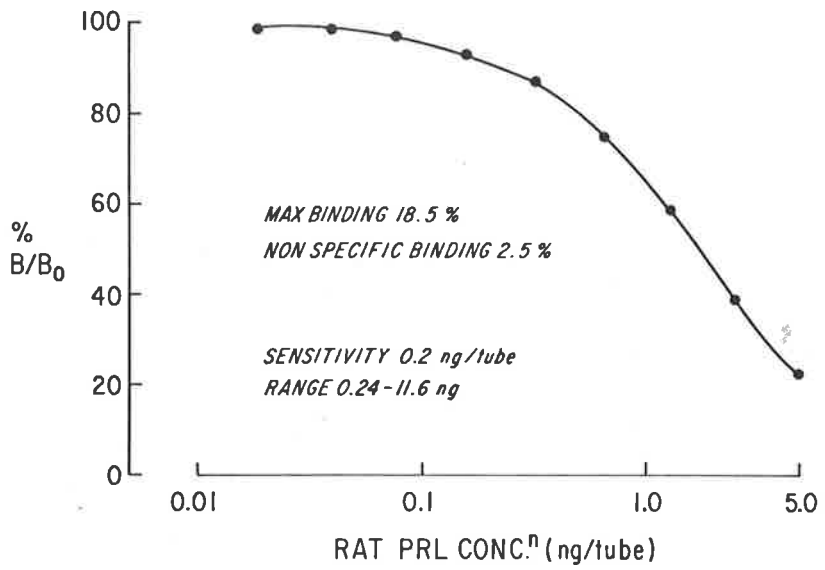
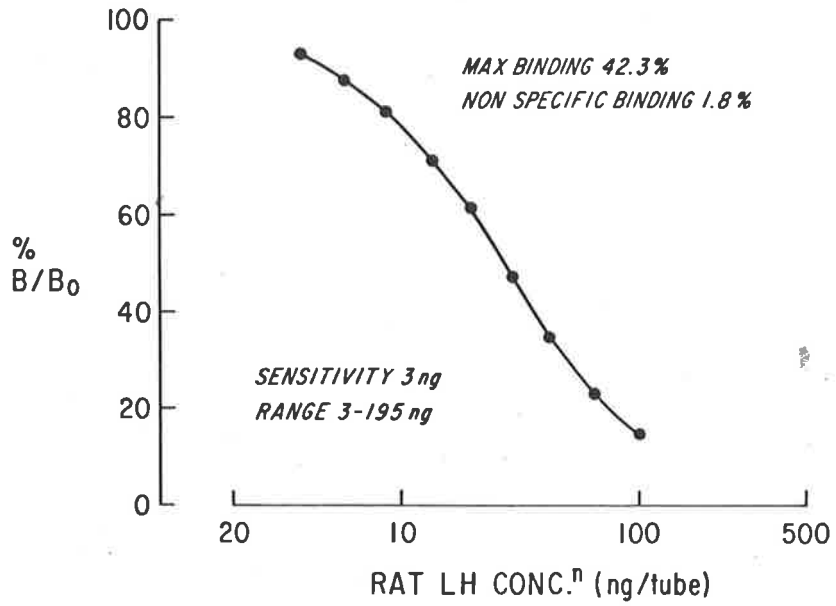


Figure 6.2

A typical standard curve for the rat LH assay showing maximum binding, non-specific binding and sensitivity and range of the assay. Each point on the standard curve is measured in triplicate.



NIAMDD anti-rat LH serum S-4, prepared in rabbits, is used in a final dilution of 1 in 5000. Incubation medium was assayed in aliquots of 50 - 100 µl. Details of the standard curve, range and sensitivity of the assay are shown in Figure 6.2.

6.30 Results

6.31 The Effect of DA on the Spontaneous Release of LH and PRL

Rat pituitaries were collected from three groups of four male rats and were hemisected and incubated in either Krebs Ringer Buffer (KRB) or KRB containing 5.3×10^{-8}M, 5.3×10^{-6} M, or 5.3×10^{-4} M DA over a period of three hours. Samples of media were collected at hourly intervals and assayed for LH and PRL.

There was a spontaneous release of LH from the paired hemipituitaries incubated in both media (Table 6.1); the mean increase in LH by the end of the third hour in the KRB group was 170%, whilst the increase in the three DA treated groups was 180%. The hourly secretion of LH from the hemipituitaries incubated in DA was not significantly different from the paired hemipituitaries incubated in KRB (Medium Alone), (Figure 6.3).

Similarly, there was a spontaneous secretion of PRL from the hemipituitaries incubated in KRB (Table 6.2). Incubation in DA at 5.3×10^{-6} M or 5.3×10^{-4} M was associated with a significant decrease, in the rate of secretion of PRL, although it was not affected by incubation in 5.3×10^{-8} M DA (Figure 6.3).

Table 6.1

EFFECT OF DA IN VARYING CONCENTRATIONS ON THE RELEASE OF LH FROM RAT HEMIPITUITARIES IN VITRO

		Mean ± SEM Rat LH (µg equivalents LH-RP-1/tube/hour)		
	No.	1 hour	2 hour	3 hour
Medium Alone	4	3.3 ± 0.6	6.7 ± 0.5	7.1 ± 1.0
Med. + DA 5 x 10^{-8} M	4	4.4 ± 0.5 (N.S.)	7.5 ± 1.4 (N.S.)	8.5 ± 1.5 (N.S.)
Medium Alone	4	5.2 ± 1.8	5.8 ± 1.7	7.4 ± 2.3
Med. + DA 5 x 10^{-6} M	4	3.8 ± 0.7 (N.S.)	4.4 ± 1.1 (N.S.)	5.6 ± 1.5 (N.S.)
Medium Alone	4	3.5 ± 0.8	5.6 ± 1.0	8.4 ± 1.6
Med. + DA 5 x 10^{-4} M	4	5.4 ± 1.2 (N.S.)	6.4 ± 0.5 (N.S.)	7.0 ± 1.2 (N.S.)

P > 0.05, compared with control.

N.S. indicates - Not significant.

TABLE 6.2

EFFECT OF DA IN VARYING CONCENTRATIONS ON THE RELEASE OF PRL FROM RAT HEMIPITUITARIES, IN VITRO

		Mean ± SEM Rat PRL μg equivalents PRL-RP-1/tube/hour		
	No.	1 hour	2 hour	3 hour
Medium Alone	4	1.9 ± 0.2	2.1 ± 0.2	2.2 ± 0.3
Med. + DA 5 x 10^{-8} M	4	1.5 ± 0.1 (N.S.)	2.0 ± 0.3 (N.S.)	2.0 ± 0.2 (N.S.)
Medium Alone	4	1.6 ± 0.1	2.3 ± 0.03	2.8 ± 0.1
Med. + DA 5 x 10^{-6} M	4	0.9 ± 0.04 (b)	1.3 ± 0.1 (b)	1.0 ± 0.02 (c)
Medium Alone	4	1.6 ± 0.2	2.4 ± 0.1	3.1 ± 0.4
Med. + DA 5 x 10^{-4} M	4	1.4 ± 0.1 (N.S.)	1.7 ± 0.1 (a)	1.4 ± 0.2 (b)

(a) $P < 0.05$ compared to control.

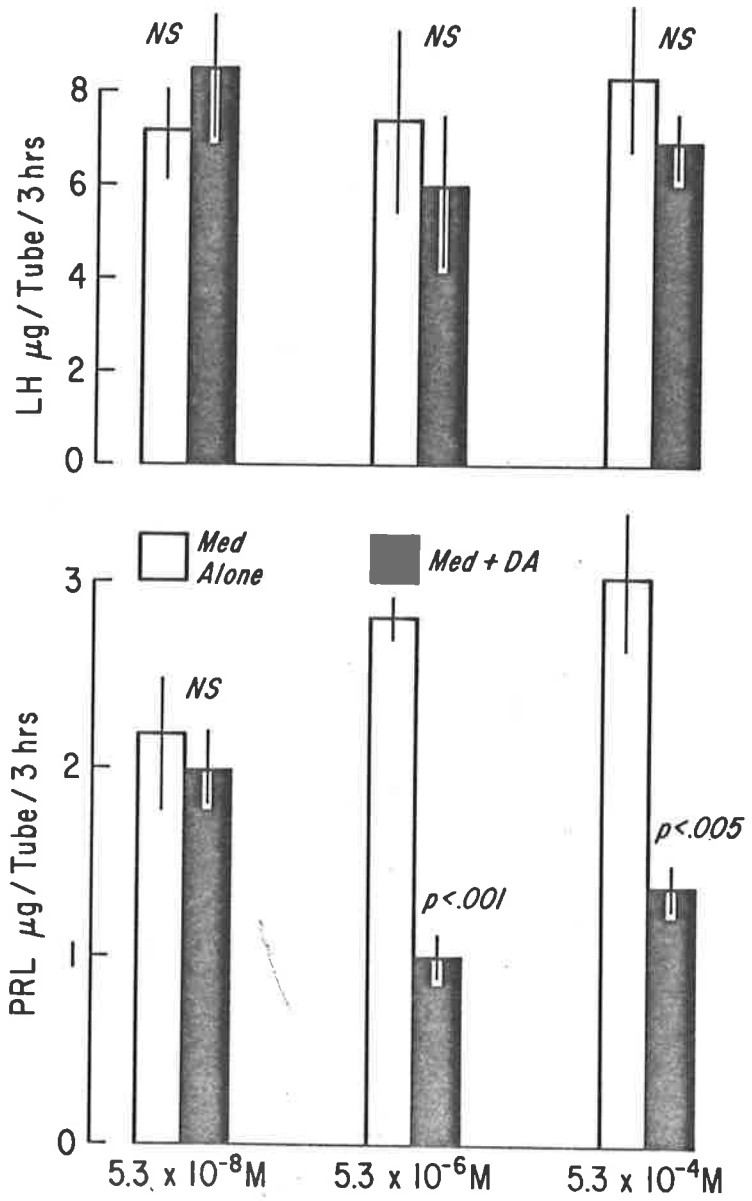
(b) $P < 0.005$ compared to control.

(c) $P < 0.001$ compared to control.

N.S. = Not significant.

Figure 6.3

The effect of varying concentrations of DA on the spontaneous secretion of LH and PRL over a period of three hours. The bars represent the mean amount of LH or PRL (± SEM) present in the incubation tubes at the end of three hours.



6.32 The Effect of DA on the Stimulated Secretion of LH

Pituitary glands were collected from three groups of either five or six male rats. The glands were hemisected and incubated for three hours in one of the following media:

Group A	KRB (Medium Alone)
Group A_1	KRB + 5×10^{-6} M DA
Group B	KRB + Hypothalamic Extract (HE)
Group B_1	KRB + HE + 5×10^{-6} M DA
Group C	KRB + 1×10^{-8} M LRF
Group C_1	KRB + LRF + 5×10^{-6} M DA

The hypothalamic extract was prepared as previously described and the equivalent of ½ hypothalamus was added to each tube. Synthetic LRF (# 14 - 136 - 06) was kindly provided by Drs. Jean Rivier and Roger Guillemin. In previous experiments, using pituitary cell cultures, 10^{-8} M of LRF produced an unequivocal but submaximal LH response (Drouin and Labrie, 1976; Vale et al., 1972).

The addition of hypothalamic extract (HE) caused an increase in the rate of secretion of LH which was significant ($P < 0.05$) by the end of the second hour of incubation, and by the end of the third hour, the LH concentration in the tubes incubated with HE was 150% of that in the control tubes containing only medium (Table 6.3). Addition of synthetic LRF caused a significant increase ($P < 0.0005$) in serum LH by the end of the first hour and by the end of the third hour, the increase was 316% compared to the tubes containing only medium (Table 6.3).

Table 6.3

EFFECT OF DA (5×10^{-6} M) ON LH SECRETION FROM RAT HEMIPITUITARIES INDUCED BY LRF OR HYPOTHALAMIC EXTRACT, IN VITRO

		Mean ± SEM Rat LH (µg equivalents LH-RP-1/tube/hour)		
	No.	1 hour	2 hour	3 hour
A. Medium Alone	5	2.6 ± 0.7	3.8 ± 0.7	4.4 ± 0.6
A_1. Med. + DA (5×10^{-6} M)	5	2.0 ± 0.7	4.1 ± 0.6	4.8 ± 0.7 (N.S.)
B. Medium + HE	6	4.7 ± 0.8	5.9 ± 0.4 (a)	6.6 ± 0.8
B_1. Med. + HE + DA (5×10^{-6} M)	6	4.6 ± 0.4	6.0 ± 0.6	6.0 ± 0.5 (N.S.)
C. Med. + LRF (1×10^{-8} M)	5	8.8 ± 0.8 (b)	11.4 ± 1.2	13.9 ± 0.8
C_1. Med. + LRF 1×10^{-8} + DA 5×10^{-6} M	5	7.7 ± 1.4	10.8 ± 1.3	12.0 ± 1.4 (N.S.)

N.S. = Not significant when compared to paired control.

(a) $P < 0.05$ compared to medium alone.

(b) $P < 0.0005$ compared to medium alone.

Figure 6.4

The effect of DA on the in vitro secretion of LH from the rat hemipituitary. The bars represent the absolute amount of LH (± SEM) present in the incubation tubes at the end of three hours.

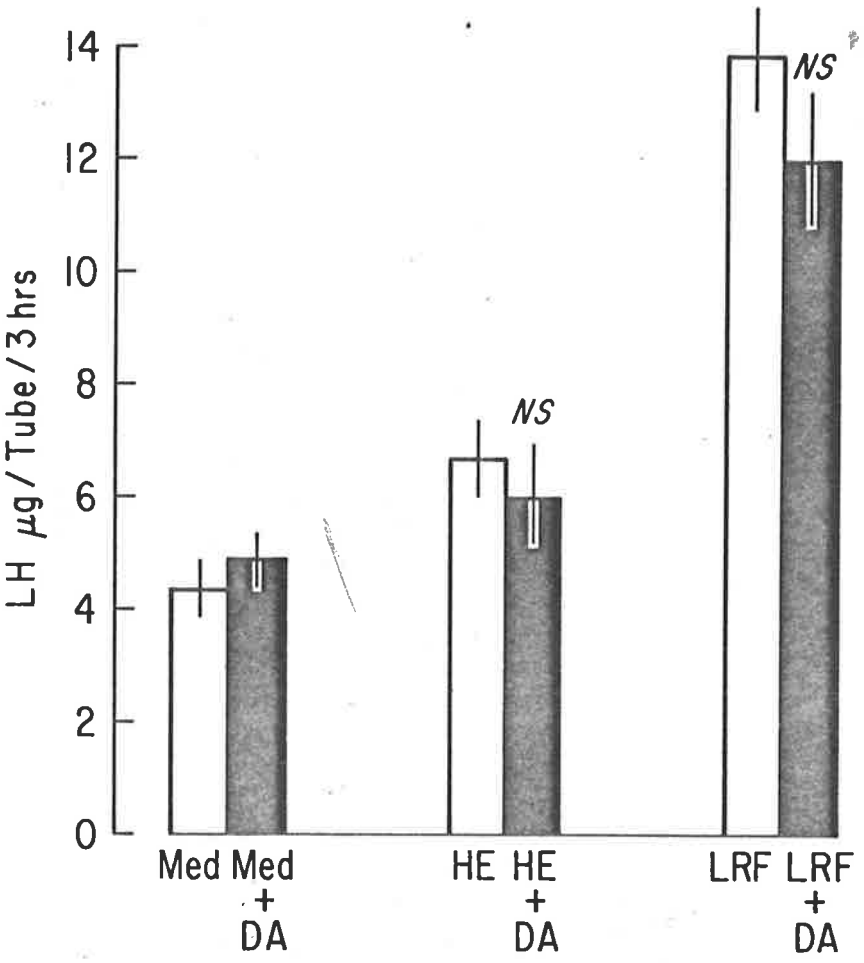
DA = Dopamine (5.3×10^{-6} M).

MED. = Krebs Ringer Bicarbonate Buffer

HE = Acidic Extract of the Hypothalamus

LRF = 1×10^{-8} M (10 ng/tube) synthetic Luteinising Hormone Releasing Hormone.

N.S. = Not Significant.



Co-incubation of hemipituitaries with DA (5.3×10^{-6} M) did not significantly alter the increase in secretory rate of LH stimulated by either HE or LRF (Figure 6.4).

6.33 The Effect of Metoclopramide on the Pituitary Gland

This experiment was designed to determine if MCP has a direct effect on the pituitary to stimulate the release of PRL and to test the interaction of MCP with DA.

Pituitary glands were collected from five groups of four male rats and were hemisected and incubated for three hours in the following media:

Group A	Krebs Ringer Buffer (Medium Alone)
Group A_1	KRB + 1×10^{-5} M MCP
Group B	KRB (Medium Alone)
Group B_1	KRB with 5.3×10^{-6} M DA
Group C	KRB with 5.3×10^{-6} M DA
Group C_1	KRB with 5.3×10^{-6} M DA and 1×10^{-6} M MCP
Group D	KRB with 5.3×10^{-6} M DA
Group D_1	KRB with 5.3×10^{-6} M DA and 1×10^{-5} M MCP
Group E	KRB with 5.3×10^{-6} M DA
Group E_1	KRB with 5.3×10^{-6} M DA and 1×10^{-4} M MCP.

The media were sampled at hourly intervals and assayed for PRL. There was a spontaneous release of PRL into the media over the three hours of the experiment. Addition of MCP alone did not alter the rate of secretion of PRL at any time during the three hours (Table 6.4). Conversely, the addition of DA (5.3×10^{-6} M) was associated with a marked decrease in the release of PRL so that after

Table 6.4

THE EFFECT OF MCP, DA AND COMBINATIONS OF MCP AND DA ON THE RELEASE OF PRL FROM RAT PITUITARIES, IN VITRO

		Mean ± SEM Rat PRL (μg equivalents PRL-RP-1/tube/three hours		
	No.	1 hour	2 hours	3 hours
A_1. Medium Alone	4	1.0 ± 0.1	2.0 ± 0.2	2.8 ± 0.3
A_2. Med. + MCP (1×10^{-5} M)	4	1.3 ± 0.1	2.0 ± 0.3	2.6 ± 0.2 (N.S.)
B_1. Medium Alone	4	1.6 ± 0.4	2.3 ± 0.3	2.8 ± 0.1
B_2. Med. + DA (5×10^{-6} M)	4	0.9 ± 0.04	1.3 ± 0.1	1.0 ± 0.3 (a)
C_1. Med. + DA (5×10^{-6} M)	4	1.1 ± 0.04	1.3 ± 0.1	1.2 ± 0.1
C_2. Med. + DA + (MCP 1×10^{-6} M)	4	0.9 ± 0.06 (N.S.)	2.0 ± 0.2	2.2 ± 0.2 (a)
D_1. Med. + DA (5×10^{-6} M)	4	1.1 ± 0.1	1.2 ± 0.05	1.1 ± 0.04
D_2. Med. + DA + (MCP 1×10^{-5} M)	4	1.8 ± 0.3 (N.S.)	2.1 ± 0.2 (a)	2.4 ± 0.2 (b)
E_1. Med. + DA (5×10^{-6} M)	4	1.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.02
E_2. Med. + DA + (MCP 1×10^{-4} M)	4	1.8 ± 0.2 (N.S.)	2.3 ± 0.2 (a)	2.6 ± 0.2 (c)

N.S. = Not significantly different from paired control.

(a) $P < 0.005$ compared with paired control.

(b) $P < 0.001$ compared with paired control.

(c) $P < 0.0005$ compared with paired control.

three hours the level of PRL in the control medium was 280% of that in the medium containing DA ($P < 0.001$).

When the pituitary halves were incubated in media containing both DA and MCP, the inhibitory action of DA was blocked. Hence, the secretion of PRL was significantly greater in media containing DA with MCP than in control media containing only DA (Table 6.4). Both MCP 1×10^{-5} M and 1×10^{-4} M completely blocked the action of DA so that there was no significant difference in the secretion of PRL after three hours when this is compared to the PRL release in media without any DA (Figure 6.5). However, the lowest concentration of MCP (1×10^{-6} M) did not completely block the inhibitory action of DA (5.3×10^{-6} M) since the level of PRL remains significantly lower at three hours than the level in media without any DA.

6.34 The Effect of MCP on the Inhibitory Action of Hypothalamic Extract on the Pituitary Release of PRL.

In this experiment, the nature of the prolactin inhibitory factor in the hypothalamus was investigated; pituitaries were collected from four groups of four male rats and were hemisected and incubated for three hours in the following media:

Group A	KRB (Medium Alone)
Group A_1	KRB with hypothalamic extract (HE)
Group B	KRB with HE
Group B_1	KRB with HE and 1×10^{-6} M MCP
Group C	KRB with HE
Group C_1	KRB with HE and 1×10^{-5} M MCP
Group D	KRB with HE
Group D_1	KRB with HE and 1×10^{-4} M MCP

Table 6.5

THE EFFECT OF HYPOTHALAMIC EXTRACT (HE) ± MCP ON THE SECRETION OF PRL FROM HEMIPITUITARIES, IN VITRO

		Mean ± SEM Rat PRL (µg equivalents PRL-RP-1/tube/hour)		
	No.	1 hour	2 hour	3 hour
A_1. Medium Alone	4	1.2 ± 0.2	1.5 ± 0.3	1.6 ± 0.3
A_2. Medium + HE	4	0.5 ± 0.2	0.5 ± 0.1	0.7 ± 0.2 (a)
B_1. Medium + HE	4	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
B_2. Med. + HE + (MCP 1 x 10^{-6} M)	4	0.3 ± 0.1 (N.S.)	0.5 ± 0.1 (N.S.)	0.8 ± 0.2 (N.S.)
C_1. Medium + HE	4	0.7 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
C_2. Med. + HE + (MCP 1 x 10^{-5} M)	4	0.5 ± 0.1 (N.S.)	0.7 ± 0.1 (N.S.)	0.8 ± 0.1 (N.S.)
D_1. Medium + HE	4	0.5 ± 0.04	0.6 ± 0.01	0.6 ± 0.03
D_2 Med. + HE + (MCP 1 x 10^{-4} M)	4	0.5 ± 0.1 (N.S.)	0.4 ± 0.1 (N.S.)	0.7 ± 0.1 (N.S.)

N.S. = Not significantly different from paired control.

(a) $P < 0.05$ compared to paired control.

Figure 6.5

The effect of Metoclopramide (MCP) in varying concentrations on the inhibition of PRL secretion by Hypothalamic Extract (HE) or Dopamine (DA). The bars represent the mean (± SEM) of the absolute amount of PRL present in the incubation tubes at the end of three hours.

HE = Hypothalamic Extract equivalent to ½ Hypothalamus per tube.

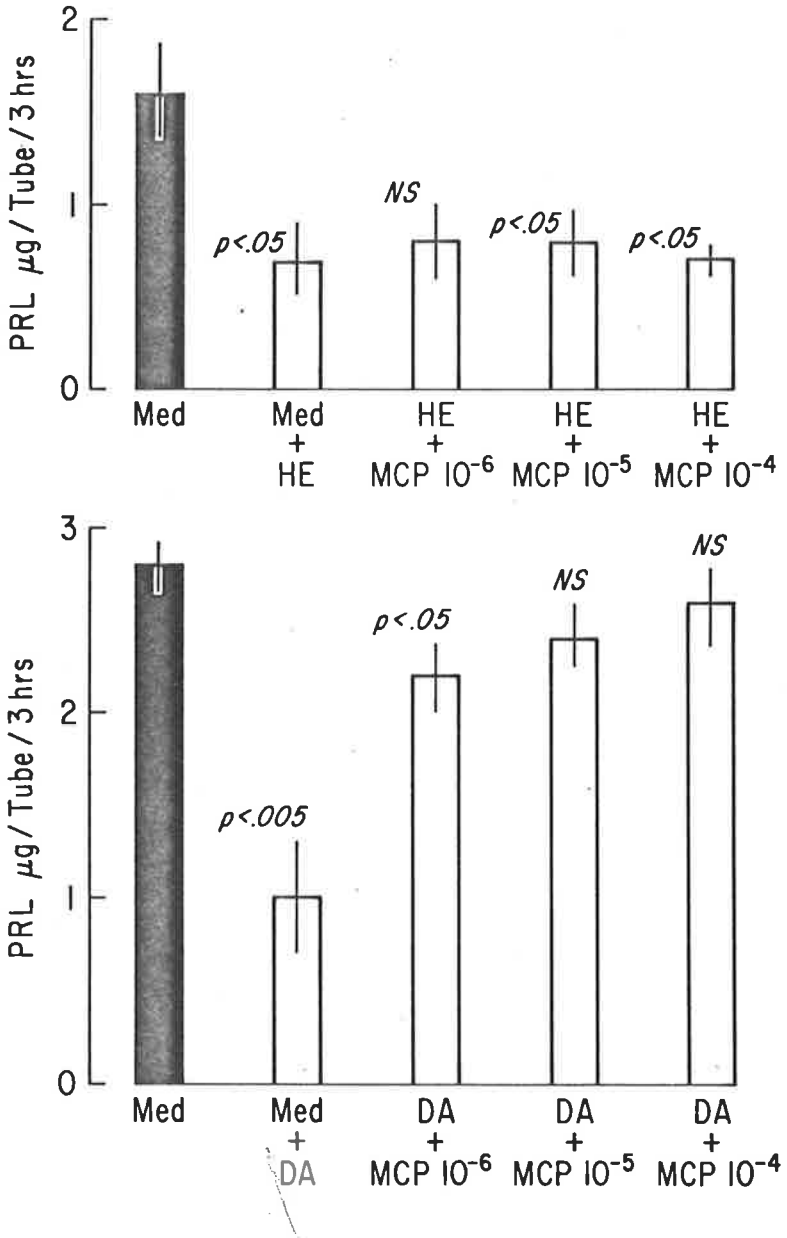
DA = Dopamine (5.3×10^{-6} M)

MED.= Krebs Ringer Buffer alone.

N.S. = Not Significant

For statistical purpose, the quantity of PRL after each treatment is compared to the quantity of PRL in incubation tubes containing Med. alone.

Figure 6.5


PRL µg / Tube / 3 hrs
2
1
0
p<.05
NS
p<.05
p<.05
Med
Med + HE
HE + MCP 10⁻⁶
HE + MCP 10⁻⁵
HE + MCP 10⁻⁴
PRL µg / Tube / 3 hrs
3
2
1
0
p<.005
p<.05
NS
NS
Med
Med + DA
DA + MCP 10⁻⁶
DA + MCP 10⁻⁵
DA + MCP 10⁻⁴

The acid extract of the hypothalamus was the same as was used in the second experiment to stimulate LH release and the equivalent of ½ hypothalamus was added to each incubation tube.

The secretion of PRL was significantly reduced by the addition of hypothalamic extract so that by the end of the third hour the level of PRL in the media containing HE was 66% of the control ($P < 0.05$) (Table 6.5). Although MCP in doses of 1×10^{-5} M and 1×10^{-4} M completely inhibited the effect of DA on PRL secretion, neither of these doses blocked the inhibition of PRL by HE (Figure 6.5). At three hours, there remained a significant difference between the amount of PRL released into medium alone and the amount of PRL released into medium containing HE and MCP 1×10^{-4} M ($P < 0.05$).

6.35 The Effect of Pre-incubation of the Pituitary in DA on the Subsequent Release of PRL

In this experiment, the mechanism of the rebound increase in PRL at the end of DA infusion was investigated by studying the release of PRL from the isolated pituitary for two hours after pre-incubation in either DA (5.3×10^{-6} M) or KRB.

The pituitaries of five male rats were collected, hemisected and incubated for three hours in either KRB or KRB with DA 5.3×10^{-6} M. At the end of three hours, the pituitary halves were removed from the incubation media, washed and returned to paired tubes containing KRB alone; the incubation was then continued for another two hours. Hourly samples of each medium were collected and assayed for PRL.

Table 6.6

THE EFFECT OF PRE-INCUBATION IN KREBS RINGER BUFFER (KRB) OR DA 5 x 10^{-6} M FOR THREE HOURS ON THE SUBSEQUENT RELEASE OF PRL FROM RAT HEMIPITUITARIES.

	Mean ± SEM Rat PRL (µg equivalents PRL-RP-1/tube/hour)				
	Pre-incubation			Re-incubation	
Media	1 hour	2 hour	3 hour	1 hour	2 hour
KRB into KRB	1.2 ± 0.2	1.8 ± 0.2	2.2 ± 0.3	0.4 ± 0.1	0.7 ± 0.2
DA (5 x 10^{-6} M) into KRB	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.2
Significance P	< .05	< .005	< .05	< .05	N.S.

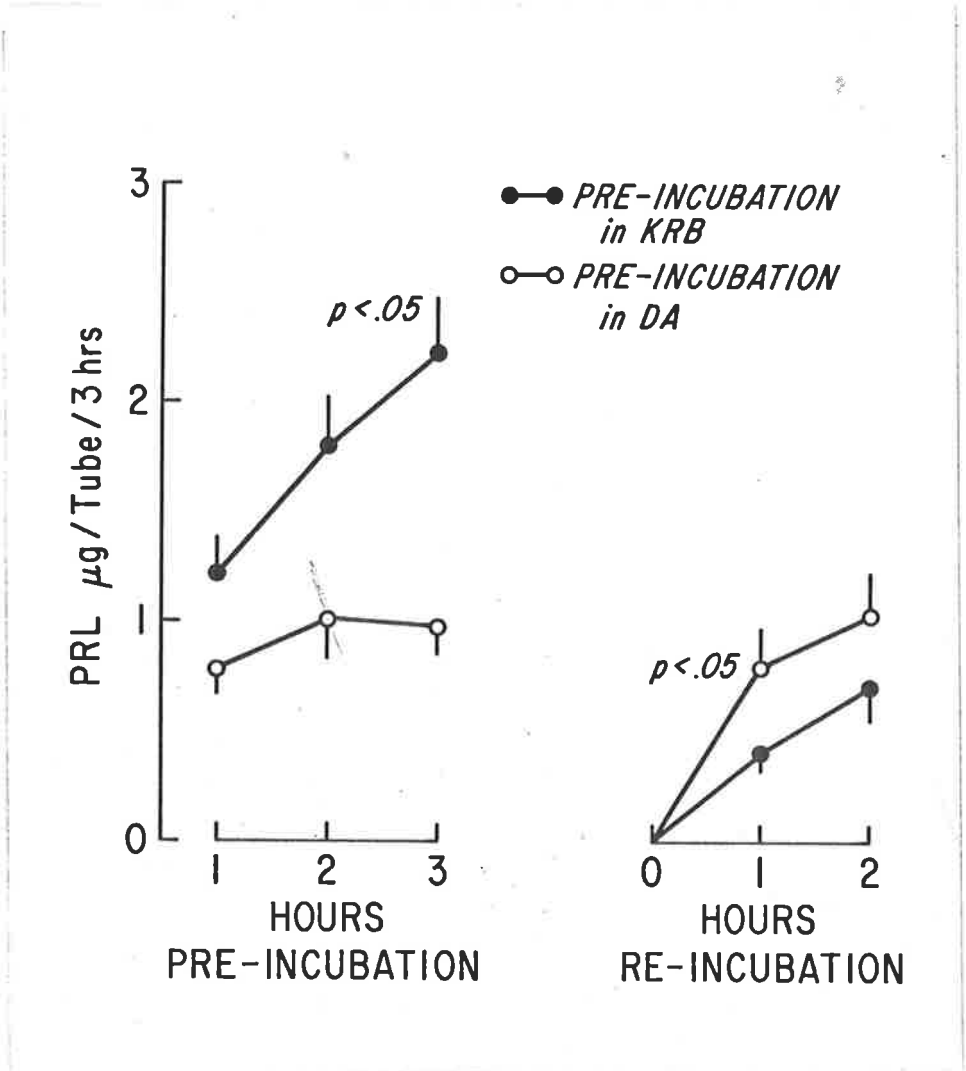
Figure 6.6

The effect of pre-incubation of rat hemipituitaries in Dopamine (DA 5 x 10^{-6} M) on the subsequent release of PRL after re-incubation.

The left panel shows the mean (± SEM) secretion of PRL in the incubation tubes (n = 5) each hour. The P value compares the two levels of PRL at three hours. Thr right panel shows the rate of secretion of PRL when the hemipituitaries are reincubated in Krebs Ringer Buffer.

O—O preincubated in DA

●—● preincubated in Krebs Ringer Buffer



During the period of pre-incubation in DA, there is a significant decrease in PRL release (Table 6.6), so that by three hours, the mean level of PRL in the medium with DA is 41% of the control. When the hemipituitaries are transferred to tubes containing KRB and are re-incubated, the hemipituitaries previously incubated in KRB containing DA have an initial increase in the rate of PRL secretion; by the end of the first hour the PRL level is twice that of the control group (Figure 6.6).

During the second hour of re-incubation, the secretion of PRL from the hemi-pituitaries initially incubated in DA remains greater than the control group incubated in medium alone, but the difference between the group is not significant (Figure 6.6).

6.40 Discussion

In the experiments described in this chapter, the effects of DA and MCP were assessed by measuring the secretion of LH and PRL from hemisected rat pituitary gland, incubated either in a test medium or an appropriate control medium. This technique is designed to overcome the problem of marked variation in secretion from individual whole pituitaries, even in rats of the same age and sex; the pattern and rate of secretion from these hemisected pituitary halves is reproducible (Appelbaum and Taleisnick, 1976).

Other methods used more refined techniques. The monolayer culture of enzymatically dispersed pituitary cells (Vale et al., 1972) has the advantage of allowing easier access of test substances to pituitary cells and has been shown to be more sensitive in detecting a response to LRF (Appelbaum and Taleisnick, 1976).

However, this technique requires enzyme treatment of the pituitary and *in vitro* culture for several days, and since these procedures may alter the number or reactivity of pituitary hormone receptors, it was not chosen.

Addition of DA in varying concentrations did not significantly affect the spontaneous release of LH from hemisected pituitaries (Figure 6.3) or the release of LH stimulated by crude hypothalamic extract or synthetic LRF (Figure 6.4); these findings confirm those of Schneider and McCann (1969). Although the *in vitro* model is undoubtedly a simplification of the *in vivo* situation, these findings are in keeping with the hypothesis, previously formulated (3.40.1) that DA infusion in women reduces serum LH by inhibiting LRF secretion rather than directly inhibiting the release of LH from the pituitary.

Although it is well known that DA is readily oxidised and inactivated to its orthoquinone derivative (MacLeod and Lehmeyer, 1972), the consistent lowering of serum PRL in the same samples of incubate demonstrate that DA added to the medium remained active. The inhibition of PRL by these concentrations of DA, *in vitro*, confirms previous reports (Birge et al., 1970; Koch et al., 1970; MacLeod and Lehmeyer, 1974). Although it is a theoretical possibility, the decrease in incubate PRL cannot be explained by an interference of DA with the radio-immunoassay of rat PRL, since it has previously been found that addition of DA over the range 10^{-9} M to 10^{-4} M to known amounts of PRL does not alter their measurement in this assay (Lu, 1972).

A large number of behavioural and pharmacological studies have led to the conclusion that metoclopramide (MCP) is an antagonist of DA reception (3.40). It was conceivable, however, that MCP also had a direct action on the pituitary, like TRH, to stimulate PRL release. This was found not to be the case when MCP was incubated *in vitro* with hemisected pituitary glands; the rate of PRL release was found to be not significantly different from the amount released after incubation with Krebs Ringer Buffer alone. Similar results have been found using the same technique of hemipituitary incubation with MCP (MacLeod, R.M., personal communication) or perfusion of dispersed pituitary cells suspended on micro-columns (Besser, G.M., personal communication).

Despite this, it has been reported that sulpiride, a substituted benzamide with a similar structure to metoclopramide, increases the secretion of PRL from pituitary incubates (Debeljuk et al., 1974). In this study, a paradoxical increase in secretion of PRL was found as the dose of sulpiride was decreased. A more recent careful study has been unable to confirm these findings and found, to the contrary, that sulpiride had no direct effect of its own on PRL secretion, but that it blocked the inhibitory action of DA (MacLeod and Robyn, 1977); this situation seems also to apply to metoclopramide.

Although metoclopramide does not increase the rate of PRL secretion *in vitro*, it is capable of blocking, in a dose dependent way, the inhibitory effect of DA (Figure 6.5). The likely mechanism for this is by a mutual antagonism at a common DA receptor. Although this has been well demonstrated for a variety of neuroleptic

drugs (Seeman, 1977), a definite study of the same kind has not been done for metoclopramide.

Crude acid extract of the rat hypothalamus causes a significant reduction in the release of PRL into the incubation medium (Table 6.5) as well as stimulating LH release (Table 6.3). The nature of the factor causing this inhibition is not known; however, it appears to be distinct from DA. Hence, MCP given in a dose which completely blocked the action of pharmacological levels of DA did not affect the action of hypothalamic extract on the pituitary gland (Figure 6.3). Similar results have been reported (Greibrokk et al., 1975; Enjalbert et al., 1977; Dupont et al., 1977), using more highly purified fractions from the hypothalamus. Schally's group later suggested that this non-dopamine suppressible inhibiting factor might be gamma-aminobutyric acid (Schally et al., 1977). These studies serve to emphasise that DA may not be the only prolactin inhibitory factor present in the median eminence.

The phenomenon of a rebound increase in serum PRL after discontinuation of the DA infusion has been previously described (3.40.2). The in vitro studies have demonstrated that this is a phenomenon which occurs in the isolated pituitary gland (Figure 6.6) and suggest that the rebound phenomenon is due to a release of stores of PRL accumulated during exposure to DA. This conclusion is in keeping with the demonstration of an acute inhibition of the release of newly synthesised 4, 5 ^{3}H Leucine labelled PRL from the pituitary gland (MacLeod and Robyn, 1977), and from the rebound phenomenon shown by DA perfused isolated pituitary cells (Besser, G.M., personal communication).

These *in vitro* studies are not considered by themselves to provide definitive answers to the site and mode of action of DA and MCP. However, the studies have provided support for some of the conclusions that have been discussed in relation to the *in vivo* human studies.

CHAPTER 7

THE ROLE OF DA NEURONS IN THE CONTROL OF LRF RELEASE :

FORMULATION AND SPECULATION

7.10 Introduction

It is not the intention of this dissertation to deny the role of neurons containing other neurotransmitter substances, apart from DA, in the regulation of LRF secretion. The evidence that noradrenaline neurons stimulate LRF secretion has been discussed (1.50.5) and there is good evidence to implicate neurons containing acetylcholine (Everett, 1964; Simonovic et al., 1974; Libertun and McCann, 1973; Libertun and McCann, 1974; Gnodde and Schuiling, 1976), pineal indoleamines (Kamberi et al., 1971; Kamberi, 1975; Domanski et al., 1975; Fraschini et al., 1968a; Gallo and Moberg, 1977) and the prostaglandins (Harms et al., 1973; Spies and Norman, 1973; Tsafriri et al., 1973; Carlson et al., 1973; Batta et al., 1974; Ojeda et al., 1975; Eskay et al., 1975; Chobsieng et al., 1975). The purpose of this dissertation, however, has been to present evidence to support the view that dopaminergic neurons of the tubero-infundibular pathway exert an inhibitory influence on the secretion of LRF in the median eminence of the human female.

7.20 DA Control of LRF Secretion

There is little doubt that systemically administered DA can enter the median eminence, if a sufficient dose is given to overcome metabolism by monoamine oxidase and catechol-o-methyl transferase (Bertler et al., 1963). The pituitary gland is also freely accessible to DA but other areas of the hypothalamus lie inside the "blood brain barrier" and only small amounts of labelled DA, administered systemically, are identified in this area (Olendorf, 1971).

Systemic infusion of DA into women consistently causes a decrease in serum LH, FSH as well as PRL. *In vitro* experiments suggest that the effect on LH and FSH secretion is not exerted directly on the pituitary gland (6.31; 6.32). *In vivo*, it is found that when endogenous secretion of LRF is increased, as in agonadal women (3.36), or in women in the pre-ovulatory phase (3.31.1), then the suppressive effect of DA on LH is augmented. Conversely, in hyperprolactinaemic women, in whom secretion of LRF is decreased, there is an attenuation of the LH response to DA. These studies, taken together, are best explained by an inhibitory effect of DA on LRF nerve terminals in the median eminence, causing a decrease in secretion of LRF.

The close intermingling of DA and LRF nerve terminals in the median eminence raises the possibility that the tubero-infundibular DA neuron may exert a physiological inhibition on LRF neurons, by axo-axonic interaction. If this is the case, then it could be reasoned that systemically administered DA enters the median eminence, is taken up by the nerve terminals of the tubero-infundibular neurons with a subsequent augmentation of the normal inhibitory effect on LRF neurons (Figure 7.1).

If DA exerts a tonic inhibitory effect on LRF secretion, it was theorised that blockade of DA activity should lead to an increase in serum LH and FSH. To test this, serum LH and FSH responses to MCP were assessed, on the assumption that DA was most likely to act through a specific receptor mechanism. Unfortunately, although MCP caused a rapid change in PRL secretion, no consistent effect was observed on gonadotropins. This implies that either DA does not

Figure 7.1

Formulation: The role of DA neurons in the control of LRF secretion.

1. LRF cell bodies in the hypothalamus can be directly influenced by the incerto-hypothalamic DA neurons as well as neurons containing other neurotransmitters.

2. LRF nerve terminals in the median eminence can be inhibited by DA released by the tubero-infundibular DA neurons.

3. When DA is infused, it penetrates the median eminence (but not the higher levels of the hypothalamus) and either directly, or after uptake by DA neurons, inhibits the LRF nerve terminals.

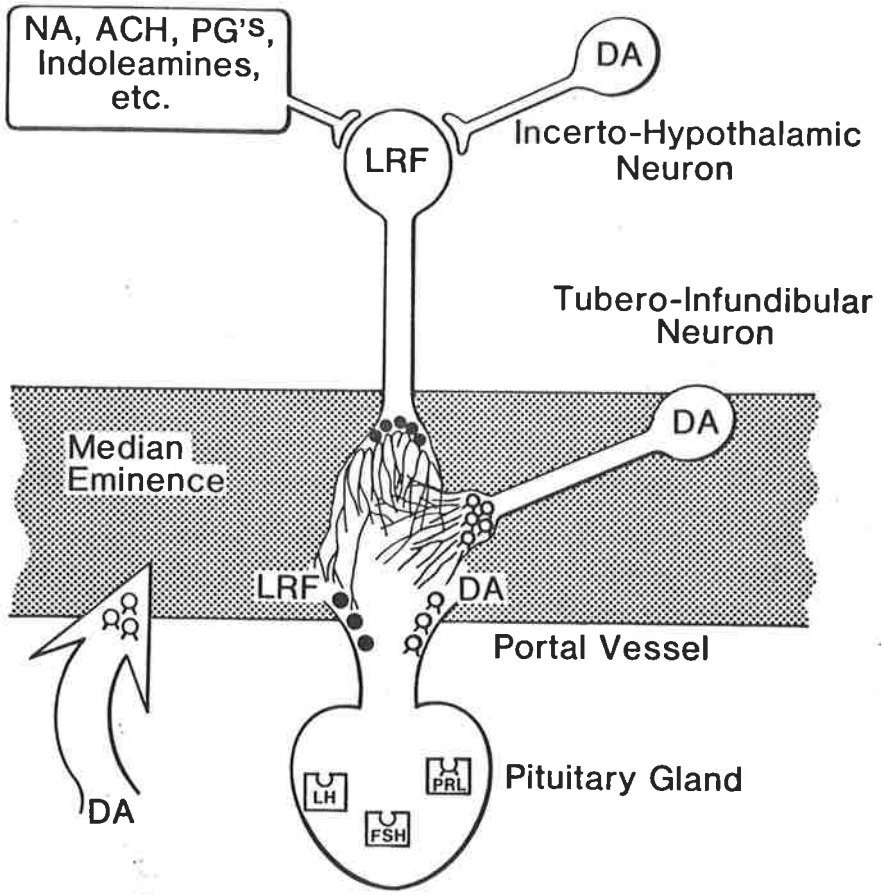
4. DA and LRF both enter the portal circulation to produce effects on pituitary cells.

NA = Noradrenaline

ACH = Acetylcholine

PG = Prostaglandin.

Figure 7.1


NA, ACH, PG's,
Indoleamines,
etc.
DA
LRF
Incerto-Hypothalamic
Neuron
Tubero-Infundibular
Neuron
DA
Median
Eminence
LRF
DA
Portal Vessel
DA
LH
PRL
FSH
Pituitary Gland

exert a tonic influence on LRF neurons or else that the effect of DA is not mediated through the same kind of DA receptors which control PRL secretion. This aspect requires further investigation, although, very recently it has been found that larger doses of MCP (10 mg given intravenously) have caused a three-fold increase in serum LH in three women with hyperprolactinaemia (Quigley and Yen, personal communication). It is possible, then, that a receptor mechanism is involved in the control of LRF secretion, but that this receptor is less sensitive to metoclopramide than those present on the lactotrope.

The hypothetical model which can be constructed on the basis of this work suggests that tubero-infundibular DA neurons act locally on LRF neurons to inhibit LRF secretion, and at the same time DA, released from the nerve terminals, enters the portal vessels where it passes to the lactotrope receptors to cause an inhibition of PRL (Figure 7.1). The model does not exclude involvement of other neurotransmitters, including DA neurons of the incerto-hypothalamic pathway, on LRF cell bodies in higher levels of the hypothalamus.

7.30 The Possible Involvement of DA Neurons in the Physiological Control of LRF Secretion.

If DA neurons of the median eminence exert an inhibitory control on endogenous LRF secretion, then it is relevant to question the activity of DA neurons at a time when secretion of LRF is influenced by oestrogen, a known regulator of LH release. It is possible, for example, that DA neurons mediate the effects of oestrogen on LRF secretion. Oestrogen binding in the median

eminence and medial pre-optic area has been well demonstrated (Maurer, 1974) and it has been suggested that some DA neurons contain receptors for oestrogen on their cell bodies (Grant and Stumpf, 1973). In addition, it has been shown that oestrogen induces cyclic AMP accumulation within the hypothalamus, and this can be blocked not only by clomiphene (Gunaga et al., 1974), but also by DA receptor antagonists (Weissman and Johnson, 1976). This latter finding has been interpreted as being consistent with a direct stimulating effect of oestrogen on DA neurons in the hypothalamus.

There is circumstantial evidence in rats that oestrogen may exert its negative feedback on LRF by increasing dopaminergic activity. Oestrogen administration to castrated male rats caused an acceleration in DA turn-over in the median eminence, which was closely correlated with the fall in serum LH (Löfström et al., 1977).

There is no clear study which has measured dopaminergic activity at a time when oestrogen is producing a positive feed-back effect on LRF secretion. Certainly, it is known that DA turn-over is acutely decreased during the pre-ovulatory phase in rats (Ahren et al., 1971; Ben-Jonathon et al., 1977), but the LH surge induced at this time may not only be related to an effect of oestrogen on LRF secretion. It is highly probable that the pre-ovulatory surge of LH is a highly complex neuro-endocrine event which involves an increase in the pituitary LH capacity induced by oestrogen (Lasley et al., 1975), as well as an increase in LRF induced by increased activity of noradrenergic neurons and decreased activity of dopaminergic neurons.

Although it is known that the biphasic action of oestrogen on LH secretion is time and dose related, the mechanism by which oestrogen might have both stimulating and inhibiting actions on the DA neuron is, at the present time, unknown.

7.40 The Possible Involvement of DA Neurons in Disorders of Human Ovulation.

If, as this dissertation suggests, DA neurons in the median eminence are capable of inhibiting LRF secretion, then the intriguing possibility exists that suppressed secretion of LRF which results in chronic anovulation in women, is mediated by an inappropriate over-activity of DA neurons in the median eminence.

In anorexia nervosa, circumstantial evidence suggests that LRF secretion is reduced; hence episodic secretion of LH is suppressed (Boyar et al., 1974), there is a reduced LH response to LRF (Beumont et al., 1976), and the gonadotropin response to clomiphene is abolished (Marshall and Russell Fraser, 1971). Several of the features associated with anorexia nervosa, including nausea, polyuria, over-activity and psychotic behaviour, have been explained on the basis of excessive dopaminergic activity in the brain (Barry and Klawans, 1976); over-activity of DA neurons in the median eminence may also explain the suppression of LRF secretion.

When patients with anorexia nervosa gain weight, the pattern of the recovery of LRF secretion closely parallels the hormonal sequence during normal puberty. In fact, it has been suggested that both these situations support the concept that cyclical secretion of LRF is triggered by attainment of a certain "critical weight"

(Kalucy et al., 1976; Frisch, 1973). The possibility could be explored that at a certain critical weight, neuro-endocrine events are triggered which lead to a decrease in DA suppression of LRF, with subsequent gonadotropin cyclicity. Weight loss such as is seen in anorexia nervosa produces a return to the earlier pattern of increased dopaminergic activity.

7.50 The Possible Interaction Between Endogenous Morphinometric Compounds and DA Neurons.

In 1973, the presence of specific opiate receptors was clearly demonstrated in the brain (Simon et al., 1973; Pert and Snyder, 1973). Shortly thereafter, a series of endogenous morphine-like substances which interacted with these opiate receptors were isolated from the brain (Hughes et al., 1975; Lazarus et al., 1976). Three of these substances are polypeptides termed α, β and γ endorphin and two others are penta-peptides, methionine and leucine enkephalin. Each of these endogenous morphine-like substances seems to be derived from the large protein hormone β lipotropin.

These compounds have proved to be a most exciting new development in neuro-endocrinology. The enkephalins have been clearly demonstrated within cell bodies of the hypothalamus and nerve terminals of the median eminence, and seem to process all the properties of neurotransmitters (Hokfelt et al., 1977). Interestingly, several previous pieces of work had suggested that morphine interacts with DA receptors in the brain. Chronic morphine administration to rats produces behavioural changes suggestive of a decrease in dopaminergic activity (Bosse and Kuchinsky, 1976); also consistent is the demonstration that chronic morphine administration induces DA receptor supersensitivity (Smee and Overstreet, 1976; Lal, 1975).

Prolactin inhibition is enhanced after withdrawal of chronic treatment with morphine (Lal et al., 1977) and it has been suggested that many of the clinical features of acute opiate withdrawal in human subjects could be explained on the basis of supersensitivity of DA receptors in the brain.

Morphine administration to rats induces an increase in PRL and a decrease in serum LH. Naloxone, an opiate receptor antagonist causes a decrease in serum PRL and an increase in both serum LH and FSH (Bruni et al., 1977). All these observations are suggestive of a close alliance between endogenous morphine-like substances and DA neurons. One possible mode of interaction, which has been suggested, is that the endogenous morphine-like substances may activate pre-synaptic receptors on the DA neuron, producing decreased release of DA (Bosse and Kuschinsky, 1976).

This interaction may be an important mechanism whereby the limbic system and higher centres of the brain influence LRF secretion in the hypothalamus to produce chronic anovulation, for example, in the clinical syndrome of "hypothalamic amenorrhoea". Moreover, increased secretion of an endogenous morphine-like substance which selectively blocks DA receptors on the lactotrope would also be expected to cause hyperprolactinaemia, with a supersensitivity of the lactotrope receptor and, by activation of a short loop feed-back, increase hypothalamic DA neuron activity. These predicted findings are those that have been described (vide supra) in patients with hyperprolactinaemic chronic anovulation.

These concepts must remain highly speculative at the present time. However, as techniques for neuro-endocrine investigation become more refined, it is likely that these hypotheses will be testable. With a clear understanding of the neuro-endocrine effects that influence LRF secretion, it may be anticipated that diagnosis and management of patients with chronic anovulation will be improved.

REFERENCES

AGHAJANIAN, G.K. and B.S. BUNNEY.
Dopamine "Autoreceptors" : Pharmacological Characterisation by Micro-Iontophoretic Single Cell Recording Studies.
Arch. Pharmacol., 297, 1 - 9, 1977.

AHREN, K., K. FUXE, L. HAMBERGER and T. HÖKFELT.
Turnover Changes in the Tubero-Infundibular Dopamine Neurons During the Ovarian Cycle of the Rat.
Endocrinol., 88, 1415 - 1424, 1971.

AJIKA, K. and T. HÖKFELT.
Ultrastructural Identification of Catecholamine Neurones in the Hypothalamic Periventricular-Arcuate Nucleus-Median Eminence Complex with Special Reference to Quantitative Aspects.
Brain Res., 57, 97 - 117, 1973.

ALLWOOD, M.J., A.F. COBBOLD and J. GINSBURG.
Peripheral Vascular Effects of Noradrenaline, Isopropyl Noradrenaline and Dopamine.
Brit. Med. Bull., 19, 132 - 136, 1963.

ALLWOOD, M.J. and J. GINSBURG.
Peripheral Vascular and Other Effects of Dopamine Infusions in Man.
Clin. Sci., 27, 271 - 281, 1964.

AMENOMORI, Y. and J. MEITES.
Effect of a Hypothalamic Extract on Serum Prolactin Levels During the Estrous Cycle and Lactation.
Proc. Soc. Exp. Biol. Med., 134, 492 - 495, 1970.

ANDÉN, N.E., A. RUBENSON, K. FUXE and T. HÖKFELT.
Evidence for Dopamine Receptor Stimulation by Apomorphine.
J. Pharm. Pharmacol., 48, 627 - 629, 1967.

ANDÉN, N.E., A. CARLSSON and J. HÄGGENDAL.
Adrenergic Mechanisms.
Ann. Rev. Pharmacol., 9, 119 - 134, 1969.

ANDÉN, N.E., S.G. BUTCHER, H. CORRODI, K. FUXE and U. UNGERSTEDT.
Receptor Activity and Turnover of Dopamine and Noradrenaline After Neuroleptics.
Europ. J. Pharmacol., 11, 303 - 314, 1970.

ANTON-TAY, F. and R.J. WURTMAN.
Norepinephrine : Turnover in Rat Brains After Gonadectomy.
Science, 159, 1245. 1968.

APPELBAUM, M.E. and S. TALEISNIK.
Interaction Between Oestrogen and Gonadotrophin Releasing Hormone on the Release and Synthesis of Luteinising Hormone and Follicle Stimulating Hormone from Incubated Pituitaries.
J. Endocrinol., 68, 127 - 136, 1976.

ARBUTHNOTT, G.W., T.J. CROW, and K. FUXE.
Depletion of Catecholamines in vivo Induced by Electrical Stimulation of Central Monoamine Pathways.
Brain Res., 24, 471 - 483, 1970.

ARIMURA, A.
Hypothalamic Gonadotropin-Releasing Hormone and Reproduction.
P. 1 - 21.
In International Review of Physiology, Vol. 13,
Edit. R.O. Greep, Pub. Univ. Park Press, 1977.

ARIMURA, A., L. DEBELJUK and A.V. SCHALLY.
Stimulation of FSH Release in vivo by Prolonged Infusions of Synthetic LHRH.
Endocrinol., 91, 529 - 532, 1972.

ARIMURA, A. and A.V. SCHALLY.
Hypothalamic LH and FSH-Releasing Hormone : Re-evaluation of the Concept That One Hypothalamic Hormone Controls the Release of LH and FSH.
in M. Kawakami (ed.).
Biological Rhythms in Neuroendocrine Activity, pp. 73 - 90.
Igaku Shoin, Tokyo, 1974.

ARIMURA, A., L. DEBELJUK and A.V. SCHALLY.
Blockade of Pre-ovulatory Surge of Gonadotropins, LH and FSH, and of Ovulation by Anti-LHRH in Rats.
Endocrinol., 95, 323 - 327, 1974(a).

ARIMURA, A., A.J. KASTIN and A.V. SCHALLY.
Immuno-reactive LH-Releasing Hormone in Plasma-Midcycle Elevation in Women.
J. Clin. Endocrinol. Metab., 38, 510 - 513, 1974(b).

ARITA, T., R. HORI, K. ITO, K. ICHIKAWA and T. UESUGI.
Transformation and Excretion of Drugs in Biological Systems.
II - Transformation of Metoclopramide in Rabbits.
Chem. Pharm. Bull., 18, 1663 - 1669, 1970.

AULSEBROOK, L.H. and R.C. HOLLAND.
Central Regulation of Oxytocin Release With and Without Vasopressin Release.
Am. J. Physiol., 216, 818 - 829, 1969.

BACQ, Z.M.
La Pharmacologie du Systeme Nerveux Autonome et Particulièrement du Sympatique, d'àpres la Théorie Neurohumorale.
Ann. Physiol., 10, 467, 1934.

BAKER, R.L., W.C. DERMODY and J.R. REEL.
Localisation of Luteinising Hormone Releasing Hormone in the Mammalian Hypothalamus.
Am. J. Anat., 179, 129 - 134, 1974.

BAKKE, O.M. and J. SEGURA.
The Absorption and Elimination of Metoclopramide in Three Animal Species.
J. Pharm. Pharmac., 28, 32 - 39, 1976.

BARBEAU, A., G.F. MURPHY and T.L. SOURKES.
Excretion of Dopamine in Diseases of Basal Ganglia.
Science, 133, 1706 - 1707, 1961.

BAROLIN, G.S., H. BERNHEIMER and O. HORNYKIEWICZ.
Seitenuerschiedenes Verhalten des Dopamins (3-hydroxytyramin) in Gehirn eines Falles von Hemiparkinsonismus.
Schweiz. Arch. Neurol. Psychiat., 94, 241 - 248, 1964.

BARRACLOUGH, C.A. and C.H. SAWYER.
Blockade of the Release of Pituitary Ovulating Hormone in the Rat by Chlorpromazine and Reserpine : Possible Mechanisms of Action.
Endocrinol., 61, 341 - 351, 1957.

BARRY, J.
Characterisation and Topography of LHRH Neurons in the Rabbit.
Neuroscience Letters, 2, 201 - 205, 1976.

BARRY, J.
Characterisation and Topography of LHRH Neurons in the Human Brain.
Neuroscience Letters, 3, 289 - 291, 1976(a).

BARRY, J., M.P. DUBOIS and P. POULAIN.
LRF Producing Cells of the Mammalian Hypothalamus.
Z. Zellforsch. M. Krosk. Anat., 146, 351 - 366, 1973.

BARRY, J. and M.P. DUBOIS.
Immunofluorescence Study of LRF Producing Neurons in the Cat and the Dog.
Neuroendocrinol., 18, 290 - 298, 1975.

BARRY, V.C. and H.L. KLAWANS.
On the Role of Dopamine in the Pathophysiology of Anorexia Nervosa.
J. Neural. Transmission, 38, 107 - 122, 1976.

BATTA, S.K., M. ZANIZI and L. MARTINI.
Prostaglandins and Gonadotropin Secretion.
Neuroendocrinol., 14, 224 - 232, 1974.

BAUDRY, M., J. COSTENTIN, H. MARCAIS, M.P. MARTRES, P. PROTAIS and J.C. SCHWARTZ.
Decreased Responsiveness to Low Doses of Apomorphine After Dopamine Agonists and the Possible Involvement of Hyposensitivity of Dopamine "Auto-Receptors".
Neuroscience Letters, 4, 203 - 207, 1977.

BAUMGARTEN, H.G., A. BJORKLUND, A.F. HOLSTEIN and A. NOBIN.
Organisation and Ultrastructural Identification of the Catecholamine Nerve Terminals in the Neural Lobe and Pars Intermedia of the Rat Pituitary.
Z. Zellforsch., 126, 483 - 517, 1972.

BEATTIE, C.W., M.I. GLUCKMAN and A. CORBIN.
A Comparison of Gamma-Butyrolactone and Pimozide on Serum Gonadotrophins and Ovulation in the Rat.
Proc. Soc. Exp. Biol. Med., 153, 147 - 150, 1976.

BECK, W. and W. WUTTKE.
Desensitisation of Dopaminergic Inhibition of Luteinising Hormone Release by Prolactin in Ovariectomised Rats.
J. Endocrinol., 74, 67 - 74, 1977.

BECKETT, A.H. and G. HUIZING.
In vitro Metabolism of Metoclopramide.
J. Pharm. Pharmac., 27, (Supp.); 42, 1975.

BELL, J.E., E. ROSENKOVICH and D. RABINOWITZ.
Blue Dextran : Influence on Chromatographic Profile and Immuno-Reactivity of Human Follicle Stimulating Hormone.
Proc. Soc. Exp. Biol. Med., 149, 565 - 569, 1975.

BEN-JONATHON, N., R.S. MICAL and J.C. PORTER.
Super-Fusion of Hemipituitaries with Portal Blood.
I - LRF Secretion in Castrated and Diestrous Rats.
Endocrinol., 93, 497 - 503, 1973.

BEN-JONATHON, N., R.S. MICAL and J.C. PORTER.
Transport of LRF from CSF to Hypophysial Portal and Systemic Blood and Release of LH.
Endocrinol., 95, 18 - 25, 1974.

BEN-JONATHON, N., N.C. OLIVER, H.J. WEINER, R.S. MICAL and J.C. PORTER.
Dopamine in Hypophysial Portal Plasma of the Rat During the Estrus Cycle and Throughout Pregnancy.
Endocrinol., 100, 452 - 458, 1977.

BERNHEIMER, H.
Distribution of Homovanillic Acid in the Human Brain.
Nature, 204, 587 - 588, 1964.

BERTLER, A. and E. ROSENGREN.
Occurrence and Distribution of Dopamine in Brain and Other Tissues.
Experientia, 15, 10 - 11, 1959.

BERTLER, A., B. FALCK and E. ROSENGREN.
The Direct Demonstration of a Barrier Mechanism in Brain Capillaries.
Acta Pharmacol. et Toxicol., 20, 317 - 321, 1963.

BESSES, G.S., G.N. BURROW, S.W. SPALDING and R.K. DONABEDIAN.
Dopamine Infusion Acutely Inhibits the TSH and Prolactin Response to TRH.
J. Clin. Endocrinol. Metab., 41, 985 - 988, 1975.

BEUMONT, P.J.V., G.C.W. GEORGE, B.L. PIMSTONE and A.I. VINIK.
Body Weight and the Pituitary Response to Hypothalamic Releasing Hormones in Patients with Anorexia Nervosa.
J. Clin. Endocrinol. Metab., 43, 487 - 496, 1976.

BHARGUVA, K.P. and M.L. GUPTA.
A Study of Central Adrenergic Mechanisms in the Regulation of the Oestrous Cycle of Albino Mice.
Brit. J. Pharmacol., 26, 601 - 605, 1966.

BIRD, E.D., S.A. CHIAPPA and G. FINK.
Brain Immuno-Reactive Gonadotropin-Releasing Hormone in Huntington's Chorea and in Non-Choreic Subjects.
Nature, 260, 536 - 538, 1976.

BIRGE, C.A., L.S. JACOBS, C.T. HAMMER and W.H. DAUGHADAY.
Catecholamine Inhibition of Prolactin Secretion by Isolated Rat Adenohypophyses.
Endocrinol., 86, 120 - 130, 1970.

BIRKMAYER, W. and O. HORNYKIEWICZ.
Der L Dioxyphenylalanin (= Dopa) - effekt bei der Parkinson-akinese.
Wien Klin. Wschr., 73, 787 - 788, 1961.

BISCHOFF, F. and A. TORRES.
Determination of Urine Dopamine.
Clin. Chem., 8, 370 - 377, 1962.

BJÖRKLUND, A., B. EHINGER and B. FALCK.
A Method for Differentiating Dopamine from Noradrenaline in Tissue Sections by Microspectrofluometry.
J. Histochem. Cytochem., 16, 263 - 270, 1968.

BJÖRKLUND, A., R.Y. MOORE, A. NOBIN and U. STENEVI.
The Organisation of Tubero-Hypophyseal and Reticulo-Infundibular Catecholamine Neuron Systems in Rat Brain.
Brain Res., 51, 171 - 191, 1973.

BJÖRKLUND, A. and O. LINDVALL.
Dopamine in Dendrites of Substantia Nigra Neurons : Suggestions for a Role in Dendritic Terminals.
Brain Res., 83, 531 - 537, 1975.

BJÖRKLUND, A., O. LINDVALL and A. NOBIN.
Evidence of an Incerto-Hypothalamic Dopamine Neurone System in the Rat.
Brain Res., 89, 29 - 42, 1975.

BLASCHKO, H.
Metabolism and Storage of Biogenic Amines.
Experientia, 13, 9 - 12, 1957.

BODEN, G., L.E. LUNDY and O.E. OWEN.
Influence of Levodopa on Serum Levels of Anterior Pituitary Hormones in Man.
Neuroendocrinol., 10, 309 - 315, 1972.

BOHNET, H.G., H.G. DAHLEN, W. WUTTKE and H.P.G. SCHNEIDER.
Hyperprolactinaemic Anovulatory Syndrome.
J. Clin. Endocrinol. Metab., 42, 132 - 143, 1976.

BORGEAT, P., G. CHAVANCY, A. DUPONT, F. LABRIE, A. ARIMURA and A.V. SCHALLY.
Stimulation of Adenosine 3', 5' Cyclic Monophosphate Accumulation in Anterior Pituitary Gland in vitro by Synthetic Luteinising Hormone-Releasing Hormone.
Proc. Natl. Acad. Sci., USA, 69, 2677 - 2681, 1972.

BOSSE, A. and K. KUSCHINSKY.
Alterations of Dopaminergic Neurotransmission After Chronic Morphine Treatment : Pre- and Post-Junctional Studies in Striatal Tissue.
Arch. Pharmacol., 294, 17 - 22, 1976.

BOYAR, R.M., J. KATZ, J.W. FINKELSTEIN, S. KAPEN, H. WEINER, E.D. WEITZMAN and L. HELLMAN.
Anorexia Nervosa. Immaturity of the 24-Hour Luteinising Hormone Secretory Pattern.
New Eng. J. Med., 291, 861 - 865, 1974.

BOYAR, R.M., S. KAPEN, J.W. FINKELSTEIN, M. PERLOW, J.F. SASSIN, D.K. FUKUSHIMA, E.D. WEITZMAN and L. HELLMAN.
Hypothalamic-Pituitary Function in Diverse Hyperprolactinaemic States.
J. Clin. Invest., 53, 1588 - 1598, 1974(a).

BRAMBLE, F.J., A.L. HOUGHTON, S.S. ECCLES, M.A.F. MURRAY and H.S. JACOBS.
Specific Control of Follicle Stimulating Hormone in the Male : Postulated Site of Action of Inhibin.
Clin. Endocrinol., 4, 443 - 449, 1975.

BROOKS, C. McC., J.S. GILBERT, H.A. LEVEY and D.R. CURTIS.
Humors, Hormones and Neurosecretions.
Page 154, Pub. State University of New York, 1962.

BROWN, G.M., P. SEEMAN and T. LEE.
Dopamine/Neuroleptic Receptors in Basal Hypothalamus and Pituitary.
Endocrinol., 99, 1407 - 1409, 1976.

BROWN, P.S.
Antigonadotrophic Effects of ∝ Methyltyrosine, Methysergide and Reserpine.
Nature, 214, 1268 - 1269, 1967.

BROWN-GRANT, K.
Physiological Aspects of the Steroid Hormone-Gonadotropin Interrelationship.
Int. Rev. Physiol., 13, 57 - 83, 1977.

BRUNI, J.F., O. VAN VUGT, S. MARSHALL and J. MEITES.
Effects of Naloxone, Morphine and Methionine Enkephalin on Serum Prolactin, Luteinising Hormone, Follicle Stimulating Hormone, Thyroid Stimulating Hormone and Growth Hormone.
Life Sci., 21, 461 - 466, 1977.

BUCKMAN, M.T. and G.T. PEAKE.
Estrogen Potentiation of Phenothiazine-Induced Prolactin Secretion in Man.
J. Clin. Endocrinol. Metab., 37, 977 - 980, 1973.

BUCKMAN, M.T., G.T. PEAKE and L.S. SRIVASTAVA.
Endogenous Estrogen Modulates Phenothiazine Stimulated Prolactin Secretion.
J. Clin. Endo. Metab., 43, 901 - 906, 1976.

BUNNEY, B.S., J.R. WALTERS, R.H. ROTH and G.K. AGHAJANIAN.
Dopaminergic Neurons : Effects of Antipsychotic Drugs and Amphetamine on Single Cell Activity.
J. Pharmac. Exp. Ther., 185, 560 - 571, 1973.

BUNNEY, B.S. and G.K. AGHAJANIAN.
d Amphetamine-Induced Inhibition of Central Dopaminergic Neurons : Mediation by a Striato-Nigral Feedback Pathway.
Science, 192, 391 - 393, 1976.

BURGUS, R., M. BUTCHER and M. AMOSS.
Primary Structure of the Ovine Hypothalamic Luteinising Hormone-Releasing Factor (LRF).
Proc. Natl. Acad. Sci., USA, 69, 278 - 282, 1972.

BURT, D.R., I. CREESE and S.H. SNYDER.
Antischizophrenic Drugs : Chronic Treatment Elevates Dopamine Receptor Binding in Brain.
Science, 196, 326 - 327, 1977.

BUTCHER, L., J. ENGEL and K. FUXE.
L Dopa Induced Changes in Central Monoamines After Peripheral Decarboxylase Inhibition.
J. Pharm. Pharmacol., 22, 313 - 316, 1970.

CANNON, W.B. and A. ROSENBLUETH.
Studies on Conditions of Activity in Endocrine Organs.
XXIX - Sympathin E and Sympathin I.
Am. J. Physiol., 104, 557 - 574, 1933.

CARLSON, H.E., L.S. JACOBS and W.H. DAUGHADAY.
Growth Hormone, Thyrotropin and Prolactin Responses to Thyrotropin-Releasing Hormone Following Diethyl-Stilbesterol Pretreatment.
J. Clin. Endocrinol. Metab., 37, 488 - 490, 1973.

CARLSON, J.C., B. BARCIKOWSKI and J. McCRACKEN.
Prostaglandin $F_2\alpha$ and the Release of LH in the Sheep.
J. Reprod. Fert., 34, 357 - 361, 1973.

CARLSSON, A., M. LINDQVIST and T. MAGNUSSON.
3, 4, Dihydroxyphenylalanine and 5 Hydroxytryptophan as Reserpine Antagonists.
Nature, 180, 1200 - 1211, 1957.

CARLSSON, A. and B. WALDECK.
A Fluorimetric Method for the Determination of Dopamine (3 Hydroxytyramine).
Acta Physiol. Scand., 44, 293 - 298, 1957.

CARLSSON, A. and N.A. HILLARP.
Formation of Phenolic Acids in Brain After Administration of 3, 4 Dihydroxyphenylalanine.
Acta Physiol. Scand., 55, 95 - 100, 1962.

CARLSSON, A. and M. LINDQVIST.
Effect of Chlorpromazine or Haloperidol on Formation of 3 Methoxytyramine and Metanephrine in Mouse Brain.
Acta Pharmacol., 20, 140 - 144, 1963.

CARLSSON, A. and B. WALDECK.
A Method for the Fluorimetric Determination of 3 Methoxytyramine in Tissues and the Occurrence of this Amine in Brain.
Scand. J. Clin. Lab. Inv., 16, 133 - 138, 1964.

CARMEL, P.C., S. ARAKI and M. FERIN.
Prolonged Stalk Portal Blood Collection in Rhesus Monkeys : Pulsatile Release of Gonadotropin - Releasing Hormone (GnRH).
Abst. 107, 57th Annual Meeting Endocrine Society, New York, 1975.

CARSON, R.S., E.J. KEOUGH, J.K. FINDLAY, M.J. McKINLEY, W.J. BREMNER, H.G. BURGER and B. HUDSON.
LH - Releasing Hormone (LH - RH) in Ovine Cerebrospinal Fluid.
Abst. 23, 19th Annual Meeting Endocrine Society of Australia, Brisbane, 1976.

CHEN, C.L., H. MINAGUCHI and J. MEITES.
Effects of Transplanted Pituitary Tumours on Host Pituitary Prolactin Secretion.
Proc. Soc. Exp. Biol. Med., 126, 317 - 320, 1967.

CHEN, C.L., Y. AMENOMORI, K.H. LU, J.L. VOOGT and J. MEITES.
Serum Prolactin Levels in Rats with Pituitary Transplants or Hypothalamic Lesions.
Neuroendocrinol., 6, 220 - 227, 1970.

CHEUNG, C.Y. and R.I. WEINER.
Supersensitivity of Anterior Pituitary Dopamine Receptors Involved in the Inhibition of Prolactin Secretion Following Destruction of the Medial Basal Hypothalamus.
Endocrinol., 99, 914 - 917, 1976.

CHOBSIENG, P., Z. NAOR, Y. KOCH, V. ZOR and H.R. LINDNER.
Stimulating Effect of Prostaglandin E_2 on LH Release in the Rat : Evidence for Hypothalamic Site of Action.
Neuroendocrinol., 17, 12 - 17, 1975.

CLEMENS, J.A. and J. MEITES.
Inhibition by Hypothalamic Prolactin Implants of Prolactin Secretion, Mammary Growth and Luteal Function.
Endocrinol., 82, 878 - 881, 1968.

CLEMENS, J.A., C.J. SHEAR, E.B. SMALSTIG and C. MATSUMOTO.
Effect of Some Psycho-active Agents on Prolactin Secretion in Rats of Different Endocrine States.
Hormone Met. Res., 6, 187 - 190, 1974.

COLLU, R., J.C. JEQUIER, G. LEBOEUF, J. LETARTE and J.R. OUCHARME.
Endocrine Effects of Pimozide, A Specific Dopaminergic Blocker.
J. Clin. Endocrinol. Metab., 41, 981 - 984, 1975.

CONNOR, J.D.
Caudate Nucleus Neurones : Correlation of the Effects of Substantia Nigra Stimulation with Ionophoretic Dopamine.
J. Physiol., 208, 691 - 703, 1970.

CONSTERTIN, J., P. PROTAIS and J.C. SCHWARTZ.
Rapid and Dissociated Changes in Sensitivities of Different Dopamine Receptors in Mouse Brain.
Nature, 257, 405 - 407, 1975.

COOLS, A.R., H.A.J. STRUYKER-BOUDIER and J.M. VAN ROSSUM.
Selective Agonists and Antagonists of Functionally Distinct Types Within the Feline Brain.
European Journ. of Pharmacol., 37, p. 283 - 293, 1976.

COPPOLA, J.A.
The Apparent Involvement of the Sympathetic Nervous System in the Gonadotrophin Secretion of Female Rats.
J. Reprod. Fert. Supp., 4, 35 - 45, 1968.

COPPOLA, J.A.
Turnover of Hypothalamic Catecholamines During Various States of Gonadotrophin Secretion.
Neuroendocrinol., 5, 75 - 80, 1969.

COPPOLA, J.A., W. LEONARDI and W. LIPPMANN.
Ovulatory Failure in Rats After Treatment with Brain Norepinephrine Depletors.
Endocrinol., 78, 225 - 228, 1966.

COSTALL, B. and R.J. NAYLOR.
Stereotyped and Circling Behaviour Induced by Dopaminergic Agonists After Lesions of the Mid-Brain Raphe Nuclei.
Europ. J. Pharmacol., 29, 206 - 222, 1974.

COTZIAS, G.C., M.H. VAN WOERT and L.M. SCHIFFER.
Aromatic Amino Acids and Modification of Parkinsonism.
New Eng. Journ. Med., 276, 374 - 379, 1967.

CRAVEN, R.R. and P.G. McDONALD.
The Effect of Intrahypothalamic Infusions of Dopamine and Noradrenaline on Ovulation in Reserpine Treated Rats.
J. Endocrinol., 58, 319 - 326, 1973.

CREESE, I. and S.D. IVERSEN.
The Role of Forebrain Dopamine Systems in Amphetamine Induced Stereotyped Behaviour in the Rat.
Psychopharmacol., 39, 8 - 24, 1974.

CRIGHTON, D.B., H.P.G. SCHNEIDER and S.M. McCANN.
Localisation of LH Releasing Factor in the Hypothalamus and Neurohypophysis as Determined by an in vitro Method.
Endocrinol., 87, 323 - 329, 1970.

CROSSLAND, I.
Chemical Transmission in the Central Nervous System.
J. Pharm. Pharmacol., 12, 1 - 36, 1960.

DAHLSTROM, A., K. FUXE, L. OLSON and U. UNGERSTEDT.
Ascending Systems of Catecholamine Neurons from the Lower Brain Stem.
Acta Physiol. Scand., 62, 485 - 486, 1962.

DALE, H.H.
Chemical Transmission of the Effects of Nerve Impulses.
Brit. Med. Journ., 1, 20, 1934.

DAVIES, C., J. JACOBI, H.M. LLOYD and J.D. MEARES.
DNA Synthesis and the Secretion of Prolactin and Growth Hormone by the Pituitary Gland of the Male Rat. Effects of Diethylstilboesterol 2 bromo-α - Ergocryptine Methanesulphonate.
J. Endo., 61, 411 - 417, 1974.

DEBELJUK, L., H. DASKAL, R. ROZADOS and A. GUITELMAN.
Effect of Sulpiride on Prolactin Release by Rat Pituitaries in vitro.
Experientia, 30, 1355 - 1356, 1974.

DEL POZO, E., L. VARGA, H. WYSS, G. TOLIS, H. FRIESEN, R. WENNER, L. VETTER and A. UETTWILER.
Clinical and Hormonal Response to Bromocriptin (CB - 154) in the Galactorrhoea Syndromes.
J. Clin. Endocrinol. Metab., 39, 18 - 26, 1974.

DEMPSEY, E.W. and U.U. UOTILA.
The Effect of Pituitary Stalk Section upon Reproductive Phenomena in the Female Rat.
Endocrinol., 27, 573 - 579, 1940.

DE VANE, G.W., N.C. CZEKALA, H.L. JUDD and S.S.C. YEN.
Circulating Gonadotropins, Estrogens and Androgens in Polycystic Ovarian Disease.
Am. J. Obstet. Gynaecol., 121, 496 - 500, 1975.

DIEFENBACH, W.P., P.W. CARMEL, A.G. FRANTZ and M. FERIN.
Suppression of Prolactin Secretion by L Dopa in Stalk Sectioned Rhesus Monkeys.
J. Clin. Endocrinol. Metab., 43, 638 - 642, 1976.

DOLPHIN, A., P. JENNER, C.D. MARSDEN, C. PYCOCK and D. TARSY.
Pharmacological Evidence for Cerebral Dopamine Receptor Blockade by Metoclopramide in Rodents.
Psychopharmacol., 41, 133 - 138, 1975.

DOMANSKI, E., F. PRZEKOP, B. SKUBISZEWSKI and E. WOLIŃSKA.
The Effect and Site of Action of Indoleamines on the Hypothalamic Centres Involved in the Control of LH Release and Ovulation in Sheep.
Neuroendocrinol., 17, 265 - 273, 1975.

DONOSO, A.O. and F.J.E. STEFANO.
Sex Hormones and Concentration of Noradrenalin and Dopamine in the Anterior Hypothalamus of Castrated Rats.
Experientia, 23, 665 - 666, 1967.

DONOSO, A.O., F.J.E. STEFANO, A.M. BISCARDI and J. CUKIER.
Effects of Castration on Hypothalamic Catecholamines.
Am. J. Physiol., 212, 737 - 739, 1967(a).

DONOSO, A.O. and R.C. SANTOLAYA.
The Effect of Monoamine Synthesis Inhibitors on the Ovarian Compensatory Hypertrophy.
Experientia, 25, 855 - 857, 1969.

DONOSO, A.O. and M.B. DE G. MOYANO.
Adrenergic Activity in Hypothalamus and Ovulation.
Proc. Soc. Exp. Biol. Med., 125, 633 - 635, 1970.

DONOSO, A.O., W. BISHOP, W. FAWCETT, C.P. KRULICH and S.M. McCANN.
Effects of Drugs that Modify Brain Monoamine Concentrations on Plasma Gonadotrophin and Prolactin Levels in the Rat.
Endocrinology, 89, 774 - 784, 1971.

DROUIN, J. and F. LABRIE.
Specificity of the Stimulatory Effect of Prostaglandins on Hormone Release in Rat Anterior Pituitary Cells in Culture.
Prostaglandins, 11, 355 - 365, 1976.

DROUVA, S.V. and R.V. GALLO.
Catecholamine Involvement in Episodic Luteinising Hormone Release in Adult Ovariectomised Rats.
Endocrinol., 99, 651 - 658, 1976.

DROUVA, S.V. and R.V. GALLO.
Further Evidence for Inhibition of Episodic Luteinising Hormone Releasing in Ovariectomised Rats by Stimulation of Dopamine Receptors.
Endocrinol., 100, 792 - 798, 1977.

DUNN, A. and J. ARDITTI.
Experimental Physiology.
Pub. Holt, Rinehart and Winston Inc., New York, 1968.

DUPONT, A. and T.W. REDDING.
Purification and Characterisation of PIF from Pig Hypothalami.
Abstract 85, Programme of 57th Meeting Endocrine Society, New York, 1975.

ECCLES, J.C.
Under the Spell of the Synapse.
The Neurosciences - Third Study Program.
Eds. F.O. Schmidt and F.G. Worden, M.I.T. Press, Cambridge, Mass. 1974.

EHARA, Y., T. SILER, G. VAN DEN BERG, Y.N. SINHA and S.S.C. YEN.
Circulating Prolactin Levels During the Menstrual Cycle : Episodic Release and Diurnal Variation.
Am. J. Obstet. Gynaecol., 117, 962 - 970, 1973.

EHRINGER, H. and O. HORNYKIEWICZ.
Distribution of Noradrenaline and Dopamine (3 Hydroxytyramine) in the Human Brain and Their Behaviour in Diseases of the Extrapyramidal System.
Klin. Wschr., 38, 1236 - 1239, 1960.

EIKENBURG, D.C., A.J. RAVITZ, G.A. GUDELSKY and K.E. MOORE.
Effects of Estrogen on Prolactin and Tubero-Infundibular Dopaminergic Neurons.
J. Neural Trans., 40, 235 - 244, 1977.

ELLIOTT, T.R.
On the Action of Adrenalin.
J. Physiol., 31, Proc. p. XX, 1904.

ENJALBERT, A., A. PRIAM and C. KORDON.
Evidence in Favour of the Existence of a Dopamine-Free Prolactin-Inhibiting Factor (PIF) in Rat Hypothalamic Extracts.
Europ. Journ. Pharmacol., 41, 243 - 244, 1977.

ESKAY, R.L., J. WARBERG, R.S. MICAL and J.C. PORTER.
Prostaglandin E_2-Induced Release of LHRH into Hypophysial Portal Blood.
Endocrinol., 97, 816 - 824, 1975.

EVERETT, J.
Luteotrophic Function of Autografts of the Rat Hypophysis.
Endocrinol., 54, 685 - 690, 1954.

EVERETT, J.W.
Central Neural Control of Reproductive Functions of the Adenohypophysis.
Physiol. Rev., 44, 373 - 431, 1964.

FALCK, B., N.A. HILLARP, G. THIEME and A. TORP.
Fluorescence of Catecholamines and Related Compounds Condensed with Formaldehyde.
J. Histochem. Cytochem., 10, 348 - 354, 1962.

FAULL, R.L.M. and J.B. CARMAN.
Ascending Projections of the Substantia Nigra in the Rat.
J. Comp. Neurol., 132, 73 - 92, 1968.

FERNSTROM, J.D. and R.J. WURTMAN.
Brain Monoamines and Reproductive Function.
P. 23 - 55. In International Review of Physiology.
Reproductive Physiology II, Vol. 13, Ed. R.O. Greep.
University Park Press, 1977.

FIBIGET, H.C., R.E. PUDRITZ, P.L. McGEER and E.G. McGEER.
Axonal Transport in Nigro-Striatal and Nigro-Thalamic Neurons : Effects of Medial Forebrain Bundle Lesions and 6 Hydroxydopamine.
J. Neurochem., 19, 1697 - 1708, 1972.

FLEISCHHAUER, K.
Some Basic Facts Concerning the Morphology of the Hypothalamus and its Constituent Elements.
Adv. Biosciences, 15, 9 - 23, 1975.

FLUCKIGER, E. and H.R. WAGNER.
2 Br-alpha-ergocryptine : Beinflussung von Fertilitat und Laktation bei der ratte.
Experientia, 24, 1130 - 1132, 1969.

FRANKS, S., M.A.F. MURRAY, A.M. JEQUIER, S.J. STEELE, J.D.N. NABARRO and H.S. JACOBS.
Incidence and Significance of Hyperprolactinaemia in Women with Amenorrhoea.
Clin. Endocrinol., 4, 597 - 607, 1975.

FRASCHINI, F., B. MESS and L. MARTINI.
Pineal Gland, Melatonin and the Control of Luteinising Hormone Secretion.
Endocrinol., 82, 919 - 924, 1968.

FRASCHINI, F., B. MESS, F. PURA and L. MARTINI.
Brain Receptors Sensitive to Indole Compounds Function in Control of Luteinizing Hormone Secretion.
Science, 159, 1104 - 1105, 1968(a).

FRISCH, R.
Critical Weight at Menarche, Initiation of the Adolescent Growth Spurt and Control of Puberty.
in The Control of the Onset of Puberty.
Ed. M. Grumbach, G. Grave and F. Mayer, 403 - 423, 1973.

FRIESEN, H., H. GUYDA, P. HWANG, J.E. TYSON and A. BARBEAU.
Functional Elevation of Prolactin Secretion : A Guide to Therapy.
J. Clin. Investig., 51, 706 - 709, 1972.

FUXE, K. and T. HÖKFELT.
Further Evidence for the Existence of Tubero-Infundibular Dopamine Neurons.
Acta Physiol. Scand., 66, 245 - 246, 1966.

FUXE, K., T. HÖKFELT and O. NILSSON.
Factors Involved in the Control of the Activity of the Tubero-Infundibular Dopamine Neurons During Pregnancy and Lactation.
Neuroendocrinol., 5, 257 - 270, 1969.

FUXE, K., T. HÖKFELT and O. NILSSON.
Castration, Sex Hormones and Tubero-Infundibular Dopamine Neurons.
Neuroendocrinol., 5, 107 - 120, 1969(a).

FUXE, K., H. CORRODI, T. HÖKFELT and G. JONSSON.
Central Monoamine Neurons and Pituitary Adrenal Activity.
Prog. Brain Res., 32, 42 - 56, 1970.

FUXE, K., T. HÖKFELT, C.D. SUNSTEDT, K. AHRÉN and L. HAMBERGER.
Amine Turnover Changes in the Tubero-Infundibular Dopamine (DA) Neurons in Immature Rats Injected with PMS.
Neuroendocrinol., 10, 282 - 300, 1972.

FUXE, K., T. HÖKFELT, I. AGNATI, A. LÖFSTRÖM, B.J. EVERITT, O. JOHANSSON, G. JONSSON, W. WUTTKE and M. GOLDSTEIN.
Role of Monoamines in the Control of Gonadotrophin Secretion.
Ed. T.C. Anand Kumar, 124 - 140.
in Neuroendocrine Regulation of Fertility, Karger-Basal, 1976.

FUXE, K., T. HÖKFELT, A. LÖFSTRÖM, O. JOHANSSON, L. AGNATI, B.J. EVERITT, M. GOLDSTEIN, S. JEFFCOATE, N. WHITE, P. ENEROTH, J.Å. GUSTAFSSON and P. SKETT.
On the Role of Neurotransmitters and Hypothalamic Hormones and Their Interactions in Hypothalamic and Extrahypothalamic Control of Pituitary Function and Sexual Behaviour.
in Subcellular Mechanisms in Reproductive Neuroendocrinology.
Eds. F. Naftolin, K.S. Ryan and J. Davies, 193 - 246.
Pub. Elsevier Sci. Pub. Co., Amsterdam, Neth., 1976 (a).

FUXE, K., P. ENEROTH, J.Å. GUSTAFSSON, A. LÖFSTRÖM and P. SKETT.
Dopamine in the Nucleus Accumbens : Preferential Increase of DA Turnover by Rat Prolactin.
Brain Res., 122, 177 - 182, 1977.

GALLO, R.V. and G.P. MOBERG.
Serotonin Mediated Inhibition of Episodic Luteinising Hormone Release During Electrical Stimulation of the Arcuate Nucleus in Ovariectomised Rats.
Endocrinol., 100, 945 - 954, 1977.

GEFFEN, L.B. and B.G. LIVETT.
Synaptic Vesicles in Sympathetic Neurons.
Physiol. Rev., 51, 98 - 157, 1971.

GERSTEN, B.E. and B.L. BAKER.
Local Action of Intrahypophyseal Implants of Estrogen as Revealed by Staining with Peroxidase-Labelled Antibody.
Am. J. Anat., 128, 1 - 20, 1970.

GIANUTSOS, G., R. DRAWBAUGH, M.D. HYNES and H. LAL.
Behavioural Evidence for Dopaminergic Supersensitivity After Chronic Haloperidol.
Life Sci., 14, 887 - 898, 1974.

GLASS, M.R., R.W. SHAW, W.R. BUTT, R.L. EDWARDS and D.R. LONDON.
An Abnormality of Oestrogen Feedback in Amenorrhoea-Galactorrhoea.
Brit. Med. Journ., 3, 274 - 275, 1975.

GNODDE, H.P. and G.A. SCHUILLING.
Involvement of Catecholaminergic and Cholinergic Mechanisms in the Pulsatile Release of LH in the Long-Term Ovariectomised Rat.
Neuroendocrinol., 20, 212 - 223, 1976.

GOLDBERG, L.I., P.P. SONNEVILLE and J.L. McNAY.
An Investigation of the Structural Requirements for Dopamine-like Renal Vasodilatation : Phenylethylamines and Apomorphine.
J. Pharmacol. Exp. Ther., 163, 188 - 197, 1968.

GOLDFINE, I.D.
Binding of Insulin to Thymocytes from Suckling and Hypophysectionised Rats. Evidence for Two Mechanisms Regulating Insulin Sensitivity.
Endocrinol., 97, 948 - 954, 1975.

GRANT, L.D. and W.E. STUMPF.
Localisation of ^{3}H Estradiol and Catecholamines in Identical Neurons in the Hypothalamus.
J. Histochem. Cytochem., 21, 404, 1973.

GREENGARD, P. and J.W. KEBABIAN.
Role of Cyclic AMP in Synaptic Transmission in the Mammalian Peripheral Nervous System.
Fed. Proc., 33, 1059 - 1067, 1974.

GREIBROKK, T., B.L. CURRIE, K.N.G. JOHANSSON, J.J. HANSEN, K. FOLKERS and C.Y. BOWERS.
Purification of a Prolactin-Inhibiting Hormone and the Revealing of a Hormone, D-GHIH, Which Inhibits the Release of Growth Hormone.
Biochem. Biophy. Res. Commun., 59, 704 - 709, 1974.

GREIBROKK, T., J. HANSEN, R. KNUDSEN, Y.K. LAM and K. FOLKERS.
On the Isolation of a Prolactin Inhibiting Factor (Hormone).
Biochem. Biophys. Res. Comm., 67, 338 - 344, 1975.

GROVES, P., C.J. WILSON, S.J. YOUNG and G.V. REBEC.
Self-Inhibition by Dopaminergic Neurons.
Science, 190, 522 - 529, 1975.

GUDELSKY, G.A., J. SIMPKINS, G.P. MUELLER, J. MEITES and K.E. MOORE.
Selective Actions of Prolactin on Catecholamine Turnover in the Hypothalamus and on Serum LH and FSH.
Neuroendocrinol., 22, 206 - 215, 1976.

GULDNER, F.H. and J.R. WOLFF.
Neurono-Glial Synaptoid Contacts in the Median Eminence of the Rat : Ultrastructure, Staining Properties and Distribution on Tanocytes.
Brain Res., 61, 217 - 234, 1973.

GUNAGA, K.P., A. KAWANO and K.M.J. MENON.
Effect of Estradiol Benzoate on the Accumulation of Adenosine 3', 5' Cyclic Monophosphate in the Rat Hypothalamus.
Neuroendocrinol., 16, 273 - 281, 1974.

HARDY, J.
Trans-Sphenoidal Microsurgical Removal of Pituitary Microadenoma.
Prog. Neurol. Surg., 6, 200, 1975.

HARMS, P.G., S.R. OJEDA and S.M. McCANN.
Prostaglandin Involvement in Hypothalamic Control of Gonadotropin and Prolactin Release.
Science, 181, 760 - 761, 1973.

HARRIS, G.W.
The Pituitary Stalk and Ovulation.
in Villie. Control of Ovulation, 56 - 74.
Pergamon Press, London, 1961.

HAUG, E. and K.M. GAUTVIK.
Effects of Sex Steroids on Prolactin Secreting Rat Pituitary Cells in Culture.
Endocrinol., 99, 1482 - 1489, 1976.

HAYEK, A. and J.D. CRAWFORD.
L Dopa and Pituitary Hormone Secretion.
Journ. Clin. Endocrinol. Metab., 34, 764 - 766, 1972.

HOFF, J.D., B.L. LASLEY, C.F. WANG and S.S.C. YEN.
The Two Pools of Pituitary Gonadontropin : Regulation During the Menstrual Cycle.
J. Clin. Endocrinol. Metab., 44, 302 - 312, 1977.

HÖKFELT, T.
The Possible Ultrastructural Identification of Tubero-Infundibular Dopamine Containing Nerve Endings in the Median Eminence of the Rat.
Brain Res., 5, 121 - 123, 1967.

HÖKFELT, T. and U. UNGERSTEDT.
Electron and Fluorescence Microscopical Studies of the Nucleus Caudate Putamen of the Rat After Unilateral Lesions of the Ascending Nigro-Neostriatal Dopamine Neurons.
Acta Physiol. Scand., 76, 415 - 426, 1969.

HÖKFELT, T. and K. FUXE.
On the Morphology and the Neuroendocrine Role of the Hypothalamic Catecholamine Neurons.
in Brain-Endocrine Interaction. Median Eminence : Structure and Function.
(Eds.) K.M. Knigge, D.E. Scott and A. Weindl, Karger-Basel, 1972.

HÖKFELT, T. and K. FUXE.
Effects of Prolactin and Ergot Alkaloids on the Tubero-Infundibular Dopamine (DA) Neurons.
Neuroendocrinol., 9, 100 - 122, 1972(a).

HÖKFELT, T., R. ELDE, O. JOHANSSON, L. TERENIUS and L. STEIN.
The distribution of Enkephalin-Immunoreactive Cell Bodies in the Rat Central Nervous System.
Neuroscience Letters, 5, 25 - 31, 1977.

HOLTZ, P.
Uber die Sympathicomimetische Winksamkiet von Gehirnextrakten.
Acta Physiol. Scand., 20, 354 - 362, 1950.

HOLTZ, P. and K. CREDNER.
Die Enzymetische Entsehung von Oxytyramin im Organismus und die Physiologische Bedeutung der Dopadecarboxylase.
Arch. Exp. Path. Pharmak., 200, 356 - 388, 1942.

HORNYKIEWICZ, O.
Dopamine in the Basal Ganglia.
Brit. Med. Bull., 29, 172 - 178, 1973.

HUCKER, H.B., A. HOCHBERG, E.A. HOFFMAN and J.K. MILLER.
Studies on the Physiological Distribution of Metoclopramide.
Pharmacologist, 8, 220, 1966.

HUGHES, J.
Isolation of an Endogenous Compound from the Brain with Pharmacological Properties Similar to Morphine.
Brain Res., 88, 295 - 308, 1975.

HYTTEL, J.
Changes in Dopamine Synthesis Rate in the Super-Sensitivity Phase After Treatment with a Single Dose of Neuroleptics.
Psychopharmacol., 51, 205 - 207, 1977.

KALUCY, R.S., A.H. CRISP, T. CHARD, A. McNEILLY, C.N. CHIEN and J.H. LACEY.
Nocturnal Hormonal Profiles in Massive Obesity, Anorexia Nervosa and Normal Females.
J. Psychosom. Res., 20, 595 - 604, 1976.

KAMBERI, I.A.
Brain Neurotransmitters and Their Interaction with the Hypothalamus-Pituitary-Gonadal Principles.
Adv. Biosci., 15, 249 - 266, 1975.

KAMBERI, I.A. and S.M. McCANN.
Effect of Biogenic Amines, FSH-Releasing Factor (FRF) and Other Substances on the Release of FSH by Pituitaries Incubated in vitro.
Endocrinol., 85, 815 - 824, 1969.

KAMBERI, I.A., R.S. MICAL and J.C. PORTER.
Effect of Anterior Pituitary Perfusion and Intraventricular Injection of Catecholamines and Indoleamines on LH Release.
Endocrinol., 87, 1 - 12, 1970.

KAMBERI, I.A., R.S. MICAL and J.C. PORTER.
Hypophysial Portal Vessel Infusion : in vivo Demonstration of LRF, FRF and PIF in Pituitary Stalk Plasma.
Endocrinol., 88, 1012 - 1020, 1971.

KAMBERI, I.S., R.S. MICAL and J.C. PORTER.
Effects of Melatonin and Serotonin on the Release of FSH and Prolactin.
Endocrinol., 88, 1288 - 1293, 1971(a).

KEBABIAN, J.W., G.L. PETZOLD and P. GREENGARD.
Dopamine-Sensitive Adenylate Cyclase in the Caudate Nucleus of the Rat Brain and Its Similarity to the "Dopamine Receptor".
Proc. Natl. Acad. Sci., USA, 69, 2145 - 2149, 1972.

KEBABIAN, J.W., Y.C. CLEMENT-CORMIER, G.L. PETZOLD and P. GREENGARD.
Chemistry of Dopamine Receptors.
in Advances in Neurol., Vol. 9, 1 - 11, Edit. D.B. Calne et al.
Raven Press, New York, 1975.

INGRAND, J. and R. BOULU.
Study of the Distribution of Tritiated Metoclopramide in the Mouse.
Thérapie, 25, 741 - 751, 1970.

IVERSEN, L.L., A.S. HORN and R.J. MILLER.
Actions of Dopaminergic Agonists on Cyclic AMP Production in Rat Brain Homogenates.
in Neurol., 197 - 212, Vol. 9, Edit. D.B. Calne et al.
Raven Press, New York, 1975.

JANSSEN, P.A.J., C.J.E. NIEMEGEERS, K. SCHELLEKENS and F.M. LENAERTS.
Is it Possible to Predict the Clinical Effects of Neuroleptic Drugs (Major Tranquillisers) from Animal Data.
Arzneimittel - Forsch., 17, 841 - 854, 1967.

JUSTIN-BESANÇON, L., C. LAVILLE and M. THOMINET.
Le Metoclopramide et ses Homologues. Introduction à Leur Étude Biologique.
C.R. Acad. Sc., Paris, 258, 4384 - 4386, 1964.

KALRA, S.P., K. AJIKA, L. KRULRICH, C.P. FAWCETT, M. QUIJADA and S.M. McCANN.
Effects of Hypothalamic and Pre-optic Electrochemical Stimulation on Gonadotropin and Prolactin Release in Pro-estrus Rats.
Endocrinol., 88, 1150 - 1158, 1971.

KALRA, P.S., S.P. KALRA, L. KRULICH, C.P. FAWCETT and S.M. McCANN.
Involvement of Norepinephrine in Transmission of the Stimulatory Influence of Progesterone on Gonadotropin Release.
Endocrinol., 90, 1168 - 1176, 1972.

KALRA, S.P. and S.M. McCANN,
Effect of Drugs Modifying Catecholamine Synthesis on LH Release Induced by Pre-optic Stimulation in the Rat.
Endocrinol., 93, 356 - 362, 1973.

KEHR, W., A. CARLSSON, M. LINDQVIST, M. MAGNUSSON and C.V. ATACK.
Evidence for a Receptor Mediated Feedback Control of Striatal Tyrosine Hydroxylase Activity.
J. Pharm. Pharmacol., 24, 744 - 747, 1972.

KING, J.A., A. ARIMURA and T.H. WILLIAMS.
Localisation of Luteinising Hormone-Releasing Hormone in Rat Hypothalamus Using Radioimmunoassay.
J. Anat., 120, 275 - 288, 1975.

KING, J.C., J.A. PARSONS, S.L. ERLANDSEN and T.H. WILLIAMS.
Luteinising Hormone-Releasing Hormone (LHRH) Pathway of the Rat Hypothalamus Revealed by the Unlabelled Antibody Peroxidase-Anti-Peroxidase Method.
Cell Tissue Res., 153, 211 - 217, 1974.

KIZER, J.S., A. ARIMURA, A.V. SCHALLY and M.J. BROWNSTEIN.
Absence of Luteinising Hormone-Releasing Hormone (LH-RH) from Catecholaminergic Neurons.
Endocrinol., 96, 523 - 525, 1975.

KLEIN, R.L., T.E. MILITELLO and C.M. BALLINGER.
Anti-Emetic Effect of Metoclopramide. Evaluation in Humans.
Anaesthesia and Analgesia, 47, 259 - 264, 1968.

KOBAYASHI, H., T. MATSUI and S. ISHII.
Functional Electron Microscopy of the Hypothalamic Median Eminence.
Int. Rev. Cytol., 29, 281 - 381, 1970.

KOCH, Y., K.H. LU and J. MEITES.
Biphasic Effects of Catecholamines on Pituitary Prolactin Release in vitro.
Endocrinol., 87, 673 - 675, 1970.

KOCH, Y., P. CHOBSIENG, R. ZOR, M. FRIDKIN and H.R. LINDNER.
Suppression of Gonadotropin Secretion and Prevention of Ovulation in the Rat by Antiserum to Synthetic Gonadotropin-Releasing Hormone.
Biochem. Biophys. Res. Commun., 55, 623 - 629, 1973.

KRAGT, C.L. and J. MEITES.
Dose Response Relationships Between Hypothalamic PIF and Prolactin Release by Rat Pituitary Tissue in vitro.
Endocrinol., 80, 1170 - 1173, 1967.

KRNJEVIC, K.
Electrophysiology of Dopamine Receptors.
in Advances in Neurology, 9, 12 - 24.
Eds. D.B. Calne, T.N. Chase and A. Barbeau, 1975.

KUEHN, D.C.G.
Claudii Galini Opera Omnia.
Pub. C. Crobloch, 4, 371. Leipzig, 1821.

KUENG, W., A. WIRZ-JUSTICE, R. MENZI and E. CHAPPUIS-ARNDT.
Regional Brain Variations of Tryptophan, Monoamines, Monoamine Oxidase Activity, Plasma Free and Total Tryptophan During the Estrous Cycle of the Rat.
Neuroendocrinol., 21, 289 - 296, 1976.

KURACHI, K. and K. HIROTA.
Catecholamine Metabolism in Rat's Brain Related with Sexual Cycle.
Endocrinol. Jap. Supp., 1, 69 - 73, 1969.

LACHELIN, G.C.L., S. ABU-FADIL and S.S.C. YEN.
Functional Delineation of Hyperprolactinaemic Amenorrhoea.
J. Clin. Endocrinol. Metab., 44, 1163 - 1174, 1977.

LACHELIN, G.C.L., H. LEBLANC and S.S.C. YEN.
The Inhibitory Effect of Dopamine Agonists on LH Release in Women.
J. Clin. Endocrinol. Metab., 44, 728 - 732, 1977(a).

LAL, H.
A Comparison of Narcotic Analgesics with Neuroleptics on Behavioural Measures of Dopaminergic Activity.
Life Science, 17, 29 - 34, 1975.

LAL, S., C.E. DE LA VEGA, T.L. SOURKES and H.G. FRIESEN.
Effect of Apomorphine on Growth Hormone, Prolactin, Luteinising Hormone and Follicle-Stimulating Hormone Levels in Human Serum.
J. Clin. Endocrinol. Metab., 37, 719 - 724, 1973.

LAL, H., W. BROWN, R. DRAWBAUGH, M. HYNES and G. BROWN.
Enhanced Prolactin Inhibition Following Chronic Treatment with Haloperidol and Morphine.
Life Science, 20, 101 - 106, 1977.

LASLEY, B.L., C.F. WANG and S.S.C. YEN.
The Effect of Estrogen and Progesterone on the Functional Capacity of the Gonadotrophs.
J. Clin. Endocrinol. Metab., 41, 820 - 826, 1975.

LAZARUS, L.H., N. LING and R. GUILLEMIN.
β Lipotropin as a Prohormone for the Morphinomimetic Peptides Endorphins and Enkephalins.
Proc. Nat. Acad. Sci., USA, 73, 2156 - 2159, 1976.

LEBLANC, H., G.C.L. LACHELIN, S. ABU-FADIL and S.S.C. YEN.
Effects of Dopamine Infusion on Pituitary Hormone Secretion in Humans.
J. Clin. Endocrinol. Metab., 43, 668 - 674, 1976.

LEVITT, M., S. SPECTOR, A. SJOEDSMA and S. UDENFRIEND.
Elucidation of the Rate Limiting Step in Norepinephrine Biosynthesis in the Perfused Guinea-Pig Heart.
J. Pharmacol., 148, 1 - 8, 1965.

L'HERMITE, M., M. VEKEMANS, A. CAUFRIEZ, J. MARTIN-COMIN and C. ROBYN.
Effects of Sulpiride-Induced Hyperprolactinaemia on Human Menstrual Cycle.
Acta Endo. Supp., 199, 322, 1975.

LIBERTUN, C. and S.M. McCANN.
Blockade of the Release of Gonadotropins and Prolactin by Subcutaneous or Intraventricular Injection of Atropin in Male and Female Rats.
Endocrinol., 92, 1714 - 1724, 1973.

LIBERTUN, C. and S.M. McCANN.
Further Evidence for Cholinergic Control of Gonadotropin and Prolactin Secretion.
Proc. Soc. Exp. Biol. Med., 147, 498 - 504, 1974.

LICHTENSTEIGER, W.
Cyclic Variations of Catecholamine Content in Hypothalamic Nerve Cells During the Estrous Cycle of the Rat, with a Concomitant Study of the Substantia Nigra.
J. Pharmacol. Exp. Therap., 165, 204 - 215, 1969.

LIEBERMAN, A., M. ZOLFAGHARI and D. BOAL.
The Antiparkinsonian Efficacy of Bromocriptine.
Neurol., 26, 405 - 409, 1976.

LIEBERMAN, A., M. KUPPERSMITH, E. ESKEY and M. GOLDSTEIN.
Treatment of Parkinson's Disease with Bromocriptine.
New Eng. J. Med., 295, 1400 - 1404, 1976.

LIPPMAN, W.
Relationship Between Hypothalamic Norepinephrine and Serotonin and Gonadotrophin Secretion in the Hamster.
Nature, 218, 173 - 174, 1968.

LLOYD, S.J., J.B. JOSIMOVICH and D.F. ARCHER.
Amenorrhoea and Galactorrhoea : Results of Therapy with 2 Bromo-α-Ergocryptine (C.B.154).
Am. J. Obstet. Gynacol., 122, 85 - 89, 1975.

LOEWI, O.
Uber Humorale Ubertragbarkeit der Hernznervenwirkung.
Pflugers. Arch., 189, 239 - 242, 1921.

LÖFSTRÖM, A., G. JONSSON and K. FUXE.
Microfluorimetric Quantitation of Catecholamine Fluorescence in Rat Median Eminence.
I - Aspects on the Distribution of Dopamine and Noradrenaline Nerve Terminals.
J. Histochem. Cytochem., 24, 415 - 429, 1976.

LÖFSTRÖM, A., P. ENEROTH, J.Å. GUSTAFSSON and P. SKETT.
Effects of Estradiol Benzoate on Catecholamine Levels and Turnover in Discrete Areas of the Median Eminence and the Limbic Forebrain, and on Plasma LH, FSH, and Prolactin Concentrations in the Ovariectomised Female Rat.
Endocrinol., 101, 1559 - 1569, 1977.

LU, K.H., Y. AMENOMORI, C.L. CHEN and J. MEITES.
Effects of Central Acting Drugs on Serum and Pituitary Prolactin Levels in Rats.
Endocrinol., 87, 667 - 672, 1970.

LU, K.H. and J. MEITES.
Effects of L Dopa on Serum Prolactin and PIF in Intact and Hypophysectomised Pituitary-Grafted Rats.
Endocrinol., 91, 868 - 972, 1972.

McCANN, S.M.
A Hypothalamic Luteinising Hormone-Releasing Factor.
Am. J. Physiol., 202, 395 - 400, 1962.

McCANN, S.M.
Luteinising-Hormone-Releasing-Hormone.
New Eng. J. Med., 296, 797 - 802, 1977.

McCANN, S.M., S. TALEISNIK and H.M. FRIEDMAN.
LH-Releasing Activity in Hypothalamic Extracts.
Proc. Soc. Exp. Biol. Med., 104, 432 - 434, 1960.

MacLEOD, R.M., E.H. FONTHAM and J.E. LEHMEYER.
Prolactin and Growth Hormone Production as Influenced by Catecholamines and Agents that Affect Brain Catecholamines.
Neuroendocrinol., 6, 283 - 294, 1970.

MacLEOD, R.M. and J.E. LEHMEYER.
Regulation of the Synthesis and Release of Prolactin.
in Lactogenic Hormones, 53 - 76.
Eds. G.E.W. Wolstenholme and J. Knight, Churchill Livingstone, Edin., 1972.

MacLEOD, R.M. and J.E. LEHMEYER.
Studies on the Mechanism of the Dopamine-Mediated Inhibition of Prolactin Secretion.
Endocrinol., 94, 1077 - 1085, 1974.

MacLEOD, R.M. and C. ROBYN.
Mechanism of Increased Prolactin Secretion by Sulpiride.
J. Endocrinol., 72, 273 - 277, 1977.

McCORMACK, J.T. and E. KNOBIL.
The Suppression, by an Antiserum to LHRH, of Tonic LH and FSH Secretion in the Rhesus Monkey.
Abst. 108. 57th Annual Meeting Endocrine Society, New York, 1975.

McNEILLY, A.S., M.O. THORNER, G. VOLANS and G.M. BESSER.
Metoclopramide and Prolactin.
Brit. Med. J., 2, 729, 1974.

MALACARA, J.M., E. SEYLER and S. REICHLIN.
Luteinising Hormone-Releasing Factor Activity in Peripheral Blood from Women During the Mid-Cycle Luteinising Hormone Ovulatory Surge.
J. Clin. Endocrinol. Metab., 34, 271 - 278, 1972.

MALARKEY, W.B.
Non-Puerperal Lactation and Normal Prolactin Regulation.
J. Clin. Endocrinol. Metab., 40, 198 - 204, 1975.

MALARKEY, W.B., L.S. JACOBS and W.H. DAUGHADAY.
Levodopa Suppression of Prolactin in Non-Puerperal Galactorrhoea.
New Eng. Journ. Med., 285, 1160 - 1163, 1971.

MALARKEY, W.B. and J.C. JOHNSON.
Pituitary Tumours and Hyperprolactinaemia.
Arch. Int. Med., 136, 40 - 44, 1976.

MALMÉJAC, J., C. LAVILLE and J. MARGARIT.
Technique pour l'Étude de l'Action Centrale de Poisons Émétiques et Anti-Émétique.
Comp. Rend. Seances Soc. Biol. Fil., 158, 964 - 965, 1964.

MANCINI, A.M., A. GUITELMAN and L. DEBEJUK.
Effect of Administration of Sulpiride on Serum Follicle Stimulating Hormone and Luteinising Hormone Levels in a Group of Post-Menopausal Women.
J. Endocrinol., 67, 127 - 128, 1975.

MANNICH, C. and W. JACOBSOHN.
Uber Oxyphenyl Alkamine und Dioxyphenyl Alkylamine.
Ber. Chem. Ges., 43, 189, 1909.

MARKEE, J.E., C.H. SAWYER and W.H. HOLLINGSHEAD.
Adrenergic Control of the Release of Luteinising Hormone from the Hypophysis of the Rabbit.
Recent Progress Hormone Res., 2, 117 - 131, 1948.

MARSHALL, J.C. and T. RUSSELL FRASER.
Amenorrhoea in Anorexia Nervosa : Assessment and Treatment with Clomiphene Citrate.
Brit. Med. J., 4, 590 - 592, 1971.

MARTIN, J.B., S. LAL and G. TOLIS.
Inhibition by Apomorphine of Prolactin Secretion in Patients with Elevated Serum Prolactin.
J. Clin. Endocrinol. Metab., 39, 180 - 182, 1974.

MATSUO, H., Y. BABA, R.M.G. NAIR, A. ARIMURA and A.V. SCHALLY.
Structure of the Porcine LH and FSH Releasing Hormone.
I - The Proposed Aminoacid Sequence.
Biochem. Biophys. Res. Comm., 43, 1334 - 1339, 1971.

MAURER, R.
^{3}H Estradiol Binding Macromolecules in the Hypothalamus and Anterior Pituitary of Normal Female, Androgenised Female and Male Rats.
Brain Res., 67, 175 - 177, 1974.

MEITES, J., R.H. KAHN and C.S. NICOLL.
Prolactin Production by Rat Pituitary in vitro.
Proc. Soc. Exp. Biol. Med., 108, 440 - 443, 1961.

MEYERSON, B. and C.H. SAWYER.
Monoamines and Ovulation in the Rat.
Endocrinol., 83, 170 - 176, 1968.

MIDGLEY, A.R. and R.B. JAFFE.
Regulation of Human Gonadotropin :
X - Episodic Fluctuation of LH During the Menstrual Cycle.
J. Clin. Endocrinol. Metab., 33, 962 - 969, 1971.

MINAGUCHI, H. and J. MEITES.
Effects of Suckling on Hypothalamic LH-Releasing Factor and Prolactin Inhibiting Factor, and on Pituitary LH and Prolactin.
Endocrinol., 80, 603 - 607, 1967.

MISHRA, R.K., E.L. GARDNER, R. KATZMAN and M.H. MAKMAN.
Enhancement of Dopamine-Stimulated Adenyl Cyclase Activity in Rat Caudate After Lesions in Substantia Nigra : Evidence for Denervation Supersensitivity.
Proc. Natl. Acad. Sci., USA, 71, 3883 - 3887, 1974.

MIYACHI, Y., R.S. MECKLENBERG and M.B. LIPSETT.
In vitro Studies of Pituitary-Median Eminence Unit.
Endocrinol., 93, 492 - 503, 1973.

MONTAGUE, K.A.
Catechol Compounds in Rat Tissues and in Brains of Different Animals.
Nature, 180, 244 - 245, 1957.

MORTIMER, C.H., G.M. BESSER and A.S. McNEILLY.
Gonadotropin Releasing Hormone Therapy in the Induction of Puberty, Potency, Spermatogenesis and Ovulation in Patients with Hypothalamic Pituitary-Gonadal Dysfunction.
in Hypothalamic Hormones, 325 - 336, Academic Press, London, 1975.
Eds. M. Motta, P.G. Crosignani and L. Martini.

MUELLER, G.P., J. SIMPKINS, J. MEITES and K.E. MOORE.
Differential Effects of Dopamine Agonists and Haloperidol on Release of Prolactin, Thyroid Stimulating Hormone, Growth Hormone and Luteinising Hormone in Rats.
Neuroendocrinol., 20, 121 - 135, 1976.

NAFTOLIN, F., S.S.C. YEN, D. PERLMAN, C.C. TSAI, D.C. PARKER and T. VARGO.
Nocturnal Patterns of Serum Gonadotropins During the Menstrual Cycle.
J. Clin. Endocrinol. Metab., 37, 6 - 10, 1973.

NAIK, D.V.
Immunoreactive LHRH Neurons in the Hypothalamus Identified by Light and Fluorescent Microscopy.
Cell Tissue Res., 157, 423 - 436, 1975.

NAKRA, B.R.S., A.J. BOND and M.H. LADER.
Comparative Psychotropic Effects of Metoclopramide and Prochlorperazine in Normal Subjects.
J. Clin. Pharmacol., 15, 449 - 454, 1975.

NETT, T.M. and T.E. ADAMS.
Further Studies on the Radioimmunoassay of Gonadotropin-Releasing Hormone : Effects of Radioiodination, Antiserum and Unextracted Serum on Levels of Immunoreactivity in Serum.
Endocrinol., 101, 1135 - 1144, 1977.

NICOLL, C.S. and MEITES, J.
Estrogen-Stimulation of Prolactin Production by Rat Adenohypophysis in vitro.
Endocrinol., 70, 272 - 277, 1962.

NOEL, G.L., R.C. DIMOND, L. WARTOFSKY, J.M. EARL and A.G. FRANTZ.
Studies of Prolactin and TSH Secretion by Continuous Infusion of Small Amounts of Thyrotropin-Releasing Hormone (TRH).
J. Clin. Endocrinol. Metab., 39, 6 - 17, 1974.

NYBACH, H. and G. SEDVALL.
Further Studies on the Accumulation and Disappearance of Catecholamines Formed from Tyrosine ^{14}C in Mouse Brain. Effect of Some Phenothiazine Analogues.
Europ. J. Pharmacol., 10, 193 - 205, 1970.

OJEDA, S.R., P.G. HARMS and S.M. McCANN.
Effect of Blockade of Dopaminergic Receptors on Prolactin and LH Release : Median Eminence and Pituitary Sites of Action.
Endocrinol., 94, 1650 - 1657, 1974.

OJEDA, S.R., P.G. HARMS and S.M. McCANN,
Effect of Inhibitors of Prostaglandin Synthesis on Gonadotropin Release in the Rat.
Endocrinol., 97, 843 - 854, 1975.

OLENDORF, W.H.
Brain Uptake of Radio-Labelled Aminoacids, Amines and Hexoses After Arterial Injection.
Am. J. Physiol., 221, 1629 - 1639, 1971.

OLIVER, G. and E.A. SCHAFFER.
On the Physiological Action of Extract of the Suprarenal Capsules.
J. Physiol., 16, Proc. p. 1, 1984.

OLSON, L., K. FUXE and T. HÖKFELT.
The Effect of Pituitary Transplants on the Tubero-Infundibular Dopamine Neurons in Various Endocrine States.
Acta Endocrinol., 71, 233 - 244, 1972.

OSLAND, R.B., R.V. GALLO and J.A. WILLIAMS.
In vitro Release of Luteinising Hormone from Anterior Pituitary Fragments Superfused with Constant or Pulsatile Amounts of Luteinising Hormone-Releasing Factor.
Endocrinol., 96, 1210 - 1215, 1975.

PALKOVITS, M., A. ARIMURA, M. BROWNSTEIN, A.V. SCHALLY and J.M. SAAVEDRA.
Luteinising Hormone-Releasing Hormone (LHRH) Content of the Hypothalamic Nuclei in the Rat.
Endocrinol., 95, 554 - 558, 1974.

PARKER, D.C., H.L. JUDD, L.G. ROSSMAN and S.S.C. YEN.
Pubertal Sleep-Wake Patterns of Episodic LH, FSH, and Testosterone Release in Twin-Boys.
J. Clin. Endocrinol. Metab., 40, 1099 - 1109, 1975.

PERINGER, E., P. JENNER and C.D. MARSDEN.
Effect of Metoclopramide on Turnover of Brain Dopamine, Noradrenaline and 5-Hydroxytryptamine.
J. Pharm. Pharmacol., 27, 442 - 444, 1975.

PERINGER, E., P. JENNER, I.M. DONALDSON, C.D. MARSDEN and R. MILLER.
Metoclopramide and Dopamine Receptor Blockade.
Neuropharmacol., 15, 463 - 469, 1976.

PERT, C.B. and S.H. SNYDER.
Opiate Receptor : Demonstration in Nervous Tissue.
Science, 179, 1011 - 1014, 1973.

PIJNENBERG, A.J.J. and J.M. VAN ROSSUM.
Stimulation of Locomotor Activity Following Injection of Dopamine into the Nucleus Accumbens.
J. Pharmac. Pharmacol., 25, 1003 - 1005, 1973.

POIRIER, L.J. and T.L. SOURKES.
Influence of the Substantia Nigra on the Catecholamine Content of the Striatum.
Brain, 88, 181 - 192, 1965.

POLANSKY, S., E. MEUCHLER and S. SORRENTINO Jr.
The Effect of L Dopa and Clomiphene Citrate on Peripheral Plasma Levels of Luteinising Hormone-Releasing Factor.
Obstet. Gynaecol., 48, 79 - 83, 1976.

PORTER, S.C., R.S. MICAL and O.M. CRAMER.
Effect of Serotonin and Other Indoles on the Release of LH, FSH and Prolactin.
Gynaecol. Invest., 2, 13 - 22, 1972.

PORTIG, P.J. and M. VOGT.
Activities of a Dopaminergic Nigro-Striatal Pathway.
J. Physiol., 197, 20 - 21, 1968.

QUIJADA, M., P. ILLNER, L. KRULICH and S.M. McCANN.
The Effect of Catecholamines on Hormone Release from Anterior Pituitaries and Ventral Hypothalami Incubated in vitro.
Neuroendocrinol., 13, 151 - 163, 1973.

RAAB, W.
Adrenaline and Related Substances in Blood and Tissue.
Biochem. Journ., 37, 470 - 473, 1943.

RAKOFF, J.S., T.M. SILER, Y.N. SINHA and S.S.C. YEN.
Prolactin and Growth Hormone Release in Response to Sequential Stimulation by Arginine and Synthetic TRF.
J. Clin. Endocrinol. Metab., 37, 641 - 644, 1973.

RIESE, W.
Milchsekretion und Zwischenhirn.
Klin. Woch., 41, 1954 - 1955, 1928.

SANO, I., T. GAMO, Y. TAKIMOTO, K. TANIGUCHI, M. TAKESDA and K. NISHINUMA.
Distribution of Catechol Compounds in Human Brain.
Biochim. Biophys. Acta, 32, 586 - 587, 1959.

SARKAR, D.K., S.A. CHIAPPA, G. FINK and N.W. SHERWOOD.
Gonadotropin-Releasing Hormone Surge in the Pro-estrus Rat.
Nature, 264, 461 - 463, 1976.

SAWYER, C.H., J.E. MARKEE and W.H. HOLLINGSHEAD.
Inhibition of Ovulation in the Rabbit by the Adrenergic-Blocking Agent Dibenamine.
Endocrinol., 41, 395 - 402, 1947.

SAWYER, C.H., J. HILLIARD, S. KANEMATSU, R. SCARAMUZZI and C.A. BLAKE.
Effects of Intraventricular Infusions of Norepinephrine and Dopamine on LH Release and Ovulation in the Rabbit.
Neuroendocrinol., 15, 328 - 337, 1974.

SCHALLY, A.V., A. KUROCHUMA, Y. ISHIDO, T.W. REDDING and C. BOWERS.
The Presence of Prolactin Inhibiting Factor (PIF) in Extracts of Beef, Sheep and Pig Hypothalamus.
Proc. Soc. Exp. Biol. Med., 118, 350 - 352, 1965.

SCHALLY, A.V., T.W. REDDING, A. ARIMURA, A. DUPONT and G.L. LINTHICUM.
Isolation of Gamma-Amino Butyric Acid from Pig Hypothalami and Demonstration of its Prolactin Release-Inhibiting (PIF) Activity in vivo and in vitro.
Endocrinol., 100, 681 - 691, 1977.

SCHNEIDER, H.P.G. and S.M. McCANN.
Possible Role of Dopamine as Transmitter to Promote Discharge of LH-Releasing Factor.
Endocrinol., 85, 121 - 132, 1969.

SCHNEIDER, H.P.G. and S.M. McCANN.
Mono and Indolamines and Control of LH Secretion.
Endocrinol., 86, 1127 - 1133, 1970.

SEEMAN, P.
Anti-Schizophrenic Drugs - Membrane Receptor Sites of Action.
Biochem. Pharmacol., 26, 1741 - 1748, 1977.

SELMANOFF, M.K., M.J. PRAMIK-HOLDAWAY and R.I. WEINER.
Concentrations of Dopamine and Norepinephrine in Discrete Hypothalamic Nuclei During the Rat Estrous Cycle.
Endocrinol., 99, 326 - 329, 1976.

SETALO, G., S. VIGH, A.V. SCHALLY, A. ARIMURA and B. FLERKO.
LHRH Containing Neural Elements in the Rat Hypothalamus.
Endocrinol., 96, 135 - 142, 1975.

SEYLER, L.E., and S. REICHLIN.
Luteinising Hormone-Releasing Factor (LRF) in Plasma of Post-Menopausal Women.
J. Clin. Endocrinol. Metab., 37, 197 - 203, 1973.

SHAAR, C.J. and J.A. CLEMENS.
The Role of Catecholamines in the Release of Anterior Pituitary Prolactin in vitro.
Endocrinol., 95, 1202 - 1212, 1974.

SHELESNYAK, M.C.
Maintenance of Gestation in Ergotoxine Treated Pregnant Rats by Exogenous Prolactin.
Acta Endocrinol., 27, 99 - 109, 1958.

SIEGEL, R.E.
Galen on Psychology, Psychopathology and Function and Diseases of the Nervous System.
Pub. Karger-Basel, p. 85, 1968.

SIMON, E.J., J.M. HILLER and I. EDELMAN.
Stereospecific Binding of the Potent Narcotic Analgesic ^{3}H-Etorphine to Rat Brain Homogenate.
Proc. Nat. Acad. Sci., USA, 70, 1947 - 1949, 1973.

SIMONOVIC, I., M. MOTTA and L. MARTINI.
Acetycholine and the Release of the Follicle - Stimulating Hormone - Releasing Factor.
Endocrinol., 95, 1373 - 1379, 1974.

SMALSTIG, B., B.D. SAWYER and J.A. CLEMENS.
Inhibition of Rat Prolactin Release by Apomorphine in vivo and in vitro.
Endocrinol., 95, 123 - 129, 1974.

SMEE, M.L. and D.H. OVERSTREET.
Alterations in the Effects of Dopamine Agonists and Antagonists on General Activity in Rats Following Chronic Morphine Treatment.
Psychopharmacol., 49, 125 - 130, 1976.

SOURKES, T.L., L.J. POIRIER and S. LAL.
Diseases of the Basal Ganglia.
in Molecular Pathology.
Eds. S.B. Day and R.A. Good.

Pub. C.C. Thomas, Springfield, Ill., 1975.

SPIES, H.G. and R.L. NORMAN.
Luteinising Hormone Release and Ovulation Induced by the Intra-ventricular Infusion of Prostaglandin E_1 into Pentobarbital-Blocked Rats.
Prostaglandins, 4, 131 - 141, 1973.

STEFANO, F.J.E. and A.O. DONOSO.
Norepinephrine Levels in the Rat Hypothalamus During the Estrus Cycle.
Endocrinol., 81, 1405 - 1406, 1967.

TAKAHARA, J., A. ARIMURA and A.V. SCHALLY.
Suppression of Prolactin Release by a Purified Porcine PIF Preparation and Catecholamines Infused into Rat Hypophysial Portal Vessels.
Endocrinol., 95, 462 - 465, 1974.

TALWALKER, P.K., A. RATNER and J. MEITES.
In vitro Inhibition of Pituitary Prolactin Synthesis and Release by Hypothalamic Extract.
Am. J. Physiol., 205, 213 - 218, 1963.

TARSY, D., J.D. PARKES and C.D. MARSDEN.
Metoclopramide and Pimozide in Parkinson's Disease and Levodopa-Induced Dyskinesias.
J. Neurol. Neurosurg. Psych., 38, 331 - 335, 1975.

THORNER, A.O., A.S. McNEILLY, C. HAGAN and G.M. BESSER.
Long-Term Treatment of Galactorrhoea and Hypogonadism with Bromocriptine.
Brit. Med. Journ., 2, 419 - 422, 1974.

TOLIS, G.E., J. PINTER and H.G. FRIESEN.
The Acute Effect of 2 Bromo-α -Ergocryptine (C.B.154) on Anterior Pituitary Hormones and Free Fatty Acids in Man.
Int. Journ. Clin. Pharmacol., 12, 281, 1973.

TSAFRIRI, A., Y. KOCH and H.R. LINDNER.
Ovulation Rate and Serum LH Levels in Rats Treated with Indomethacin or Prostaglandin E_2.
Prostaglandins, 3, 461 - 467, 1973.

UEMURA, H. and H. KOBAYASHI.
Effects of Dopamine Implanted in the Median Eminence on the Estrous Cycle of the Rat.
Endocrinol., Japan, 18, 91 - 100, 1971.

UNGERSTEDT, U.
Post-Synaptic Super-Sensitivity After 6-Hydroxydopamine-Induced Degeneration of the Nigro-Striatal Dopamine System.
Acta Physiol. Scand., Supp. 367, 69 - 93, 1971.

UNGERSTEDT, U.
Stereotaxic Mapping of the Monoamine Pathways in the Rat Brain.
Acta Physiol. Scand., Supp. 367, 1 - 48, 1971(a).

VAKIL, S.D., D.B. CALNE and J.L. REID.
Pyridyl-Piperonyl-Piperazine (E.T.495) in Parkinsonism.
Adv. Neurol., 3, 121 - 125, 1973.

VALE, W., G. GRANT, M. AMOSS, R. BLACKWELL and R. GUILLEMIN.
Culture of Enzymatically Dispersed Anterior Pituitary Cells : Functional Validation of a Method.
Endocrinol., 91, 562 - 572, 1972.

VON ESSEN, C.
Effects of Dopamine on Cerebral Blood Flow in the Dog.
Acta Neurol. Scand., 50, 39 - 52, 1974.

VOGT, M.
The Concentration of Sympathin in Different Parts of the Central Nervous System Under Normal Conditions and After Administration of Drugs.
J. Physiol., 123, 451 - 481, 1954.

VON EULER, U.S.
A Specific Sympathominetic Ergone in Adrenergic Nerve Fibres (Sympathin) and its Relation to Adrenaline and Noradrenaline.
Acta Physiol. Scand., 12, 73 - 97, 1946.

WALTERS, J.R. and R.H. ROTH.
Dopaminergic Neurons : Drug Induced Antagonism of the Increase in Tyrosine Hydroxylase Activity Produced by Cessation of Impulse Flow.
J. Pharmac. Exp. Therap., 191, 82 - 91, 1974.

WATANABE, S. and S.M. McCANN.
Localisation of FSH-Releasing Factor in the Hypothalamus and Neurohypophysis as Determined by in vitro Assay.
Endocrinol., 82, 664 - 673, 1968.

WATSON, J.T., L. KRULICH and S.M. McCANN.
Effect of Crude Rat Hypothalamic Extract on Serum Gonadotropin and Prolactin Levels in Normal and Orchidectomised Male Rats.
Endocrinol., 89, 1412 - 1418, 1971.

WEIL-MALHERBE, H., J. AXELROD and R. TOMCHICK.
Blood-Brain Barrier for Adrenaline.
Science, 129, 1226 - 1227, 1959.

WEISSMAN, B.A. and D.F. JOHNSON.
Possible Role of Dopamine in Diethyl-Stilbesterol — Elicited Accumulation of Cyclic AMP in Incubated Male Rat Hypothalamus.
Neuroendocrinol., 21, 1 - 9, 1976.

WELSCH, C.W., M.D. SQUIERS, E. CASSELL, C.L. CHEN and J. MEITES.
Median Eminence Lesions and Serum Prolactin : Influence of Ovariectomy and Ergocornine.
Am. J. Physiol., 221, 1714 - 1717, 1971.

WILKES, M.M., K.H. LU and S.S.C. YEN.
Reduction of Dopamine Levels in the Median Eminence of the Hypothalamus in Aging Rats.
Abstract 384, Proc. 59th Annual Meeting Endocrine Society, Chicago, 1977.

WILSON, C.A.
Hypothalamic Amines and the Release of Gonadotrophins and Other Anterior Pituitary Hormones.
Adv. Drug. Res., 8, 119 - 204, 1974.

WILSON, C.W., A.W. MURRAY and E. TITUS.
The Effects of Reserpine on Uptake of Epinephrine in Brain and Certain Areas Outside the Blood-Brain Barrier.
J. Pharmacol., 135, 11 - 16, 1962.

WISLOCKI, G.B. and E.H. LEDUC.
Vital Staining of the Hemato-Encephalic Barrier by Silver Nitrate and Trypan Blue, and Cytological Comparisons of the Neurophysis, Pineal Body, Area Postrema, Inter-Columnar Tubercule and Supra-Optic Crest.
J. Comp. Neurol., 96, 371 - 398, 1952.

WOODRUFF, G.N. and R.J. WALKER.
The Effect of Dopamine and Other Compounds on the Activity of Neurons of Helix Aspersa : Structure Activity Relationships.
Int. J. Neuropharmacol., 8, 279 - 286, 1969.

WOODS, W.H., R.C. HOLLAND and E.W. POWELL.
Connections of Cerebral Structures Functioning in Neurohypophysial Hormone Release.
Brain Res., 12, 26 - 46, 1969.

WUTTKE, W., E. CASSELL and J. MEITES.
Effects of Ergocornine on Serum Prolactin and LH, and on Hypothalamic Content of PIF and LRF.
Endocrinol., 88, 737 - 741, 1971.

YEN, S.S.C.
Neuroendocrine Aspects of the Regulation of Cyclic Gonadotropin Release in Women.
Prog. Reprod. Biol., 2, 150, 1977.

YEN, S.S.C.
Chronic Anovulation Due to Inappropriate Feedback System. Chapter 14.
in Reproductive Endocrinology, Physiology, Pathophysiology and Clinical Management.
Eds. S.S.C. Yen and R.B. Jaffe. Pub. W.B. Saunders, 1978.

YEN, S.S.C., O. LLERENA, B. LITTLE and O.H. PEARSON.
Disappearance Rates of Endogenous Luteinising Hormone and Chorionic Gonadotropin in Man.
J. Clin. Endocrinol. Metab., 28, 1763 - 1767, 1968.

YEN, S.S.C., L.A. LLERENA, O.H. PEARSON and A.S. LITTELL.
Disappearance Rates of Endogenous Follicle Stimulating Hormone in Serum Following Surgical Hypophysectomy in Man.
J. Clin. Endocrinol. Metab., 30, 325 - 329, 1970.

YEN, S.S.C., P. VELA, J. RANKIN and A.S. LITTELL.
Hormonal Relationships During the Menstrual Cycle.
J. Am. Med. Assoc., 211, 1513 - 1517, 1970(a).

YEN, S.S.C., C.C. TSAI, F. NAFTOLIN, G. VAN DEN BERG and L. AJABOR.
Pulsatile Patterns of Gonadotropin Release in Subjects With and Without Ovarian Function.
J. Clin. Endocrinol. Metab., 34, 671 - 675, 1972.

YEN, S.S.C., B.L. LASLEY, C.F. WANG, H. LEBLANC and T.M. SILER.
The Operating Characteristics of the Hypothalamic - Pituitary System During the Menstrual Cycle and Observations of the Biological Action of Somatostatin.
Recent Prog. Horm. Res., 31, 321 - 357, 1975.

YEN, S.S.C. and A. LEIN.
The Apparent Paradox of the Negative and Positive Feedback Control System on Gonadotropin Secretion.
Am. J. Ob. Gyn., 126, 942 - 954, 1976.

YOUNG, R.L., E.M. BRADLEY, J.W. GOLDZIEHER, P.W. MYERS and F.R. LECOCQ.
Spectrum of Non-Puerperal Galactorrhoea : Report of Two Cases Evolving Through Various Syndromes.
J. Clin. Endocrinol. Metab., 27, 461 - 466, 1967.

ZARATE, A.E., S. CANALES, J. SORIA, P.J. MANEIRO and C. MacGREGOR.
Effect of Acute Administration of L Dopa on Serum Concentrations of Follicle Stimulating Hormone (FSH) and Luteinising Hormone (LH) in Patients With Amenorrhoea - Galactorrhoea Syndrome.
Neuroendocrinol., 12, 362 - 365, 1973.

ZARATE, A.E., L.S. JACOBS, E.S. CANALES, A.V. SCHALLY, A. DE LA CRUZ, J. SORIA and W.H. DAUGHADAY.
Functional Evaluation of Pituitary Reserve in Patients With the Amenorrhoea - Galactorrhoea Syndrome Utilising Luteinising Hormone - Releasing Hormone (LHRH), L Dopa and Chlorpromazine.
J. Clin. Endocrinol. Metab., 37, 855 - 859, 1973(a).

ZIMMERMAN, E.A., K.C. HSU, M. FERIN and G.P. KOZLOWSKI.
Localisation of Gonadotropin-Releasing Hormone (Gn RH) in the Hypothalamus of the Mouse by Immuno-Peroxidase.
Endocrinol., 95, 1 - 8, 1974.