THE ACTIONS OF 5-HYDROXYTRYPTAMINE ON THE MARMOSET VASCULATURE

Suzanne Marie Dyer BSc(Hons)

Thesis submitted for the degree of

Doctor of Philosophy

in the

Department of Clinical and Experimental Pharmacology
The University of Adelaide
(Faculty of Medicine)
CONTENTS

ABSTRACT ............................................................................................................. ix

DECLARATION ................................................................................................. xi

PUBLICATIONS IN SUPPORT OF THIS THESIS .............................................. xii

ACKNOWLEDGEMENTS ............................................................................... xiii

ABBREVIATIONS ........................................................................................ xiv

CHAPTER 1

INTRODUCTION ................................................................................................. 1

1.1 Epidemiology of cardiovascular disease .................................................. 1

1.2 Vasospasm .................................................................................................. 2

1.3 Thromboxane A2 ...................................................................................... 3

1.4 5-Hydroxytryptamine .............................................................................. 5

1.4.1 5-Hydroxytryptamine Receptors ....................................................... 6

1.4.2 In Vivo Vascular Responses to 5-HT .................................................. 12

1.4.3 In Vitro Vascular Responses to 5-HT .................................................. 14

1.4.4 General Vascular Effects of 5-HT ....................................................... 18

1.5 Vascular Responses to Aggregating Platelets ......................................... 19

1.6 Vascular Morphology ............................................................................... 20

1.6.1 The Vascular Endothelium ................................................................. 21

1.7 Vascular Disease States .......................................................................... 23
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7.1 Atherosclerosis and Plasma Lipids</td>
<td>23</td>
</tr>
<tr>
<td>1.7.2 Atherosclerosis and Vascular Morphology</td>
<td>25</td>
</tr>
<tr>
<td>1.7.3 The Response-to-Injury Hypothesis</td>
<td>26</td>
</tr>
<tr>
<td>1.7.4 Balloon Angioplasty</td>
<td>27</td>
</tr>
<tr>
<td>1.8 Vascular Responses in Cardiovascular Disease States</td>
<td>29</td>
</tr>
<tr>
<td>1.8.1 Vascular Responses to 5-HT in Cardiovascular Disease States</td>
<td>30</td>
</tr>
<tr>
<td>1.9 Summary and Aims</td>
<td>35</td>
</tr>
<tr>
<td>CHAPTER 2</td>
<td>37</td>
</tr>
<tr>
<td>GENERAL METHODS</td>
<td></td>
</tr>
<tr>
<td>2.1 Animals</td>
<td>37</td>
</tr>
<tr>
<td>2.1.1 Tissue Collection</td>
<td>38</td>
</tr>
<tr>
<td>2.1.2 Blood Sampling</td>
<td>38</td>
</tr>
<tr>
<td>2.2 Organ Bath Preparations</td>
<td>39</td>
</tr>
<tr>
<td>2.2.1 Aortic Ring Preparation</td>
<td>39</td>
</tr>
<tr>
<td>2.2.2 Carotid Artery Preparations</td>
<td>41</td>
</tr>
<tr>
<td>2.2.3 Mesenteric Artery Preparations</td>
<td>41</td>
</tr>
<tr>
<td>2.2.4 Coronary Artery Preparations</td>
<td>41</td>
</tr>
<tr>
<td>2.3 Cyclic AMP Formation</td>
<td>44</td>
</tr>
<tr>
<td>2.4 Protein Measurement</td>
<td>45</td>
</tr>
<tr>
<td>2.5 Plasma Lipids</td>
<td>46</td>
</tr>
<tr>
<td>2.5.1 Plasma Cholesterol</td>
<td>46</td>
</tr>
<tr>
<td>2.5.2 Plasma Triglycerides</td>
<td>47</td>
</tr>
<tr>
<td>2.6 Diet Analysis</td>
<td>48</td>
</tr>
<tr>
<td>2.6.1 Total fat</td>
<td>48</td>
</tr>
<tr>
<td>2.6.2 Cholesterol</td>
<td>49</td>
</tr>
</tbody>
</table>
CHAPTER 3

GENERAL REACTIVITY OF THE MARMOSET VASCULATURE .......................... 53

3.1 Introduction ........................................................................................................ 53
3.2 Methods ................................................................................................................ 54
  3.2.1 Animals .......................................................................................................... 54
  3.2.2 Vascular Preparations .................................................................................... 54
  3.2.3 Data Analysis .................................................................................................. 55
3.3 Results .................................................................................................................. 56
  3.3.1 Aorta .............................................................................................................. 56
  3.3.2 Common Carotid Artery ................................................................................. 62
  3.3.3 Superior Mesenteric Artery ............................................................................ 65
  3.3.4 Coronary artery ............................................................................................... 65
3.4 Discussion .............................................................................................................. 68

CHAPTER 4

EXCITATORY ACTIONS OF 5-HYDROXYTRYPTAMINE IN THE
MARMOSET VASCULATURE ...................................................................................... 74

4.1 Introduction ........................................................................................................... 74
4.2 Methods .................................................................................................................. 74
  4.2.1 Animals .......................................................................................................... 74
4.2.2 Vascular Preparations .......................................... 75
4.2.3 Cyclic AMP Formation ......................................... 76
4.2.4 Data Analysis .................................................... 76
4.3 Results .............................................................. 77
4.3.1 Aortic Rings Precontracted with U44069 ...................... 77
4.3.2 Aortic Rings at Basal Tone ...................................... 80
4.3.3 Coronary Arteries Precontracted with U44069 ................ 82
4.3.4 Cyclic AMP ....................................................... 84
4.4 Discussion .......................................................... 86

CHAPTER 5
INHIBITORY ACTIONS OF 5-HYDROXYTRYPTAMINE IN THE MARMOSET
VASCULATURE .......................................................... 91
5.1 Introduction .......................................................... 91
5.2 Methods ............................................................. 91
5.2.1 Animals .......................................................... 91
5.2.2 Vascular Preparations .......................................... 92
5.2.3 Cyclic AMP Formation .......................................... 93
5.2.4 Data Analysis ................................................... 93
5.3 Results .............................................................. 94
5.3.1 Aortic Relaxation ............................................... 94
5.3.2 Carotid Relaxation ............................................. 99
5.3.3 Cyclic AMP Formation .......................................... 99
5.4 Discussion .......................................................... 101
CHAPTER 6
ANALYSIS OF SEROTONERGIC AMPLIFYING INTERACTIONS IN THE
MARMOSET AORTA .............................................. 105
6.1 Introduction .............................................. 105
6.2 Methods .................................................... 105
  6.2.1 Animals .............................................. 105
  6.2.2 Aortic Ring Preparations ......................... 106
  6.2.3 Cyclic AMP Formation ............................. 106
  6.2.4 Data Analysis ...................................... 107
6.3 Results ....................................................... 107
  6.3.1 Organ Bath Preparations ......................... 107
  6.3.2 Cyclic AMP Formation ............................. 110
6.4 Discussion ................................................. 112

CHAPTER 7
EFFECTS OF AN ATEROGENIC DIET ON SEROTONERGIC RESPONSES OF
THE MARMOSET VASCULATURE .............................. 115
7.1 Introduction .............................................. 115
7.2 Methods .................................................... 116
Atherogenic Dietary Experiment #1 ......................... 117
  7.2.1 Animals .............................................. 117
  7.2.2 Diets ................................................. 118
  7.2.3 Vascular Preparations ............................. 118
Atherogenic Dietary Experiment #2 ......................... 119
  7.2.4 Animals, Experiment #2 ........................... 119
7.2.5 Det, Experiment #2 ........................................... 120
7.2.6 Vascular Preparations, Experiment #2 .................. 120
7.2.7 Data Analysis ..................................................... 131

7.3 Results ...................................................................... 122

Atherogenic Dietary Experiment #1 ................................. 122
7.3.1 Diets .................................................................... 122
7.3.2 Plasma Lipids ....................................................... 122
7.3.3 Histology and Vascular Responses ...................... 124

Atherogenic Dietary Experiment #2 ................................. 125
7.3.4 Plasma Lipids, Experiment #2 .............................. 125
7.3.5 Morphology, Experiment #2 ............................... 125
7.3.6 Vascular Preparations, Experiment #2 ............... 127

7.4 Discussion .............................................................. 135
7.4.1 Plasma Lipids ...................................................... 135
7.4.2 Vascular Reactivity ............................................. 137
7.4.3 Summary ............................................................ 140

CHAPTER 8

EFFECTS OF BALLOON ANGIOPLASTY ON SEROTONERGIC RESPONSES OF
THE MAMMOSER AORTA .................................................. 142

8.1 Introduction ........................................................... 142
8.2 Methods ............................................................... 143
8.2.1 Animals ............................................................. 143
8.2.2 Surgery (Angioplasty) ......................................... 143
8.2.3 Aortic Ring Preparations ...................................... 144
8.2.4 Data Analysis ..................................................... 145
The principle aim of the current studies was to characterise the responses to 5-
Hydroxytryptamine (5-HT) occurring in the aorta of a primate species, the common
marmoset. Further aims were to determine whether the 5-HT-induced response of the
aorta is representative of the serotoninergic responses of other vessels from the marmoset, in
particular the coronary artery. Finally, studies were extended to vascular disease states
following a high lipid diet and aortic balloon-catheterisation.

In the marmoset aorta, responses to 5-HT differed greatly from those described in the
aorta of commonly used rodent species. In the marmoset aorta with intact endothelium
and other agents absent, 5-HT is inactive. However, when the tone is increased with a
secondary contractile agent both contractile and relaxant responses become apparent. It
appears that three 5-HT-induced responses are present in the marmoset aorta. At low
concentrations (0.001 - 9.1µM), 5-HT produced an endothelium-dependent relaxant
response. At high concentrations (0.1 - 10µM), 5-HT induces a strong relaxant response
which is unaffected by endothelial integrity and is likely to be mediated by a 5-HT$_7$
receptor. Under conditions of reduced relaxation, a 5-HT$_7$-like receptor mediates a weak
contractile response which exhibits synergism with the thromboxane (Tx) mimetic
U44069 but not noradrenaline (NA). The amplification response was greatest when
endothelial function was impaired and the lack of a synergistic interaction of 5-HT with
NA was not due to 3-receptor activation on the part of NA. Importantly, the interaction
between U44069 and 5-HT was also present in marmoset carotid, mesenteric and coronary
arteries and the 5-HT$_7$-like receptor also plays a role in the contractile effect in the
coronary artery.

In response to dietary lipid loading, marmosets exhibited a large variation in
responsiveness of plasma lipid levels, such that there were 'hyper-responding' and 'hyp-
responding" maranosets. Hypercholesterolaemia for 16 months did not result in the development of atheromatous lesions. The subset of animals that were hyper-responsive to dietary lipid had reduced aortic contractile sensitivity to sumatriptan and aortic basal NO production was reduced by comparison with the hypo-responders. In addition, hypo-responders exhibited an enhanced relaxant response to the endothelium-independent component of 5-HT-induced relaxation (possibly 5-HT1-mediated) in both the aorta and carotid arteries.

In aortae subjected to balloon angioplasty, endothelium-dependent relaxation was reduced. Serotonergic responses in angioplastied arteries were characterised by increased 5-HT1-like-mediated contraction and reduced relaxation. These responses exemplify the pathophysiological significance of serotonergic responses in vascular disease; ie, when endothelial function is impaired, Tx A2 and 5-HT will interact and the vasoconstrictive effects of 5-HT will predominate, impeding flow in the damaged artery.