An investigation into the role of central catecholamines in the antihypertensive action of clonidine.

Grimes, D.

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AN INVESTIGATION
INTO THE ROLE OF CENTRAL CATECHOLAMINES
IN THE ANTIHYPERTENSIVE ACTION OF CLONIDINE

Submitted by D. GRIMES B.Sc.

for the degree of

Doctor of Philosophy

of the

UNIVERSITY OF BATH,

1977

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This thesis may be made available for consultation within the University Library and may be photocopied or lent to other libraries for the purposes of consultation.
I wish to thank Professor R.J. Ancill for making available the facilities for this research to be carried out and also Dr. P.H. Redfern and Mr. A.J. Draper for their invaluable help, advice and discussions while jointly supervising this work.

I wish to acknowledge the University of Bath for providing the financial support for this project from the University of Bath Research Fund.

I also wish to thank Dr. J.W. Bell (Boehringer-Ingelheim) and Dr. G. Beaumont (Geigy Pharmaceuticals) for donating samples of clonidine and desmethyldimipramine respectively.

Finally, I would like to express my sincere thanks to my wife, Catherine, for typing the manuscript and also for solace in times of despair.
Cara Louise
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUMMARY</td>
<td></td>
<td>viii</td>
</tr>
<tr>
<td>Chapter 1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>1.1</td>
<td>A Brief Historical Outline of the Discovery of the Circulation</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>Hypertension</td>
<td>7</td>
</tr>
<tr>
<td>1.2.1</td>
<td>Blood Pressure</td>
<td>7</td>
</tr>
<tr>
<td>1.2.2</td>
<td>What is Hypertension?</td>
<td>7</td>
</tr>
<tr>
<td>1.2.3</td>
<td>The Aetiology of Hypertension</td>
<td>11</td>
</tr>
<tr>
<td>1.3</td>
<td>The Development of Antihypertensive Therapy</td>
<td>14</td>
</tr>
<tr>
<td>1.4</td>
<td>The Central Nervous System and Blood Pressure Control</td>
<td>19</td>
</tr>
<tr>
<td>1.4.1</td>
<td>The Central Regulation of Blood Pressure</td>
<td>19</td>
</tr>
<tr>
<td>1.4.2</td>
<td>Central Adrenergic Mechanisms affecting Blood Pressure</td>
<td>23</td>
</tr>
<tr>
<td>1.4.3</td>
<td>Centrally Acting Antihypertensive Drugs</td>
<td>28</td>
</tr>
<tr>
<td>1.4.3.1</td>
<td>α-methyldopa</td>
<td>32</td>
</tr>
<tr>
<td>1.4.3.2</td>
<td>Clonidine</td>
<td>35</td>
</tr>
<tr>
<td>1.4.3.3</td>
<td>Other Drugs exerting an Antihypertensive Effect by a Central Action</td>
<td>39</td>
</tr>
<tr>
<td>1.5</td>
<td>Basis and Aims of the Project</td>
<td>44</td>
</tr>
<tr>
<td>Chapter</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>METHODS</td>
<td>46</td>
</tr>
<tr>
<td>2.1</td>
<td>Animals</td>
<td>47</td>
</tr>
<tr>
<td>2.1.1</td>
<td>Animals and Environmental Conditions</td>
<td>47</td>
</tr>
<tr>
<td>2.1.2</td>
<td>Choice of Species</td>
<td>47</td>
</tr>
<tr>
<td>2.2</td>
<td>The Measurement of Blood Pressure</td>
<td>48</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Introduction</td>
<td>48</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Method</td>
<td>51</td>
</tr>
<tr>
<td>2.2.3</td>
<td>Discussion on the Results obtained from Tail Cuff Blood Pressure Measurements</td>
<td>52</td>
</tr>
<tr>
<td>2.3</td>
<td>Experimental Hypertension</td>
<td>59</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Introduction</td>
<td>59</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Method</td>
<td>61</td>
</tr>
<tr>
<td>2.3.3</td>
<td>The Development of the Hypertension</td>
<td>64</td>
</tr>
<tr>
<td>2.3.4</td>
<td>Observations on the Renal/DOCA Hypertensive Rats</td>
<td>65</td>
</tr>
<tr>
<td>2.4</td>
<td>The Estimation of Noradrenaline and Dopamine in Rat Brain</td>
<td>67</td>
</tr>
<tr>
<td>2.4.1</td>
<td>Introduction</td>
<td>67</td>
</tr>
<tr>
<td>2.4.2</td>
<td>General Consideration of Methodology</td>
<td>70</td>
</tr>
<tr>
<td>2.4.3</td>
<td>Brain Removal and Dissection</td>
<td>74</td>
</tr>
<tr>
<td>2.4.4</td>
<td>Extraction and Assay of Noradrenaline and Dopamine</td>
<td>77</td>
</tr>
<tr>
<td>2.4.5</td>
<td>Materials</td>
<td>81</td>
</tr>
<tr>
<td>Chapter</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Chapter 2 (cont'd.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>The Estimation of the Turnover of Noradrenaline and Dopamine in Rat Brain</td>
<td>82</td>
</tr>
<tr>
<td>2.5.1</td>
<td>Introduction</td>
<td>82</td>
</tr>
<tr>
<td>2.5.2</td>
<td>Method</td>
<td>83</td>
</tr>
<tr>
<td>2.5.3</td>
<td>Results and Discussion</td>
<td>84</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>DIFFERENCES BETWEEN NORMOTENSIVE AND HYPERTENSIVE ANIMALS</td>
<td>87</td>
</tr>
<tr>
<td>3.1</td>
<td>Introduction</td>
<td>88</td>
</tr>
<tr>
<td>3.2</td>
<td>Differences in Organ and Tissue Weight</td>
<td>91</td>
</tr>
<tr>
<td>3.3</td>
<td>Differences in Catecholamine Levels in Brain Regions</td>
<td>95</td>
</tr>
<tr>
<td>3.4</td>
<td>Differences in Central Catecholamine Turnover</td>
<td>95</td>
</tr>
<tr>
<td>3.5</td>
<td>Discussion</td>
<td>103</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>THE EFFECTS OF CLONIDINE ON BLOOD PRESSURE AND ON THE LEVELS AND TURNOVER OF CENTRAL CATECHOLAMINES</td>
<td>107</td>
</tr>
<tr>
<td>4.1</td>
<td>Introduction</td>
<td>108</td>
</tr>
<tr>
<td>4.2</td>
<td>Effects on Blood Pressure</td>
<td>109</td>
</tr>
<tr>
<td>4.2.1</td>
<td>Method</td>
<td>109</td>
</tr>
</tbody>
</table>
## Chapter 4 (cont'd.)

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.2</td>
<td>Results</td>
<td>110</td>
</tr>
<tr>
<td>4.2.3</td>
<td>Discussion</td>
<td>112</td>
</tr>
<tr>
<td>4.3</td>
<td>Effects on Endogenous Catecholamine Levels in Brain Regions</td>
<td>112</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Introduction</td>
<td>112</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Method</td>
<td>115</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Results</td>
<td>116</td>
</tr>
<tr>
<td>4.3.4</td>
<td>Discussion</td>
<td>120</td>
</tr>
<tr>
<td>4.4</td>
<td>Effects on Central Catecholamine Turnover</td>
<td>121</td>
</tr>
<tr>
<td>4.4.1</td>
<td>Introduction</td>
<td>121</td>
</tr>
<tr>
<td>4.4.2</td>
<td>Method</td>
<td>122</td>
</tr>
<tr>
<td>4.4.3</td>
<td>Results</td>
<td>123</td>
</tr>
<tr>
<td>4.4.3.1</td>
<td>Effects on the Turnover of Catecholamines in Whole Brain</td>
<td>123</td>
</tr>
<tr>
<td>4.4.3.2</td>
<td>Effects on Catecholamine Turnover in Brain Regions</td>
<td>123</td>
</tr>
<tr>
<td>4.4.4</td>
<td>Discussion</td>
<td>128</td>
</tr>
<tr>
<td>4.5</td>
<td>Effects of Clonidine on Blood Pressure and Central Catecholamine Turnover in the Spontaneously Hypertensive Rat</td>
<td>131</td>
</tr>
<tr>
<td>4.5.1</td>
<td>Introduction</td>
<td>131</td>
</tr>
<tr>
<td>4.5.2</td>
<td>Method and Results</td>
<td>132</td>
</tr>
<tr>
<td>4.5.3</td>
<td>Discussion</td>
<td>133</td>
</tr>
<tr>
<td>4.6</td>
<td>General Discussion</td>
<td>135</td>
</tr>
<tr>
<td>Chapter</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>AN EXAMINATION OF THE INTERACTION BETWEEN CLONIDINE AND DESMETHYLIMIPRAMINE: EFFECTS ON BLOOD PRESSURE AND CENTRAL NORADRENALINE TURNOVER</td>
<td>136</td>
</tr>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>137</td>
</tr>
<tr>
<td>5.2</td>
<td>Method</td>
<td>139</td>
</tr>
<tr>
<td>5.3</td>
<td>Results</td>
<td>142</td>
</tr>
<tr>
<td>5.3.1</td>
<td>Effects on Blood Pressure</td>
<td>142</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Effects on the Turnover of Noradrenaline in Whole Brain</td>
<td>147</td>
</tr>
<tr>
<td>5.3.3</td>
<td>Effects on Noradrenaline Turnover in Medulla and Hypothalamus</td>
<td>149</td>
</tr>
<tr>
<td>5.4</td>
<td>Discussion</td>
<td>153</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>THE EFFECTS OF OTHER DRUGS, BOTH ALONE AND IN COMBINATION, ON BLOOD PRESSURE AND CENTRAL NORADRENALINE TURNOVER</td>
<td>157</td>
</tr>
<tr>
<td>6.1</td>
<td>Introduction</td>
<td>158</td>
</tr>
<tr>
<td>6.2</td>
<td>Guanethidine and α-methyldopa</td>
<td>159</td>
</tr>
<tr>
<td>6.2.1</td>
<td>Guanethidine</td>
<td>159</td>
</tr>
<tr>
<td>6.2.1.1</td>
<td>Introduction</td>
<td>159</td>
</tr>
<tr>
<td>6.2.1.2</td>
<td>Method</td>
<td>161</td>
</tr>
<tr>
<td>6.2.1.3</td>
<td>Results</td>
<td>162</td>
</tr>
<tr>
<td>6.2.1.3.1</td>
<td>Effects on Blood Pressure</td>
<td>162</td>
</tr>
<tr>
<td>6.2.1.3.2</td>
<td>Effects on Central Noradrenaline Turnover</td>
<td>162</td>
</tr>
<tr>
<td>6.2.1.4</td>
<td>Discussion</td>
<td>165</td>
</tr>
<tr>
<td>Chapter</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Chapter 6 (cont'd.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.2.2</td>
<td>α-methyldopa</td>
<td>169</td>
</tr>
<tr>
<td>6.2.2.1</td>
<td>Introduction</td>
<td>169</td>
</tr>
<tr>
<td>6.2.2.2</td>
<td>Method</td>
<td>169</td>
</tr>
<tr>
<td>6.2.2.3</td>
<td>Results</td>
<td>170</td>
</tr>
<tr>
<td>6.2.2.3.1</td>
<td>Effects on Blood Pressure</td>
<td>170</td>
</tr>
<tr>
<td>6.2.2.3.2</td>
<td>Effects on Noradrenaline Turnover</td>
<td>170</td>
</tr>
<tr>
<td>6.2.2.4</td>
<td>Discussion</td>
<td>172</td>
</tr>
<tr>
<td>6.2.3</td>
<td>Discussion</td>
<td>175</td>
</tr>
<tr>
<td>6.3</td>
<td>Phenoxybenzamine, an α-blocking Agent: Effects on Central Noradrenaline Turnover</td>
<td>176</td>
</tr>
<tr>
<td>6.3.1</td>
<td>Introduction</td>
<td>176</td>
</tr>
<tr>
<td>6.3.2</td>
<td>Method</td>
<td>176</td>
</tr>
<tr>
<td>6.3.3</td>
<td>Results</td>
<td>177</td>
</tr>
<tr>
<td>6.3.4</td>
<td>Discussion</td>
<td>177</td>
</tr>
<tr>
<td>6.4</td>
<td>An Interaction between Clonidine and the β-blocking Antihypertensive Drug Sotalol</td>
<td>179</td>
</tr>
<tr>
<td>6.4.1</td>
<td>Introduction</td>
<td>179</td>
</tr>
<tr>
<td>6.4.2</td>
<td>Method</td>
<td>181</td>
</tr>
<tr>
<td>6.4.3</td>
<td>Results</td>
<td>182</td>
</tr>
<tr>
<td>6.4.3.1</td>
<td>Effects on Blood Pressure</td>
<td>182</td>
</tr>
<tr>
<td>6.4.3.2</td>
<td>Effects on Noradrenaline Turnover</td>
<td>185</td>
</tr>
<tr>
<td>6.4.4</td>
<td>Discussion</td>
<td>187</td>
</tr>
<tr>
<td>Chapter 7</td>
<td>DISCUSSION</td>
<td>190</td>
</tr>
<tr>
<td>7.1</td>
<td>Introduction</td>
<td>191</td>
</tr>
<tr>
<td>Chapter</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>7.2</td>
<td>Central Catecholamines and Blood Pressure Regulation</td>
<td>193</td>
</tr>
<tr>
<td>7.3</td>
<td>Comments on the Measurement of Noradrenaline in Gross Regions of the Brain</td>
<td>196</td>
</tr>
<tr>
<td>7.4</td>
<td>The Mechanism of Action of Clonidine</td>
<td>198</td>
</tr>
<tr>
<td>7.5</td>
<td>The Interaction between Clonidine and DMI</td>
<td>207</td>
</tr>
<tr>
<td>7.6</td>
<td>Effects of other Drugs</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td>Guanethidine &amp; α-methyldopa</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td>Phenoxybenzamine</td>
<td>218</td>
</tr>
<tr>
<td></td>
<td>Clonidine and Sotalol</td>
<td>219</td>
</tr>
<tr>
<td>7.7</td>
<td>The Localisation of the Central Effects of Clonidine</td>
<td>222</td>
</tr>
<tr>
<td>7.8</td>
<td>Clinical Implications of the Effects of Clonidine reported in this Thesis</td>
<td>225</td>
</tr>
<tr>
<td>7.9</td>
<td>Conclusions</td>
<td>228</td>
</tr>
<tr>
<td>7.10</td>
<td>Suggestions for Further Work</td>
<td>229</td>
</tr>
</tbody>
</table>

REFERENCES 232

PUBLICATIONS 253
SUMMARY
The main aim of this thesis was to investigate the role of central catecholamines in the antihypertensive action of clonidine. This was examined by attempting to correlate changes in central noradrenaline levels and turnover in specific brain regions with the hypotensive action of the drug. The investigation looked for these effects in three rat models, normotensives, renal/DOCA hypertensives and spontaneously hypertensives. The interaction between clonidine and desmethylimipramine was also examined. When given twice daily for seven days, clonidine produced a dose related decrease in blood pressure and a dose related decrease in noradrenaline turnover in the medulla and, to a lesser extent, in the hypothalamus of all three animal models studied. Combined therapy with clonidine and DMI resulted in an antagonism of the hypotensive effect of clonidine, yet a potentiation of the turnover effects. Little significant change was found on endogenous noradrenaline levels after any of the above treatments.

Based on the hypothesis that hypertension and elevated blood pressure is associated with decreased central noradrenaline turnover, the major conclusion drawn from the results of the thesis is that, although the decreases in blood pressure and central noradrenaline turnover are both brought about by clonidine treatment, the turnover effects are not directly related to the drug's hypotensive action. It is proposed that the turnover effect is by incidental stimulation of α-receptors located on a pre-synaptic
neurone, probably at the same synapse at which clonidine produces its hypotensive action by the stimulation of post-synaptic \( \alpha \)-receptors.

In the course of the experiments, other drugs were also examined for effects on blood pressure and/or central catecholamines. The drugs examined included \( \alpha \)-methyldopa, guanethidine, phenoxybenzamine and sotalol. A discussion is presented on the mechanisms by which the above effects may be produced and their possible relevance in clinical treatment with clonidine.
CHAPTER 1

INTRODUCTION
Introduction

An attempt is made in this thesis to relate the mechanism of action of centrally acting antihypertensive agents with changes in the central adrenergic mechanisms said to play an important role in the regulation of blood pressure. The particular antihypertensive agent studied was the imidazoline derivative, clonidine.

Also presented are the results of an investigation into the interaction between clonidine and certain other drugs, for example the tricyclic antidepressant, desmethylimipramine (DMI).

The thesis starts, therefore, with an outline of the discovery of the circulatory system and the disease of hypertension. It continues with a description of the role of central adrenergic mechanisms in blood pressure control and also their implication in hypertension and goes on to describe the drugs which have been developed for the treatment of the disease, in particular those with a central site of action.
1.1 A Brief Historical Outline of the Discovery of the Circulation

Although there are numerous references in ancient medical writings to the heart, the pulse and blood flow in general (for references see Leake, 1962), the credit for the discovery of the circulation of blood around the body is usually given to an Englishman, William Harvey (1578-1657), sometime lecturer in anatomy at the Royal College of Physicians. His treatise "De mortu cordis et sanguinis", published in 1628, provides the basis for modern thinking on cardiovascular physiology (Parkyn, 1952).

As early as 1700BC the ancient Egyptians and the Chinese realised that the heart was one of the major and important organs of the body and that the pulse, detected in the extremities, could be used as a guide to the condition of the heart. With these findings and the discoveries of later physicians and anatomists, notably people such as Ambriose Paré (1510-1590), Jacapo Berengaris de Corpi (1470-1550), Guido Guidi (1500-1569), Carolus Stephanus (1500-1564), Leonardo da Vinci (1452-1519) and Andreas Vesalius (1514-1564), the understanding of the functions of the heart and the blood was greatly increased.

However, it was left to William Harvey to postulate the idea of blood, driven by the heart, being circulated around the body. Yet, even Harvey was not able to explain how the blood passed from the arteries to the veins. It was not until the microscope had been perfected that, in
1661, an Italian anatomist, Malpighi, was able to observe and describe the networks of capillary vessels uniting the arteries and veins, thus providing the "missing link" in Harvey's hypothesis.

Although further work over the following years gave more and more insight into the structure and functions of the cardiovascular system, the next real advance in the understanding of the circulation came with the reporting of experiments performed by the Reverend Stephen Hales (1677-1761). His experiments concerned the direct measurement of blood pressure in a variety of animals, in particular horses, using brass catheters and long glass tubes. In these experiments, he was able to demonstrate pressure relationships between arteries and veins and, as a result, estimated systolic blood pressure in humans to be around seven and a half (7½) feet of blood, a figure that compares favourably with that obtained by modern blood pressure measurements.

The invention in 1896 by Scipione Riva-Rocci (1863-1937) of the mercury sphygmanometer, the forerunner of modern blood pressure measuring instruments, helped to confirm that blood pressure varied with heart beat and respiration.

It later became clear that blood pressure had some clinical significance and that it could be shown to be a useful adjunct to the diagnosis of disturbances of the cardiovascular system and also certain other disease states.
This led to further analysis of the factors involved in the control of blood pressure and the discovery of, among other things:

1. the cardioinhibitory effect of the vagus;

2. the accelerator nerves to the heart (Bezold, 1836-68);

3. baroreceptor reflex function of the carotid sinus (Hering, 1924).

More recent work has indicated that there are many factors which may be involved in the control and regulation of blood pressure. Figure 1.1 gives a diagrammatic representation of many of the factors.

There appear, however, to be two major control mechanisms, one humoral, centred upon the kidney and associated with the production of renin, the other neural, involving mechanisms sited in the brain. Of the two, the latter appears to be the more important and dominant mechanism.

It is with these central mechanisms that this thesis is primarily concerned. The involvement of these mechanisms in blood pressure regulation will be discussed further in a later section.
FIGURE 1.1: A simplified scheme of the various factors and mechanisms involved in the regulation of arterial blood pressure.
1.2 Hypertension

1.2.1 Blood Pressure

The importance of blood pressure in the body is a subject in itself and no attempt will be made in this thesis to discuss its implications. Instead, this thesis will be concerned more with the central controlling mechanisms involved in blood pressure maintenance and possible aberrations of these mechanisms which may result in elevated blood pressure or hypertension. The effects of drug treatment on these mechanisms will also be considered.

It has been suggested that the diastolic pressure is a better guide to the state of systemic blood pressure than systolic pressure. This is due to the shape of the pulse pressure wave which puts the mean blood pressure closer to the diastolic value. The diastolic pressure also reflects more the condition of the peripheral resistance vessels (i.e. whether narrowing or decreased elasticity has occurred) and is less variable than systolic.

1.2.2 What is Hypertension?

Many medical textbooks state that "normal" blood pressure lies within the ranges systolic 110-120mmHg and diastolic 70-80mmHg. In practice, this is not so. For
example, it is known that blood pressure increases with age. In a 50-60-year old patient, the blood pressure may be substantially higher than the figures quoted above. However, when age is taken into consideration, the blood pressure may be considered "normal". Conversely, in athletes, blood pressure may be lower than the "norm" for age, etc. (Pickering, 1974).

Considerable variation in blood pressure can occur in the "normal" person as a result of a variety of factors, excluding disease states. These include time of day (diurnal variation), environmental conditions and stress factors (see Pickering, 1974; Chapter 2). It is difficult, therefore, to state categorically a level of blood pressure above which a patient may be classed as hypertensive.

Single blood pressure measurements can be meaningless for the diagnosis of hypertension, unless supported by other clinical evidence. However, patients who display a persistent elevation in blood pressure over a period of time and repeated observations may be classed as hypertensive after age and other factors have been considered.

The question which now becomes relevant is what level of increase in blood pressure is considered outside "normal" limits and therefore unacceptable?

There now is considerable evidence that increasing arterial pressure is associated with a higher incidence of mortality. This is shown in Table 1.1 which is compiled from observations collected by the Actuarial Society
TABLE 1.1: Mortality ratios* for men according to groups of systolic and diastolic blood pressure readings with no breakdown for age. (From Actuarial Society of America and The Association of Life Insurance Medical Directors, 1941)

<table>
<thead>
<tr>
<th>Systolic Reading (mmHg)</th>
<th>Diastolic Reading (mmHg)</th>
<th>64-83</th>
<th>84-88</th>
<th>89-93</th>
<th>94-103</th>
<th>ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>118 - 132</td>
<td></td>
<td>90</td>
<td>91</td>
<td>99</td>
<td>97</td>
<td>92</td>
</tr>
<tr>
<td>133 - 142</td>
<td></td>
<td>99</td>
<td>107</td>
<td>118</td>
<td>134</td>
<td>110</td>
</tr>
<tr>
<td>143 - 152</td>
<td></td>
<td>133</td>
<td>137</td>
<td>141</td>
<td>173</td>
<td>148</td>
</tr>
<tr>
<td>153 - 167</td>
<td></td>
<td>186</td>
<td>178</td>
<td>189</td>
<td>237</td>
<td>210</td>
</tr>
<tr>
<td>ALL</td>
<td></td>
<td>95</td>
<td>100</td>
<td>141</td>
<td>151</td>
<td></td>
</tr>
</tbody>
</table>

* Actual to expected deaths (expected = 100)
of America. From the lowest to the highest values of systolic and diastolic pressure the incidence of mortality increases with increasing pressure. There is no sudden break in the increase and the relationship is quantitative.

Although it is obvious that treatment of hypertension will reduce distressing symptoms of the disease, Freis (1970) has indicated that treatment of hypertensive patients may also result in a reduction of the expected mortality rate.

Expectation of life was improved by treatment in people with diastolic pressures between 95-110mmHg, with greater success at the higher pressure. Hence, reduction of blood pressure, even in the symptomless patient, may produce greater benefit to the patient in terms of increased life expectancy. When a doctor has to decide, therefore, whether a patient is hypertensive, he is really deciding whether or not the patient needs, and will benefit from, the application of therapy. Indeed, the idea may be put forward that everyone should receive prophylactic treatment against hypertension, at least until it is possible to predict those persons who will develop and suffer from the disease in later life.

This, however, poses the question of whether the possible benefit accrued from prophylactic treatment would outweigh the probable side effects of long term drug therapy of normal people. As this idea is therefore really unacceptable, basic guidelines have to be drawn to enable decision-making on when to treat hypertension.
Pickering (1974, p34), accepting that females tolerate higher pressures than males, states that the pressure at which benefit is conferred to the patient by hypotensive therapy is higher in females than in males. Hence, he puts forward three divisions of hypertensive patient:

1. those with diastolic pressures persistently over 110mmHg, making hypotensive therapy mandatory;

2. female patients with diastolic pressures between 100-110mmHg who may benefit from treatment;

3. male patients with diastolic pressures between 95-100mmHg who also may benefit from treatment.

It would therefore appear that, to be on the safe side, the generality of treating all patients (allowing for age) presenting diastolic pressures of 95mmHg or over, whether presenting other clinical symptoms or not, may be a sound approach.

1.2.3 The Aetiology of Hypertension

Clinically, hypertension is customarily classified into two main divisions:
1. primary or "essential" hypertension, implying an unknown cause;

2. secondary hypertension, referring to a number of other conditions which are known to result or manifest in hypertension.

In the primary or "essential" hypertensive state, clinical symptoms such as retinopathy and retinal exudates are usually manifested as a result of the elevated blood pressure. The cause of the hypertension is not easily determined and can result from factors as far-ranging as heredity, changes in environmental conditions (Ledingham, 1971; Pickering, 1974), or an imbalance of the normally well-balanced systems that maintain body haemodynamics (i.e. the mosaic theory, Page, 1974).

"Essential" hypertension is one of the "chicken or the egg" problems of cardiovascular pharmacology as many factors have been shown to be associated with the hypertension but no direct evidence is available to show whether these factors are a result, or a cause, of the hypertension. For example, there is controversy about the roles of renin and sodium in hypertension with some workers showing correlations of increased renin and/or sodium with hypertension, yet other workers showing no correlation (see editorial, The Lancet 1, 345, 1976).

Secondary hypertension is usually a manifestation or
symptom of a known disease or clinical abnormality, of which probably the most common are of renal origin or endocrine imbalance (Ledingham, 1971). Other diseases and clinical states which may manifest as hypertension include diabetes, connective tissue diseases, co-arction of the aorta, phaeochromocytoma, Cushing's syndrome and drug therapy such as contraceptive pills and steroids.

In the experimental animal, mechanical methods of hypertension induction are the most commonly used and involve manipulation of the kidneys (see Chapter 2). The administration of mineralocorticoid excess is also a frequently used technique of hypertension induction.

These experimentally induced hypertensive states resemble more closely secondary hypertension, in that they are precipitated by a known cause or action. "Essential" hypertension is probably more closely represented in the experimental situation by the spontaneously hypertensive animals (Grollman, 1972).

It is clear, therefore, that hypertension is a very complex disease and research into its cause and treatment a well subscribed topic. Two major areas of research in the field of hypertension are:

1. the central regulation of blood pressure and possible imbalances of these regulating systems which may result in hypertension; also the possible modification by, and effects of, drug
therapy on these systems (for instance, see Davies and Reid, 1975; Onesti et al., 1973 & 1976); and

2. the involvement of the renin-angiotensin system (see, for example, Lebel et al., 1974; Eide, 1975).

This thesis is more concerned with the former area of research.

1.3 The Development of Antihypertensive Therapy

Early compounds used to treat hypertension were quite strong in their actions and possibly had side effects almost as distressing as the clinical symptoms of the disease. However, with the advent of modern routine screening of blood pressure, the disease can now be spotted during its development, when diastolic values may be raised above "normal" limits, but not excessively. This type of hypertension is termed 'moderate hypertension' and many drugs, along with more powerful and acceptable drugs with which to treat the more severe cases, are now available to control it.

Even so, many of the available antihypertensive drugs unfortunately display unwanted and sometimes distressing side effects (e.g. postural hypotension with neurone
blockers, sedation and "dry mouth" with clonidine and α-methyldopa, and diabetes with diazoxide). Another factor is that tolerance to many of the drugs nearly always develops to some extent.

To overcome or lessen the side effects and the tolerance effect, combinations of two or more antihypertensive drugs are used at doses lower than those used if the drugs were administered separately.

So the search for even more effective drugs, yet with less side effects, continues.

The discovery of the majority of the available antihypertensive agents has occurred over the last 50 years or so. The discoveries have not progressed smoothly but have had alternating periods of activity and dormancy.

Some of the earliest used antihypertensive drugs were the veratrum alkaloids and thiocyanate, both used to treat the symptoms of a disease of which the cause at the time was unknown. Some compounds were known to have antihypertensive activity many years before they were used in therapy. An example is the Rauwolfia alkaloids, reported to show a hypotensive effect as early as 1918 (Kivtikar & Basu, 1918), while reserpine was not used clinically in western medicine until the late 1940's.

With the development of the idea that the sympathetic nervous system was involved in blood pressure control, the 1940's saw the production and development of compounds to produce effects on the sympathetic nervous system. This led to the promotion of the neurone-blocking agents as
antihypertensives.

The majority of research until quite recently concentrated on the discovery and development of drugs which affected the peripheral autonomic nervous system or the periphery in general. Research still progresses in this area producing compounds such as the vasodilators, e.g. diazoxide and minoxidil, and new α-receptor blocking agents, such as prazosin and indoramin (see Wilhelm & De Stevens, 1976; Francis, 1976). However, much research is now being carried out to determine the role of central control mechanisms on blood pressure and the possible modification of these central mechanisms by drugs (see later section).

Table 1.2 gives a short and by no means exhaustive outline of the development of peripheral antihypertensive therapy up to the early 60's. More detailed information on the development and mechanism of action of the compounds can be found in reviews of the topic by Laverty (1973), Goldberg (1975) and Wilhelm and de Stevens (1976).

Figure 1.2 gives, in diagrammatic form, the probable sites of action and mechanisms of a variety of drugs, both peripherally and centrally acting, which are used in the treatment of hypertension. A number of interesting facts are apparent from this Figure. Certain drugs, originally thought to act by one mechanism at a particular site, are now known to act at a different site and sometimes by a different mechanism. Examples of these are:
### TABLE 1.2: The Development of Peripheral Antihypertensive Therapy

<table>
<thead>
<tr>
<th>Date of Discovery</th>
<th>Drug</th>
<th>Rationale for Use</th>
<th>In Clinical Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1859</td>
<td>Veratrum Alkaloids</td>
<td>Treat symptoms</td>
<td>-</td>
</tr>
<tr>
<td>1903</td>
<td>Thiocyanate</td>
<td>Treat symptoms</td>
<td>-</td>
</tr>
<tr>
<td>1947</td>
<td>Ganglion Blockers</td>
<td>Chemical Sympathectomy</td>
<td>1950-55</td>
</tr>
<tr>
<td>1949</td>
<td>Hydralazine</td>
<td>Serendipitous</td>
<td>1953</td>
</tr>
<tr>
<td>1949</td>
<td>Rauwolfia</td>
<td>Synthesis of plant drug</td>
<td>1953</td>
</tr>
<tr>
<td>1957</td>
<td>Thiazide Diuretics</td>
<td>Regulation of Renal Function</td>
<td>1958</td>
</tr>
<tr>
<td>1959</td>
<td>Adrenergic Neurone Blockers</td>
<td>Moderation of Autonomic N.S.</td>
<td>1960</td>
</tr>
<tr>
<td>1960</td>
<td>M.A.O. Inhibitors</td>
<td>Interference with Peripheral N.S.</td>
<td>1963-70</td>
</tr>
<tr>
<td>1960</td>
<td>α-methyldopa</td>
<td>Interference with Noradrenaline regulatory mechanisms</td>
<td>1963</td>
</tr>
<tr>
<td>1964</td>
<td>β-blockers</td>
<td>β-blockade of heart, etc.</td>
<td>-</td>
</tr>
</tbody>
</table>
FIGURE 1.2: The possible mechanisms and site of action in the Autonomic Nervous System of various antihypertensive drugs.
(Arrows indicate the primary site of action of the drug.)
(Modified from Van Zwieten, 1975a.)
1. α-methyldopa, thought originally to exert its hypotensive effect by the production of a false transmitter at peripheral synapses (Day & Rand, 1963), has now been demonstrated to have its primary site of action centrally;

2. β-blockers, shown to produce most of their action by effects on the heart, are now shown also to be, in some cases, active via a central mechanism (e.g. Day & Roach, 1975) as well as having possible side effects on the renin-angiotensin system (Clarkson, 1976).

1.4 The Central Nervous System and Blood Pressure Control

1.4.1 Central Regulation of Blood Pressure

Over the past 20 years there has been a plethora of evidence which demonstrates that control of the circulation is by some central mechanism and that this central control involves catecholaminergic neurones (see Hilton, 1975; Chalmers, 1975; Korner, 1976).

As early as the 1850's, it was recognised that, for the maintenance of normal cardiovascular function, the central nervous system was important. It has been shown
that sectioning of the spinal cord in the lower cervical region would result in falls in blood pressure.

Recently, many papers have demonstrated that blood pressure can be modified (increased or decreased) by either the lesioning of certain areas of the brain, or the application of electrical stimulation or a variety of drugs (Gagnon & Melville, 1968; Reis, Nathan & Doba, 1975; Bhargava, 1976; Philippu, 1976). There are two major areas in the brain, the hypothalamus and the medulla, which appear to be important in blood pressure regulation. The stimulation of many points within these areas, by drugs or electrical stimuli, will, depending on the exact location of the point of stimulation, produce either pressor or depressor effects on blood pressure, along with possible effects on heart rate and respiration. The consideration of the results obtained from both the above experiments and others indicates that the hypothalamus and medulla appear to be interconnected by a number of pathways which seems to have the result of these two areas working in conjunction to control blood pressure (Calaresu & Thomas, 1975). However, there are certainly other areas in the brain that are also implicated in blood pressure control, areas such as the orbito-frontal cortex, the cerebellum and parts of the amygdala (see Korner, 1976).

Figure 1.3 shows a schematic diagram of the possible interconnections between the hypothalamic and medullary regions and their possible connections to higher control centres in the brain and also to the periphery.
FIGURE 1.3: Diagram of possible functional connections between the posteromedial hypothalamus and the medulla.
(After Calaresu & Thomas, 1975)

Influences from Higher Centres

Key to abbreviations on next page

+ = stimulatory neurone
- = inhibitory neurone
Abbreviations used in Figure 1.3

BR - Baroreceptors
CR - Chemoreceptors
NTS - Nucleus tractus solitarius
MOC - Medullae oblangatae centralis
NA - Nucleus ambiguus
PHA - Para hypoglossal area
LRN - Lateral reticular nucleus
ION - Inferior olivary nucleus
1.4.2 Central Adrenergic Mechanisms affecting Blood Pressure

The presence of noradrenaline-containing neurones in the central nervous system has been confirmed both biochemically and by histofluorescence and there is also considerable evidence that the amine functions as a neurotransmitter (for review see Andén et al., 1969). There is also evidence that some of these noradrenaline-containing neurones are involved in blood pressure control (see Henning, 1975; Fuxe et al., 1975). Other monoamines, such as serotonin, dopamine and adrenaline, have also been implicated in cardiovascular control (see Chalmers, 1975).

Anatomically, central adrenergic and serotonergic neurones are well suited for the central control of the circulation. Both transmitter systems have their cell bodies situated predominantly in the brain stem with ramifications extending upwards to higher centres in the brain, particularly to the hypothalamus, and downwards to the spinal cord, especially in the lateral horns, giving rise to the sympathetic pre-ganglionic neurones (Figure 1.4).

As well as considerable evidence to indicate that blood pressure control is elicited by central adrenergic mechanisms, there is increasing evidence that these
FIGURE 1.4: Simplified schematic representation of the organisation of central mono-aminergic neurones.
(Chalmers, 1975)
mechanisms are implicated in hypertension (Haeusler, 1976; Henning, 1975; Korner, 1976; Reid & Dollery, 1976). Modification of these central adrenergic mechanisms by a variety of ways produces effects on the cardiovascular system. Denervation of the carotid and aortic sinuses in the rabbit results in an increase in blood pressure with a concommitant increase in the turnover of noradrenaline in the thoracic spinal cord segments (Chalmers & Wurtman, 1971). Destruction of noradrenaline-containing nerve endings in the brain with 6-hydroxydopamine (6-OHDA, Uretsky & Iversen, 1970) prevents the rise in blood pressure normally resulting from baroreceptor denervation (Chalmers & Reid, 1972). Selective depletion of brain noradrenaline by this agent also produces a profound effect on the development of hypertension of experimental origin in both rats and rabbits. Haeusler et al (1972) showed that injection of 6-OHDA intraventricularly prevents the development of hypertension in rats, after such procedures as mineralocorticoid treatment plus saline feeding, and also in the spontaneously hypertensive rat. However, established hypertension was unaffected by intraventricular 6-OHDA.

The application of exogenous noradrenaline to the brain can be shown to produce pressor or depressor responses depending on the area of the brain to which it is applied (Gagnon & Melville, 1968; De Jong, 1974; Chalmers, 1975; Philippu et al, 1971; Philippu, 1976). However, it is now widely accepted that, in terms of central blood pressure
control, noradrenaline displays a predominantly inhibitory influence (Gagnon & Melville, 1968; De Jong, 1974; Struyker-Boudier et al, 1975).

Attempts to correlate disturbances of these central noradrenergic mechanisms with variation in blood pressure and hypertension has led to a variety of interesting results.

Yamori et al (1970) intimated that concentrations of noradrenaline in the lower brain stem and hypothalamus of genetically hypertensive rats were significantly lower than in controls. However, Yamabe et al (1973) disputed these results on the grounds that, although a difference may have been present, it was not due to the hypertension but was due to a strain difference.

Later work on other animal models has shown that, although levels of endogenous noradrenaline may not be related to hypertension, there does appear to be a correlation between the rate of turnover of noradrenaline in certain brain areas. For example, Nakamura, Gerold and Thoenen (1971) have shown that, in the DOCA/saline hypertensive rat, associated with the hypertension is a decrease in the turnover rate of noradrenaline in the hypothalamic and medullary brain areas. Also, along with this decrease in central noradrenaline turnover, there is a reciprocal increase in noradrenaline turnover in certain peripheral organs.

These results appear to provide evidence that central noradrenaline has an inhibitory effect on blood pressure.
Decreased noradrenaline turnover produces a reduction in the availability of noradrenaline at central inhibitory noradrenergic synapses, hence allowing increased sympathetic outflow and activity resulting in elevated blood pressure.

However, the above result only shows an association between turnover and hypertension. It does not clarify whether these changes in noradrenaline turnover play a causative role in, or are a result of, the hypertension.

Recent work by Ito, Tanaka and Omae (1975) has shown that intracisternal administration of guanethidine in rats produced decreases in the turnover rate of central noradrenaline along with concurrent increases in blood pressure. These results would seem to imply a causative role for the decrease in central noradrenaline turnover in the hypertensive rat.

A different experiment performed by Elliott and Clark (1976), which involved the administration of noradrenaline intracerebroventricularly, has shown that associated with this treatment is a vasodilatation of peripheral vascular beds, resulting in a fall in blood pressure. So, from the above work, it would appear that noradrenaline centrally has, on the whole, an inhibitory function in controlling blood pressure and, as a result, it is now well established that the predominant central mechanism controlling blood pressure involves noradrenergic neurones. The application of exogenous noradrenaline to various brain areas has an inhibitory effect on blood pressure and it has also been demonstrated that this inhibitory effect of noradrenaline
can be blocked by α-adrenoceptor blocking agents, such as
yohimbine and piperoxan. The implication of this result
is that the inhibitory effect of central noradrenaline is
mediated by the stimulation of central α-receptors
(Bhargava et al., 1972; Haeusler, 1973; Van Zwieten,
1973, 1975a; Day & Roach, 1974; De Jong, 1974; Trolin,
1975).

There also recently has been an indication that
β-receptors may also play some part in the central regulation
of blood pressure (Day & Roach, 1974; Share, 1973).

The connections between central α- and β-receptor
stimulation and central blood pressure regulation has
become even clearer with the advent of the centrally acting
antihypertensives, which are discussed in the following
sections.

1.4.3 Centrally Acting Antihypertensive Drugs

At present, there are two major antihypertensive drugs
used clinically which are said to exert their effects by
a central mechanism. These are α-methyldopa and clonidine
(Figure 1.5). The development and background of these and
other antihypertensives will be discussed in later,
separate sections.

The first indication that a drug may possess central
antihypertensive properties may be obtained by systemic
administration of the drug. In the case of a centrally
acting compound, the resultant reduction in blood pressure
FIGURE 1.5: Structural formulae of α-methyldopa and clonidine.

α-methyldopa
Aldomet

Clonidine
Catapres
would be accompanied by a bradycardia and not a reflex
tachycardia as might be expected. This test by no means
provides firm evidence of a central action but certainly
provides a justification of a more detailed investigation
of the drug.

Conclusive evidence of a central mechanism can only
be obtained if the drug being tested is administered
directly to the brain.

A variety of methods are available for this type of
investigation. Some involve direct application of the
drug to brain structures, while others present the drug to
the brain via the blood stream. Examples of direct
application of the drug to brain tissue include:

i) microinjection, by stereo-taxic procedures,
allowing precise localised administration
of the drug;

ii) iontophoresis, on to single neurones; or

iii) administration of the drug into the
cerebrospinal fluid, by injection
into, or perfusion of, the brain
ventricles.

For example, the perfusion of the third and fourth
ventricles of the cat with α-methyldopa was one of the
techniques used to demonstrate a central site of action of
the drug (Heise & Kroneberg, 1972).

The main advantage of the above direct techniques is that they all circumvent the Blood Brain Barrier. However, this might be a disadvantage as administration of drugs by this route does not allow any real comparison with the clinical administration of the drug.

Methods of presenting the drug to the brain using the blood stream involve surgery and cannulation of the arteries leading to the brain. The two major routes are via the carotid and vertebral arteries. However, the carotid arteries supply mainly the higher brain regions, whereas the brain centres involved in the regulation of blood pressure are localised in the rhombencephalon and hypothalamus. These two areas are mainly supplied by the vertebral arteries.

Injection via the vertebral arteries has been used by a number of workers to demonstrate a central site of action of α-methyldopa (e.g. Henning & Van Zwieten, 1968; Bock & Van Zwieten, 1971) and clonidine (Sattler & Van Zwieten, 1967).

The technique used is a modification of a method first demonstrated by Sir Henry Dale in 1927.

The route provides direct access to lower brain structures without distribution into the peripheral circulatory system, as happens with normal intravenous injections.

The use of the above techniques has demonstrated that both α-methyldopa and clonidine have predominantly a central
site of action and that they exert their effect by the stimulation of centrally located \( \alpha \)-receptors, resulting in a reduction of sympathetic outflow to peripheral tissues and hence a fall in blood pressure. This has been supported by considerable evidence from a number of sources (e.g. Andén et al, 1970; Bolme & Fuxe, 1971; Day, Roach & Whiting, 1973; Van Zwieten, 1973, 1975a; Kobinger & Pichler, 1974; Andén & Strömbom, 1975; Finch et al, 1975). A more detailed account of the evidence for a central site of action and \( \alpha \)-receptor stimulation by \( \alpha \)-methyldopa and clonidine is presented in the following sections.

1.4.3.1 \( \alpha \)-methyldopa

This drug was originally developed as an L-dopa-decarboxylase inhibitor to prevent the conversion of dopa-decarboxylase to dopamine and subsequently to noradrenaline in the hope that this would result in decreased peripheral sympathetic activity and hence lowered blood pressure. Indeed, the drug proved to be a potent inhibitor of the enzyme and was also found to display a significant clinical hypotensive effect. However, the hypotensive effect was found not to be causally related to the drug's enzyme inhibitory action as the duration of the hypotension persisted far longer than the duration of the enzyme inhibition. As a result, the above hypothesis for a mode of action of \( \alpha \)-methyldopa was rejected.
An alternative theory for the mechanism of action of \( \alpha \)-methyldopa was put forward by Day and Rand (1963, 1964). This was the false transmitter theory based on the idea that \textit{in vivo}, in the periphery, \( \alpha \)-methyldopa is converted to \( \alpha \)-methylnoradrenaline (Figure 1.6) which replaced noradrenaline in the storage sites in the adrenergic neurone. On nerve stimulation, this \( \alpha \)-methylnoradrenaline is released and was supposed to be less active than the natural compound at post synaptic receptors, resulting in a decreased effect of sympathetic activity. Although a valid theory based on the available evidence at the time, the idea is in conflict with a number of later experimental findings (Henning, 1969a & b). For example, even after peripheral blockade of L-dopa-decarboxylase with other drugs, preventing the formation of even \( \alpha \)-methylnoradrenaline, the hypotensive action of \( \alpha \)-methyldopa is still evident (Henning, 1969b). This implied another site of action which appeared to be centrally located.

The application of techniques discussed in the previous section confirmed that the major site of action of \( \alpha \)-methyldopa was, in fact, located in the brain and in the medullary region (Henning & Van Zwieten, 1968; Bock & Van Zwieten, 1971; Heise & Kroneberg, 1972). Heise and Kroneberg (1972) showed that perfusion of the third and fourth ventricles of the cat with \( \alpha \)-methyldopa or \( \alpha \)-methylnoradrenaline caused falls in blood pressure and this action was inhibited by the application of \( \alpha \)-blocking
FIGURE 1.6: The conversion of α-methyldopa to α-methylnoradrenaline.

L-α-methyldopa

\[ \text{Decarboxylation} \]

L-α-methyldopamine

\[ \text{Hydroxylation} \]

L-α-methylnoradrenaline
compounds such as phentolamine and yohimbine, thus suggesting that, as well as being due to a central site of action, the blood pressure lowering effect of α-methyldopa was mediated by the stimulation of α-receptors.

It is now well accepted that α-methyldopa is itself not the principal active compound but that its metabolite, α-methylnoradrenaline (the conversion and action suggested by Day and Rand and said to occur in the periphery), is responsible for the hypotensive effect. Reports indicate that this conversion occurs centrally and that α-methylnoradrenaline is as potent as noradrenaline in lowering blood pressure on central application (Heise & Kroneberg, 1972; Finch & Haeusler, 1973; Nijkamp & De Jong, 1975; Nijkamp et al, 1975; Henning, 1975).

Although it is now widely accepted that α-methyldopa exerts its action predominantly by the stimulation of central α-receptors (see Van Zwieten, 1973, 1975a), a peripheral component cannot be excluded. Wepierre et al (1973) suggest that α-methylnoradrenaline possesses some peripheral β-activity which may contribute, through vasodilation, to the hypotensive effect of α-methyldopa.

1.4.3.2 Clonidine

The antihypertensive drug clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, ST 155, or catepressan) was originally produced as one of a series of compounds intended for use as decongestive agents in
the treatment of Rhinitis. However, its potential as an antihypertensive agent was soon realised (Graubner & Wolf, 1966).

In view of its structural similarity to various α-sympathomimetic agents, it is not surprising that clonidine also produces effects by α-receptor stimulation, both in the periphery and in the central nervous system (Andén et al., 1970; Schmitt, Schmitt & Penard, 1971; Van Zwieten, 1973, 1975a). As a result of its hypotensive activity and α-sympathomimetic effects, clonidine has been the topic of a great deal of research (for reviews see Haeusler, 1973; Van Zwieten, 1973, 1975a; Kobinger, 1975, 1976).

Clonidine has been shown to exert a dual action on blood pressure when injected intravenously into anaesthetised animals of most species (Van Zwieten, 1975a) producing a short-lasting increase in blood pressure followed by a prolonged fall. The initial increase is a result of peripheral α-receptor stimulation causing vasoconstriction and increased vascular resistance (Zaimis, 1970), while the fall in blood pressure is a result of stimulation of central α-receptors (Kobinger, 1975).

Although it is now widely accepted that clonidine's antihypertensive action is mediated by the stimulation of α-receptors located predominantly in the medullary area in the brain (see Kobinger, 1975, 1976; Van Zwieten, 1975a), the possibility of other sites of action contributing to the antihypertensive effect cannot be fully excluded.
Other proposed sites of action include, for example, the ventral surface of the brain stem (Bousquet \textit{et al}, 1975), the forebrain (Klevans \textit{et al}, 1973), the heart (Scriabine \textit{et al}, 1970) and the peripheral vascular system (Shaw, Hunyor & Korner, 1971).

If the above evidence as to a central site of action of clonidine in stimulating \(\alpha\)-receptors is sound, two major questions on the mechanism of action must be answered:

1. Where are the central \(\alpha\)-receptors stimulated by clonidine? and

2. Are these receptors situated on the "pre-" or "post-synaptic" membrane?

In answer to the first question, the available evidence points to the medulla as the most probable site of action of clonidine (for review see Kobinger, 1975), in particular the pathways associated with the Nucleus Tractus Solitari and baroreceptor reflexes (Haeusler, 1973; Laubie \textit{et al}, 1976). However, Struyker-Boudier and Van Rossum (1972) have demonstrated that injection of clonidine, using stereo-taxic techniques, into the hypothalamus will also result in hypotension and bradycardia, a result that may fit in with the postulated interconnections between the hypothalamus and the medulla (Figure 1.3). Decreases in blood pressure have also resulted after application of
clonidine to the ventral surface of the brain stem (Bloch et al., 1973, 1974).

The answer to the second question is more complex. Briant et al. (1973) suggested that, as the tricyclic antidepressant desmethylimipramine (DMI) had a proposed mode of action of uptake blockade of noradrenaline at a pre-synaptic neurone site and as a clinical interaction occurred between DMI and clonidine resulting in a reduction in the hypotensive action of clonidine, the α-receptors stimulated by clonidine to bring about a fall in blood pressure were situated on the pre-synaptic neurone.

It has been suggested that, in the periphery, clonidine displays a higher affinity for pre-synaptic than post-synaptic binding sites (Starke et al., 1974). The above would imply that the mechanism of action of clonidine is associated with the release of endogenous noradrenaline. However, evidence is available to show that clonidine still has a hypotensive effect even after central depletion of noradrenaline by reserpine and/or turnover inhibition by α-methyl-p-tyrosine (Haeusler, 1973, 1974; Kobinger & Pichler, 1975, 1976).

The interaction between clonidine and DMI also appears, from more recent evidence, not to involve the central pre-synaptic neurone but to be a result of either the α-adrenolytic properties of DMI at a post-synaptic central site or the possible uptake blockade of peripheral noradrenaline resulting in a "physiological" interaction (Hoefke & Warnke-Sachs, 1974; Haeusler, 1974; Van Spanning
It would therefore appear that the site of the α-receptors stimulated by clonidine is on a post-synaptic membrane. A further and more detailed discussion on this topic will be presented in the final Chapter of this thesis.

Clonidine has been used clinically with some success (Conolly, 1975) but has displayed side effects which have made it unacceptable to some patients. The antihypertensive effect of clonidine is comparable to that of α-methyldopa and both display the same principal side effects of sedation and dry mouth, with the severity of the side effects greater with clonidine treatment (Conolly et al, 1972). One other major drawback in the use of clonidine is the danger that abrupt cessation of clonidine therapy can result in a hypertensive crisis with blood pressures exceeding original pre-treatment levels (Hansson et al, 1973; Gorchein, 1974; Bailey, 1975). However, this crisis can be controlled by recommencing the clonidine therapy or by treatment with α-blocking drugs such as phentolamine. A possible explanation of this rebound hypertension will be presented in the final discussion of this thesis.

1.4.3.3 Other Drugs exerting an Antihypertensive Effect by a Central Action

A number of other drugs can be shown to display
central hypotensive activity but not all are used clinically as antihypertensive agents. Examples of such drugs are reserpine, cocaine, amphetamine, tricyclic antidepressants, monoamine oxidase inhibitors and L-dopa. Their mechanism is probably indirect in character involving effects on endogenous noradrenaline and causing it to be displaced from the neurone and hence to stimulate central α-receptors inhibitory to blood pressure. Figure 1.7 shows a schematic representation of the probable mechanisms of action of various drugs producing effects via the stimulation of central α-receptors. A fuller explanation of their mechanisms of action is discussed by Van Zwieten (1975a).

Apart from research into compounds which are related to, or analogues of, clonidine, such as tolonidine (Cosnier et al, 1975), BAY 1470 (Finch, 1974; Kobinger & Pichler, 1975) and BS 100-141 (Scholtysik et al, 1975) (structures are shown in Figure 1.8), and which appear to exert their hypotensive action in a similar way to clonidine, a new development in connection with centrally acting antihypertensives is the synthesis of certain derivatives of neuroleptic agents.

One notable compound of this series is R.28935 (Erythro-1-(1-(2-(1,4-benzodioxan-2-y1)-2-OH-ethyl)-4-piperidy1)-2-benzimidazolinone, a derivative of pimozide (Wellens et al, 1975a; Figure 1.9). This compound is said to be almost as potent as an antihypertensive agent as clonidine, yet with a central mechanism of action which appears to be unrelated to α-receptor stimulation (Wellens
FIGURE 1.7: Schematic representation of the mechanism of action of various drugs acting via central α-receptors to bring about a fall in blood pressure.

(After Van Zwieten, 1975a.)
FIGURE 1.8: Clonidine and related compounds.

Clonidine HCl

Tolonidine Nitrate

BAY 1470, Xylazine

B.S. 100-141
FIGURE 1.9: Structures of Pimozide & R.28935.
et al, 1975b; Van Zwieten, 1975 a & c) and, as yet, has not been fully elucidated.

Another series of compounds that has recently become the centre of research interest regarding central anti-hypertensive activity are the β-blocking drugs. Although these drugs are already extensively used in the treatment of hypertension (Conway & Amery, 1975), the rationale behind their use was based on a peripheral site of action.

Recent work has indicated that, in the central regulation of blood pressure, as well as α-receptors, a role may be played by β-receptors (Share, 1973; Day & Roach, 1974). Subsequently, it has been demonstrated that certain β-blockers can exert a hypotensive action via a central mechanism and that this effect may work in conjunction with their peripheral effects to lower blood pressure (Clarkson, 1976; Day et al, 1976; Klevans, Kovacs & Kelly, 1976).

1.5 Basis and Aims of the Project

In the first instance, this project involved the comparison of normotensive and experimental hypertensive rats for differences in central catecholamine disposition and turnover, differences said to exist, and reported by other workers (e.g. Nakamura, Gerold & Thoenen, 1971).

Secondly, the major topic of the project was to
examine the effects of prolonged clonidine administration on catecholamine levels and turnover rates in the brains of normotensive and hypertensive rats and relate any effects to the hypotensive action of clonidine.

Thirdly, it was decided to examine, in closer detail, the reported interaction between clonidine and DMI (see section 1.4.3.2 for references) in respect to blood pressure changes and effects on central catecholamines.

It was hoped that the results obtained from the above investigations would help to clarify how this interaction occurs and, indirectly, help to identify the site and mechanism of action of clonidine.

In the course of the experiments, other drugs were also examined for effects on blood pressure and/or central catecholamines.
CHAPTER 2

METHODS
2.1 Animals

2.1.1 Animals and Environmental Conditions

The animals used in all the experiments described in this thesis, with the exception of the spontaneously hypertensive rats (see Chapter 4), were male albino rats of the CFY strain, supplied by Anglia Laboratory Animals. They were delivered by rail at either 200-250g or 80-90g weight and kept for 3-4 days in an animal holding room to allow recovery from stress, resulting from travel, and food and water deprivation before being used for experimental purposes.

The environment in the animal holding room was controlled to give a 24-hour diurnal cycle (light on 06.00h, light off 18.00h) with temperature normally maintained at 20-21°C. Groups of five rats were housed in plastic animal cages (14" x 21" x 6") with wire tops. Standard Oxoid Breeding Diet (41B) rat chow and tap water were available ad libitum.

All experimental procedures were carried out during the light phase of the diurnal cycle.

2.1.2 Choice of Species

In the planning of these experiments, the main factor governing the choice of animal was that of cost.

Having settled on the rat as the most reasonable animal for experiments involving large number of animals, it is possible to offer some rationalisation of the choice.
The rat is used as an experimental animal in a wide variety of procedures and, as it is probably one of the most commonly used laboratory animals, there is a lot of information available on the species. The study of hypertension, and antihypertensive drugs in particular, utilizes the rat, as it is a relatively easy model in which to induce hypertension and record blood pressure (Freyburger, 1968).

The albino rat is, on the whole, a placid animal and is therefore easy to handle for the purpose of administering drugs by various routes.

Rats are also freely available from a number of suppliers in a variety of consistent strains. The CFY strain was chosen as earlier work had indicated that it appeared to be a suitable strain for the experiments to be carried out (Grimes, unpublished observations).

2.2 The Measurement of Blood Pressure

2.2.1 Introduction

In all investigations into the effects of drugs on the cardiovascular system, one of the most important parameters is that of blood pressure. To determine blood pressure and other haemodynamic parameters in acute experiments, the anaesthetised animal is normally used, and here it is relatively easy to cannulate one of the major arteries
(most commonly the carotid or femoral artery) and to record blood pressure directly by means of a pressure transducer and recording system.

As the basis of this thesis was an examination of the effects of prolonged administration of certain anti-hypertensive drugs on blood pressure and central catecholamine disposition, it was obviously essential to be able to measure blood pressure in the conscious animal, thus preventing any possible interference by anaesthetics on both blood pressure and central catecholamine levels and turnover.

Two common methods are available for the measurement of blood pressure in the conscious rat. One is an invasive technique involving cannulation (with exteriorisation of the cannula) of either the abdominal aorta (Weeks & Jones, 1960; Weeks, 1976) or one of the carotid arteries into the aortic arch (Popovic & Popovic, 1960). A short-term invasive method of blood pressure measurement is direct cannulation of one of the caudal arteries using flexible plastic cannulae (Ertama, 1976). However, this technique does not lend itself to repetitive blood pressure measurements over more than one day because the cannulae only remain open for a few hours due to blood clot formation.

The other major method available for routine use is a non-invasive technique, measuring blood pressure in the caudal arteries using a tail sphygomanometer cuff (Gerold & Tschirky, 1968), a technique which can be performed repeatedly in conscious rats. The basic principle
is the same as that underlying sphygmanometer blood pressure measurements in humans.

The use of the invasive techniques was rejected for a number of reasons. Firstly, there could be stress associated with the surgery necessary to insert the cannulae. This is possibly particularly relevant when applied to animals already having undergone surgery for hypertension induction (as described later).

Secondly, in any invasive technique, there is a constant danger of clot formation in the cannulae with consequent failure to record blood pressure. To prevent this, daily flushing of the cannulae with an anti-coagulant solution is necessary. As, in the following experiments, only two blood pressure measurements were required, separated by a period of one week, it was thought that the handling of the animal and cannulae flushing would have subjected the animal to unnecessary additional stress.

The non-invasive tail cuff method was, therefore, considered more appropriate. This system has been used successfully by a number of workers (Gerold & Tschirky, 1968; Finch & Leach, 1970; Nakamura, Gerold & Thoenen, 1971). However, it was recognised that certain criticisms could be levelled against it (Fregly, 1963; Bunag, 1971). These deficiencies will be discussed in a later section together with the methods employed in an attempt to minimise their effects.
2.2.2 Method

The system used for measuring blood pressure in this project utilised a Rat Blood Pressure Monitor manufactured by Huntingdon Instruments. This was a manually operated monitor as opposed to automatic machines used by some workers (Gerold & Tschirky, 1968). The monitor utilises a cuff/detector unit, comprising an inflatable cuff and a photo-electric pulse detector combined in one. This is placed around the rat tail, the inflatable cuff being used to occlude the caudal arteries in the tail. The pressure inside the cuff, and hence the pressure inside the artery, is measured by a pressure transducer in the monitor unit. The photo-electric pulse detector responds to changes in light intensity from a controlled light source incorporated in the cuff unit. The changes in light intensity occur as a result of variation in blood density which is caused by arterial pulsing through the occlusion created by the inflated cuff.

Recording of the pulse and pressure signals was achieved by connection of the monitor to a Devices M2 two-channel recording system. The pressure signal was amplified by a Devices DC2A pre-amp (sub-unit 1c) and the pulse signal by a Devices DC6 pre-amp (sub-unit 02). Trace recordings were produced on two-channel heat-sensitive paper.

To measure blood pressure, the rat was placed for five minutes in a wire restraining cage in a warming oven, set at 40°C, after which the cage, still containing the animal,
was removed, wrapped in cotton wool and placed on a warming plate maintained at 35°C.

The cuff-detector unit was slipped on to the tail until a snug fit was achieved. A measure of blood pressure was obtained by inflating the tail cuff until the pulse signal disappeared. The cuff was then allowed to deflate slowly, until reappearance of the signal occurred. The pulse signal increased in amplitude until a plateau was attained on the trace (Figure 2.1). Reading down from the pulse to the pressure trace gave that pressure in the cuff at which the pulse signal reappeared and also when it plateaued. The pressure at which the pulse reappeared was taken as an indication of systolic blood pressure, while the pressure at which the pulse plateaued was taken as diastolic pressure. These two points correspond to the changes in the karotkov sounds heard in conventional clinical sphygmanometer cuff systems. Indeed, other tail cuff measuring systems employ a microphone to listen for these sounds, instead of using the photo-electric pulse detector system.

The above procedure was repeated at least three times for each animal, with the cuff on the tail being relocated each time. The mean value of the results obtained was taken as the measure of blood pressure.

2.2.3 Discussion on the Results obtained from Tail Cuff Blood Pressure Measurements

Although the makers of the blood pressure monitoring
FIGURE 2.1: Example of Blood Pressure Traces.

Trace 1 - normotensive animal
systolic 135mmHg
diastolic 80mmHg

Trace 2 - hypertensive animal
systolic 205mmHg
diastolic 145mmHg

PULSE RECORDING
system used in this thesis claim that both systolic and diastolic blood pressure measurements can be ascertained. It was found that, in practice, only systolic pressure was consistently and reliably measured.

There are a number of reasons for this. Firstly, it was found that many animals would not remain passive throughout the blood pressure measuring cycle. Any struggling was picked up by the detector unit and reproduced on the trace, obscuring the point at which the pulse signal plateaued. Secondly, in some instances, although clear indications of systolic pressure were obtained, an accurate diastolic reading was not possible, either because shallowness of the trace resulted in an ill-defined plateau or because of variation in the pulse signal itself. Hence, all the blood pressure values quoted in this thesis refer to systolic pressures only.

However, using the monitor as described above, it was found that the strain of rat used gave quite reproducible results. As can be seen from the table below, the blood pressure results obtained from three groups of control normotensive rats, measured at different times, are very similar and show remarkably small variation within the groups.
<table>
<thead>
<tr>
<th>Group Number</th>
<th>Number of Animals</th>
<th>Mean Blood Pressure (+S.E.M.)mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>$140.8 \pm 1.9$</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>$144.0 \pm 2.0$</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>$142.3 \pm 2.2$</td>
</tr>
</tbody>
</table>

Over the period of time encompassed by this thesis, it was found that the mean recorded blood pressure of control normotensive animals did vary, but consistently lay in the range 125-145mmHg. Possible explanations of this variation include differences in environmental conditions or seasonal variation in blood pressure.

However, the values obtained agree with those reported by some workers using similar systems (for example, Bunag, 1973) but appear higher than mean values reported by others (for example, Finch & Leach, 1970 - approximately 120mmHg; Nakamura, Gerold & Thoenen, 1971 - 130mmHg; Lew, 1976 - 130mmHg). There may be a number of reasons for this lack of agreement.

Firstly, it is possible that blood pressure may vary between strains of animals. Indeed, the results mentioned above are from a variety of strains including Sprague-Dawley, Wistar and CFY's.

Secondly, environmental factors may also be responsible for differences in blood pressure, as may be handling, travelling, caging conditions and noise level. Certain forms of environmental stress have been used specifically
for the induction of increased blood pressure. Amongst these are sound-withdrawal (Marwood & Lockett, 1973) and overcrowding and psychosocial stimulation (Henry et al., 1975a).

Thirdly, some workers have measured blood pressure during recovery from light anaesthesia (Robertson et al., 1968; Friedman & Dahl, 1975; Freis & Ragan, 1975). This is supposed to elevate any blood pressure changes that may occur as a result of the animal being stressed by, or struggling during, the measuring procedure. However, in my opinion, any reduction in stress is likely to be more than offset by the possible variations that may occur due to the anaesthetic. Anaesthesia may lower blood pressure anyway and the degree of anaesthesia could vary markedly.

It is possible that the incidence of struggling of the restrained animal could be reduced by training the animal, before commencement of experiments involving blood pressure estimations, to accept the restraining apparatus.

In an attempt to reduce errors arising from the above factors, such as struggling and environment, control groups were included in all experiments, and were subjected to the same conditions as those experienced by experimental groups. This allowed direct comparison of drug-treated animals with matched controls, so that any changes in blood pressure between the groups could be attributed to drug action and not to the experimental conditions.

Another important factor in blood pressure estimations using the rat tail is that, in the rat, the tail is an
important organ for heat regulation (Rand et al., 1965), dilatation or constriction of tail blood vessels increasing or decreasing blood flow through the tail and hence regulating heat loss. In order to obtain an adequate pulse flow through the tail, the vessels of the tail must be fully dilated. Hence, to achieve this, the animal must be warmed to induce heat loss. According to Fregly (1963), for the tail vessels to be fully dilated, the core temperature of the rat must be raised by an average of 0.8°C and that, to achieve this, the animal must be pre-heated at 40°C for a minimum of five minutes and this heat maintained during the blood pressure measurement.

This increase in temperature may itself affect blood pressure, so it is again important that control animals are included in the experiments.

Bunag & Riley (1974) have shown that the tail cuff method cannot be relied upon to reflect accurately rapid changes in blood pressure due to acute drug administration. However, steady state levels (i.e. after prolonged drug administration) appear to be well reflected.

It can be shown that there is a good correlation between blood pressure measurements taken using the tail cuff method and those obtained by direct cannulation of the carotid artery or aorta.

Table 2.1 shows the results obtained from four anaesthetised rats in which blood pressure was measured simultaneously using the tail cuff and the cannulated carotid artery over a period of 12 minutes. Blood pressure
TABLE 2.1: Blood Pressure Measurements

A comparison between values obtained using the tail cuff method and simultaneous measurement by cannulation of the carotid artery.

(Rats anaesthetised with 25% Urethane (0.6ml/100g))

(Values represent the mean of three measurements taken every three minutes starting 10 minutes after preparation of the animal to allow for blood pressure to stabilise)

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Tail Cuff</th>
<th>Carotid</th>
<th>Difference (Tail-Carotid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>105</td>
<td>95</td>
<td>+10</td>
</tr>
<tr>
<td></td>
<td>115</td>
<td>100</td>
<td>+15</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>105</td>
<td>+5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>110</td>
<td>-10</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>100</td>
<td>+5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>105</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>105</td>
<td>+5</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>105</td>
<td>+5</td>
</tr>
<tr>
<td>3</td>
<td>155</td>
<td>145</td>
<td>+10</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>135</td>
<td>+5</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>135</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>130</td>
<td>+5</td>
</tr>
<tr>
<td>4</td>
<td>135</td>
<td>130</td>
<td>+5</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>130</td>
<td>-10</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>135</td>
<td>+5</td>
</tr>
<tr>
<td></td>
<td>115</td>
<td>110</td>
<td>+5</td>
</tr>
</tbody>
</table>
measurements from the tail cuff were consistently higher than the values obtained from the carotid artery, the mean difference being $3.44 \pm 1.69\text{mmHg (mean } \pm \text{S.E.})$. However, the difference on the whole was constant and it therefore appeared that the tail cuff method was more than adequate for our requirements.

2.3 Experimental Hypertension

2.3.1 Introduction

It would seem logical that the screening for, and examination of, the mechanisms of action of antihypertensive drugs should involve the use of animals that have elevated blood pressure.

A variety of methods have been used to produce chronic elevations in the normal blood pressure of animals, particularly rats. The methods available include:

1. renal hypertension (Grollman, 1973; Ledingham, 1975) which can be induced by manipulation of the kidneys; for example, by the constriction of one renal artery and removal of the contralateral kidney (Goldblatt et al., 1934) or by encapsulation of a kidney in cellophane (Page, 1939), producing renal compression.
2. mineralocorticoid excess hypertension, involving the administration of mineralocorticoids such as Deoxycorticosterone acetate or Aldosterone with subsequent loading of the animal with saline (Finch & Leach, 1970; Hall & Hall, 1973; Gavras et al., 1975).

3. subjection of the animals to changes in environment, noxious stimuli or stress, such as the use of sound withdrawal (Marwood & Lockett, 1973) or overcrowding and social interaction (Henry et al., 1975a).

4. neurogenic hypertension, involving lesioning of areas of the brain, such as the Nucleus Tractus Solitari (Nathan & Reis, 1977) or the hypothalamus (Nathan & Reis, 1975), or by deafferentation of the arterial baro-receptors themselves (Korner, 1971).

5. spontaneous or genetic hypertension produced by selective inbreeding of specific strains of rats for increased blood pressure (Okamoto et al., 1972; Phelan et al., 1972).

The method chosen in this thesis is a modification of the DOCA/saline method of Finch and Leach (1970). This method was decided upon partly because it appears that there
is some indication for the involvement of central adrenergic neurones (Nakamura, Gerold & Thoenen, 1971; Chalmers, 1975, 1976). Originally, an attempt was made to produce hypertension by the constriction of one renal artery, leaving the contralateral kidney untouched and loading the animal with saline (the so-called "two kidney Goldblatt"). It was hoped that this method would have produced a significant hypertension without stressing the animal too much. However, as can be seen in Figure 2.2, this method was not entirely successful, producing only a mild hypertension approximately 30mmHg above control values.

Reinforcement of this method by the inclusion of a DOCA implant produced a consistent and substantial hypertension four weeks after surgery (Figure 2.2).

2.3.2 Method

All animals were starved by the removal of food the night before surgery. Anaesthesia was induced by the application of ether and the animal placed on a small operating table. An area of skin, extending caudally from the level of the rib cage and to the left of the spine, was shaved of fur and swabbed with 70% ethanol. An incision, approximately half an inch in length, was made through the skin and abdominal muscles, just to the left of the spine and below the level of the last rib. The left kidney was exposed and brought out of the abdominal cavity and kept moist with cotton wool soaked in isotonic saline. The
FIGURE 2.2: Development of Hypertension after Surgery

Mean Systolic Blood Pressure (mmHg)

Surgery

Control - no treatment (n = 14)
Sham operated - no saline (n = 6)
Unilateral clip + saline (n = 6)
Renal/DOCA + saline (n = 11)

*** P<0.001  ** P<0.005  * P<0.01
capsule was cleared from the kidney and the renal artery also cleared of tissue. The artery was then occluded by the application of a silver clip of internal diameter 0.2mm. The silver clips used were made from pieces of silver tape, approximately 2mm x 5mm x 0.2mm (tape supplied by Johnson Mathey & Co. Ltd.). The gap between the internal surfaces of the clip were set by bending two pieces of tape over one another, as shown below, using the thickness of one to set the gap on the other:

The kidney was then replaced in the abdominal cavity and the abdominal muscle layers sutured with plain cat gut (Ethicon size 3/0). Before the skin incision was closed, a tunnel was made through the connective tissue under the skin of the back into the loose area of skin on the neck using a pair of blunt forceps. Into this area of loose skin was inserted a 25mg pellet of Deoxycorticosterone Acetate (DOCA, Organon Labs.) after which the skin was finally sutured with plain cat gut.

The animals were placed in separate cages during recovery, after which they were housed, five animals to a cage, in normal animal house conditions. Food was made available ad libitum but drinking water was replaced by a
1% solution of sodium chloride.

Animals were used for experimental purposes not earlier than four weeks after surgery, by which time they had developed a blood pressure that was, on average, 60-70 mmHg higher than that of untreated or sham-operated animals (Figure 2.2).

This type of hypertensive animal will be referred to throughout this thesis as renal/DOCA hypertensives.

2.3.3 The Development of the Hypertension

In order to see how the hypertension produced by the above procedure developed with time, four groups of animals were manipulated in various ways and their blood pressures measured every three or four days for approximately five weeks after surgery. The groups comprised the following:

i) control - no surgery;
ii) sham-operated - no saline;
iii) unilateral clip plus saline;
iv) renal/DOCA plus saline.

As can be seen from Figure 2.2, after four weeks the blood pressure of sham-operated animals did not significantly differ from that of control animals. The unilateral clip animals, group iii, showed a slight increase over control values while the renal/DOCA animals showed a relatively steady increase in blood pressure to around
210mmHg systolic, after four weeks, at which the blood pressure increase appeared to stabilise.

2.3.4 Observations on the Renal/DOCA Hypertensive Rats

It was noted that, if the hypertensive rats produced by the above methods were allowed to survive past six to eight weeks, a large percentage of the animals displayed symptoms of severe chronic hypertension. These symptoms included marked weight loss, seizures, strokes, etc. On dissection, haemorrhages were noted throughout the brain tissue. In some cases, the brain tissue was obviously oedematous and had become very pale in colour and spongy in texture.

Even some of the animals that did not display severe symptoms still had very oedematous brains. Since the oedema could cause significant changes in the wet weight of tissue, these brain samples were not included in the final results. Out of a sample of 35 hypertensive rat brains, five displayed severe oedema, increasing the weights of the brains to over 2g, which was substantially greater than the mean brain weight of the apparently unaffected animals (Table 2.2).

The incidence of oedematous brains, together with the number of hypertensive animals that died during experiments, resulted in some groups of animals being smaller than originally intended and smaller than would ideally have been wanted.
<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Mean Weight in g (± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>30</td>
<td>1.674 ± 0.010</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>30</td>
<td>1.716 ± 0.018</td>
</tr>
<tr>
<td>Hypertensive (oedematous brains)</td>
<td>5</td>
<td>2.254 ± 0.060***</td>
</tr>
</tbody>
</table>

* Significantly different from normotensive value  \( P < 0.05 \)

*** Significantly different from hypertensive value  \( P < 0.001 \)
2.4 The Estimation of Noradrenaline and Dopamine in Rat Brain

2.4.1 Introduction

As has been stated in the introduction to this thesis, one of the purposes of the investigation was to examine the effects of clonidine and other antihypertensive agents on the disposition of noradrenaline and dopamine in the central nervous system. Consequently, a reliable and reproduceable method of estimating the concentration of these catecholamines in brain tissue was required.

Two main steps are involved in the estimation of catecholamines in any tissue. Firstly, it is important to be able to extract the required catecholamine from the tissue, removing metabolites and other substances that may interfere with the assay. Secondly, the assay method chosen needs to be specific for the catecholamine to be estimated and also needs to be relatively sensitive to allow measurement of small differences between low concentrations.

At the commencement of this research project, apart from available fluorimetric methods of assay (e.g. Shellenburger & Gordon, 1971), newer methods of catecholamine estimation were becoming available, utilising enzymatic-isotopic techniques. One technique is based on the O-methylation of catecholamines by catechol-O-methyltransferase enzyme. However, although this method is reputed to have a sensitivity of around 15pg for noradrenaline.
drenaline, its main drawback is that it cannot distinguish between noradrenaline and adrenaline (Palkovits et al, 1974). Another enzymatic-isotopic method involves the conversion of noradrenaline to adrenaline using partially purified Bovine adrenal phenylethanolamine-N-methyltransferase (PNMT) and tritiated S-adenosyl-methionine, which is then selectively isolated and measured (Henry et al, 1975b). This method also has a sensitivity of around 15-25pg. However, efficiency is decreased if concentrations of noradrenaline rise above 15ng due to substrate inhibition by noradrenaline of the enzyme.

The decision not to use these radioenzymatic assays for noradrenaline was based on a number of factors. Firstly, as the assays were new, a lot of methodology would have had to be mastered. As methodology was not one of the main subjects of the project, it was thought that more well-tried methods would be more suitable. Secondly, there was the cost factor. Radiolabelled compounds are expensive and the cost of these could not be justified against the research budget. Thirdly, as we were interested primarily in gross areas of the brain, such as the hypothalamus and medulla, the sensitivity of the above assays was thought to be far in excess of requirements.

Palkovits (1973) has developed a technique that allows the removal of isolated nuclei of rat brain. Combination of this technique with, for example, the PNMT assay may now, and in future research projects, allow the examination of changes of catecholamine concentration in very
discrete and possibly more meaningful areas than the gross regions examined in this project. As these techniques were only just becoming available at the start of this project, it was again felt that more established techniques would be of value at first.

As only noradrenaline and dopamine were to be measured and not other amines, such as 5-hydroxytryptamine, a method was found in the literature that would enable easy extraction of catecholamines from brain tissue and subsequent assay by fluorimetric means (Shellenburger & Gordon, 1971). This method was examined and was found to be a relatively rapid and simple method of assaying noradrenaline and dopamine in discrete brain areas, with sufficient specificity and reproducibility to enable accurate and meaningful results to be obtained. It permits the simultaneous development and reading of fluorescence of both catecholamines in one sample and allows the detection of noradrenaline to values as low as 10ng.

The extraction of catecholamines from the brain homogenate was achieved by adsorption on to alumina at alkali pH with subsequent elution in acid media (Anton & Sayre, 1962, as modified by Shellenburger & Gordon, 1971, to deal with small quantities of tissue). This gave extraction efficiencies of 70-90%. Alumina was used instead of ion-exchange resins, as reported by other workers, as it is said to give higher recoveries (Anton & Sayre, 1962).

Catecholamines were assayed by oxidation to the
fluorescent Trihydroxyindole derivatives (Figure 2.3) which show greater fluorescence than the natural fluorescence of the untreated catecholamine. Oxidation is brought about by the use of iodine, whereas, in earlier methods, the agent was ferricyanide (Anton & Sayre, 1962). The use of iodine produces a quicker oxidation time, therefore reducing assay time, and also reduces blank values (Shellenburger & Gordon, 1971).

The actual methodology used is described in section 2.4.4.

2.4.2 General Consideration of Methodology

A number of experimental variables have to be considered which may affect the reproduceability of catecholamine estimations.

Probably one of the most important factors to consider is that of circadian variation in the concentration of catecholamine in the brain. Variation certainly does occur in the rat brain producing, depending on the time of the circadian cycle, significant differences in estimated values (Sheving et al, 1968; Lew, 1976). In attempting to overcome variation from this source, it is important to perform experiments, that are to be related to one another, at the same clock hour.

Another consideration particularly applicable to catecholamine estimations is that noradrenaline and dopamine are unstable in the presence of oxygen and non-acid
FIGURE 2.3: Simplified diagram of the production of the fluorescent derivatives of catecholamines.
pH (Heacock & Powell, 1972) and so have to be stabilised in an acid medium as soon as possible. Coupled with this is the possibility of post mortem changes occurring in the tissues. Some workers (Faiman et al., 1973; Sloviter & Connor, 1977) found that the rate of degradation of noradrenaline changes with time after death. Hence, it can be seen that it is important to have a method of brain removal and dissection that can be performed rapidly and reproducibly to allow rapid stabilisation of the catecholamines.

The method of brain dissection developed by Glowinski and Iversen (1965) allows removal and subsequent dissection of a rat brain into a minimum of five regions in under five minutes with relative ease. This allows groups of up to 20 animals to be killed, have their brains dissected and the tissue homogenised within a period of 100 minutes. In the experimental situation, control animals are included in every experiment to further reduce circadian variation, that may occur even over this time period, and are killed alternately with experimental animals. It is also obvious that long periods of time between the killing of an animal and the removal of its brain and subsequent stabilisation of the catecholamines may lead to erroneous results. Therefore, to standardise any degradation that may occur, as well as requiring a rapid dissection technique, the animal must be killed and its brain removed swiftly.

One method of stabilising catecholamines in the brain,
while the animal is being killed, involves the quick freezing of the whole animal, or its chest and head, in liquid nitrogen. This freezes the tissue in situ which can then be removed by chipping the skull away. This method is satisfactory for the examination of whole brain, but, due to the brittleness of the frozen tissue, does not allow quick or accurate dissection of the brain into regions. The time required for dissection of tissue in this state usually exceeds 10 minutes (Buxton, 1974). The procedure is also expensive in its use of liquid nitrogen.

Decapitation of the animal, by a guillotine, combined with the dissection technique of Glowinski and Iversen, ensures, however, that the time from the instant of killing to final dissection of the required brain regions never exceeds five minutes. Although this technique may allow some variation as a result of fear, stress, or shock, plus also some enzyme degradation, this is at least reduced by the consistency of the technique and the relatively short time it takes.

Buxton (1974), on comparing the above two methods, suggests that the decapitation technique is superior, in that it gives greater reproducibility of tissue weights and can be performed quickly.

Another method, which at least deserves a mention, is the use of microwave radiation to kill animals and "fix" the catecholamines in brain tissue. However, reports on this method indicate that higher values of catecholamines are detected than when conventional killing methods are
used (Merritt, Medina & Frazer, 1975). It remains for an explanation of this phenomena to be found before the possible value of this technique; in catecholamine research, can be assessed.

2.4.3 Brain Removal and Dissection

Five minutes were found to be ample time for the removal of a rat brain, its dissection into regions and subsequent homogenisation in acid medium.

Rats were first stunned by a blow on the head and then decapitated. The spinal cord and extraneous tissue were trimmed level with the base of the skull. The top of the skull was then cleared of skin and removed by two parallel cuts through the bone along the side of the cranium. The bone flap produced was bent forward over the snout of the animal exposing the brain. The brain was then cleared of dura and removed intact using a small, blunt, rounded spatula and placed on a filter paper soaked in cooled 0.32M sucrose in preparation for dissection.

Areas of the brain containing the cortex, midbrain, hypothalamus and medulla were dissected out according to the method of Glowinski and Iversen (1966), the lines of dissection are shown in Figure 2.4, and the dissection carried out as follows.

Firstly, the cerebellum was teased away from the medulla and discarded and a transverse section made through the brain to remove the medulla-pons region (section 1).
FIGURE 2.4: Diagrammatic representation of dissection procedure for rat brain. Dotted lines indicate positions of initial sections.

- c - cortex
- m - midbrain
- hi - hippocampus
- hy - hypothalamus
- me - medulla
- s - striatum
- ce - cerebellum
A second, transverse section was made at the level of the optic chiasma passing also through the level of the anterior commissure (section 2). This gave two parts - A and B. Part A contained cortex and some striata. The striata were removed and discarded. The striatal areas remaining in part B were then dissected out and discarded and the cortical portions peeled off and combined with part A. The hippocampal regions were also dissected away and discarded. The region containing the hypothalamus was then dissected out using the anterior commissure as a horizontal reference and the line between the posterior hypothalamus and the mammillary bodies as the caudal limit. The remaining tissue was designated midbrain.

The tissue sections were then weighed on a top pan balance and homogenised for 30 seconds in 8ml of 0.4N perchloric acid containing 0.1% sodium metabisulphite and 0.05% disodium EDTA using a "TRI-R" homogeniser with a teflon/glass pestle and mortar. The homogenate was poured into a Beckman 13.5ml centrifuge tube and stored in ice until all the samples had been prepared. Homogenates were then centrifuged in a Beckman Highspeed L.50 at 17,500 r.p.m. for 10 minutes, after which 6ml aliquots of the particle-free supernatent were removed and stored in screw-capped tubes (Flow-labs) at -18°C until required for extraction and assay.

Studies on standard solutions of catecholamines and brain homogenate had previously shown that there was negligible reduction in the measurable catecholamine content.
of the supernatent over two to three weeks, if stored in this way.

2.4.4 Extraction and Assay of Noradrenaline and Dopamine

The assay of noradrenaline and dopamine was performed essentially according to the method of Shellenburger and Gordon (1971).

The first step was purification of the catecholamine by adsorption on to 300mg of alumina (prepared by the method of Anton & Sayre, 1962) at pH7.5, buffered with tricine buffer (17.9g tricine, 25g disodium EDTA in 1 litre of 0.525N sodium hydroxide). The alumina was subsequently washed three times with distilled water, the washings being discarded each time, and the adsorbed catecholamines finally eluted with 2.5ml of 0.05N perchloric acid. The samples could be stored at this stage overnight in screw-capped tubes at -18°C without any loss of measurable catecholamine concentration.

One ml aliquots of the eluate were then used to assay for noradrenaline and dopamine. To each aliquot was added 1.5ml of phosphate buffer at pH7.0 (4.27g disodium hydrogen phosphate, 9.52g potassium dihydrogen phosphate, 9.0g disodium EDTA in 1 litre of distilled water and adjusted to pH7.0 with 5N sodium hydroxide), the sample being shaken to ensure mixing. This was followed by the addition of 0.2ml of iodine reagent (0.5g iodine,
2.0g potassium iodide in 40ml of distilled water), the tube shaken again and then allowed to stand for two minutes at room temperature, after which 0.5ml of alkaline sulphite (1g sodium sulphite, 4ml distilled water in 36ml 5N sodium hydroxide) were added, the tube again shaken and allowed to stand for a further two minutes at room temperature. This was followed by the addition of 0.3ml of glacial acetic acid and the tube shaken again.

The samples were placed in an oven at 100°C for five minutes, after which they were placed on ice for a further five minutes and the relative fluorescence of the samples read at excitation 380mu, emission 495mu (uncorrected - maximum fluorescence peaks for noradrenaline) to estimate the concentration of noradrenaline present. The samples were then returned to the oven for a further 35 minutes followed by five minutes on ice, after which the relative fluorescence at excitation 325mu and emission 380mu (maximum fluorescence peaks for dopamine) was measured to give levels of dopamine in the sample.

An indication of the efficiency of recovery of catecholamines from alumina was obtained by the adsorption and elution of aqueous catecholamine standards included in all assays of brain samples. Normally, the standards consisted of 200ng each of noradrenaline and dopamine in one sample. The relative fluorescence (RF) values obtained from brain extract samples and extracted standards were then applied to the following formula, allowing direct calculation of the concentration of noradrenaline and dopamine in the original
tissue samples in ng/g tissue:

\[
\text{concentration of catecholamine} = \frac{\text{RF sample}}{\text{RF standard}} \times \chi \times \left(\frac{8 + w}{6}\right) \times w
\]

\( \chi \) = concentration of aqueous extracted standard in ng.

\( w \) = wet weight of tissue in g.

The use of the above equation and aqueous standards included in all assays made the constant reference to standard curves of noradrenaline and dopamine unnecessary. Previous experiments had shown that, in the concentrations encountered in the experiments, the relationship of RF to concentration of catecholamine displayed a linear relationship (Figure 2.5).

Typical percentage recoveries of standards from aqueous solution were noradrenaline 75% and dopamine 67%. The recoveries of internal standards of catecholamines from brain tissue were found to be lower than the values quoted above. However, the difference was shown to be constant and reproducible. As changes in catecholamine levels, and not the absolute values of concentration, were the main subject of interest, the discrepancy of value resulting from the use of aqueous standards to calculate recovery was thought acceptable.

Two types of blank samples were included in every
FIGURE 2.5: Standard fluorescence curves of Catecholamine Fluorophores.

- Noradrenaline 380-495μu
- Dopamine 320-380μu
assay:

i) reagent blank - taken through the whole procedure,

ii) reversed sample blank - brain extract sample with the order of addition of iodine reagent and alkaline sulphite reversed.

RF values for both types of blank were similar so the values obtained from reversed blanks were subtracted from sample RF values before application of the formula.

The values obtained for the concentrations of noradrenaline in various brain regions using the above method can be found elsewhere in this thesis and will be seen to be in general agreement with other published values (see Chapter 3).

2.4.5 Materials

perchloric acid (S.G. 1.70)
sodium metabisulphite
ethylenediaminetetra acetic acid disodium salt (EDTA)
sodium hydroxide
disodium hydrogen phosphate
potassium dihydrogen phosphate
iodine
potassium iodide
sodium sulphite

All the above chemicals were of ANALAR grade and obtained from B.D.H. Ltd.

tricine, (N-tris(hydroxymethyl)methylglycine) — obtained from B.D.H. Biochemicals Ltd.

alumina oxide (neutral), Brockmann Grade I — obtained from B.D.H. Ltd.

noradrenaline, DL-arterenol — obtained from Sigma Chemical Co.

dopamine, 3-hydroxy tyramine — obtained from Koch-Light Laboratories

2.5 The Estimation of the Turnover of Noradrenaline and Dopamine in Rat Brain

2.5.1 Introduction

In some of the experiments reported in this thesis the effect of antihypertensive drugs on the turnover of central noradrenaline and dopamine was also assessed.

Turnover may be defined as the phenomenon by which catecholamines are metabolised (and/or released) and then replaced with newly synthesised molecules. The rate limiting
step in the synthesis of noradrenaline and dopamine is the conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase (Kopin, 1968). Blockade of this enzyme by a tyrosine hydroxylase inhibitor, such as α-methyl-p-tyrosine (α-MT), will prevent the synthesis of endogenous noradrenaline (Spector et al., 1965), and the subsequent decline of the endogenous concentration of noradrenaline and dopamine over a period of time can be equated with metabolism and release of the amines and, hence, an indication of "turnover" assessed. It has been shown that the decline of catecholamine concentration after synthesis inhibition occurs exponentially (Brodie et al., 1966). Hence, estimation of the rate of decline of the levels of noradrenaline and dopamine in various regions of the rat brain by measuring catecholamine levels at time zero and a specific time interval after synthesis inhibition with α-MT will give an indication of the rate of turnover of the catecholamines in the tissue (Brodie et al., 1966).

2.5.2 Method

In experiments involving the estimation of the rate of turnover of noradrenaline and dopamine in brain tissue, animals were dosed with the tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine (α-MT), at 250mg/kg i.p. (as the methyl ester hydrochloride) dissolved in 0.9% saline. The animals were then killed at various times after injection,
their brains removed and catecholamine concentration estimated by methods previously described (section 2.4).

Turnover was expressed as the decline in catecholamine concentration, estimated by measuring the levels of catecholamine remaining in the brain tissue at various time points after injection of α-MT. The mean value (usually of five samples) for each time point was plotted as log. concentration against time after injection and the slope of the line produced used as an expression of rate of decline.

2.5.3 Results and Discussion

Using the above method, it was found that up to four hours after injection of α-MT the decline in the concentrations of noradrenaline and dopamine was linear, both in whole brains (Figure 2.6) and in certain brain regions, such as the hypothalamus and medulla (Figure 2.7). Consequently, during routine experiments, catecholamine levels were only estimated at two time points, those of zero and four hours after α-MT.

To estimate the effects of drug treatment on catecholamine turnover, the slopes produced from the results of control and treated animals were compared and any difference in the drug-treated result expressed as a percentage change of the control value. Tests for significance were performed by application of the t-test for comparing slopes (Bailey, 1959, p99).
FIGURE 2.6: Decline of Noradrenaline and Dopamine in whole brains of rats after synthesis inhibition with α-MT 250mg/Kg i.p. at various time intervals. Five animals per point.
FIGURE 2.7: Decline of Noradrenaline in medulla and hypothalamus after synthesis inhibition with α-MT at various time intervals.
CHAPTER 3

DIFFERENCES BETWEEN
NORMOTENSIVE AND HYPERTENSIVE ANIMALS
3.1 Introduction

Research into the causes of hypertension has shown that there are a large number of differences between normotensive and hypertensive animals, probably the most striking of which are changes in the vascular system (Somylo & Somylo, 1968, 1970; Jones, 1976) and differences in central adrenergic and peripheral sympathetic activity (Nakamura, Gerold & Thoenen, 1971; De Champlain & Van Ameringen, 1975). The DOCA hypertensive rat, an experimental model of hypertension used by many workers (e.g. Finch & Leach, 1970; Nakamura, Gerold & Thoenen, 1971; Haeusler & Finch, 1972; Hall & Hall, 1973; Jones, 1976), displays a number of differences when compared to normotensive rats of the same strain. These differences appear to be related to the hypertension and not to other factors, such as environmental changes (Somylo & Somylo, 1968; Nakamura, Gerold & Thoenen, 1971). However, as to whether these changes play a causative role in the hypertension, or are a result of it, is still a matter for discussion.

Histological examination demonstrates differences in the tissues of normotensive and hypertensive animals. The hypertensive animal displays signs of fibroid deposition and vascular necrosis in the kidney (Gavras et al, 1975) and narrowing and constriction of the lumen of the arteries.
It is well documented that the hypertensive animal shows increased vascular resistance and increased vascular reactivity to both endogenous and exogenous vasoconstrictor stimuli (Somylo & Somylo, 1970; Haeusler & Finch, 1972; Dupont & Sassard, 1974; Berecek & Bohr, 1976; Jones, 1976). Dupont and Sassard (1974), in fact, suggest that, in the spontaneous hypertensive rat, increased sensitivity to catecholamines might be one of the possible causes of hypertension, while Hansen & Bohr (1975) suggest that altered contractility and sensitivity of vascular smooth muscle may precede the increase in arterial blood pressure produced in DOCA hypertension.

It is perhaps feasible that the increased vascular resistance of hypertensive animals, thought to result from the functional changes in the vascular smooth muscle (Jones, 1976), may impose a greater work load on the heart, resulting in hypertrophy of the heart muscle. De Champlain et al (1967) and Nakamura, Gerold and Thoenen (1971) have indicated that, in the DOCA hypertensive animal, there is a significant correlation between the rise in systolic blood pressure and an increase in heart weight. Other organs have also been shown to hypertrophy in the hypertensive animal. Hall and Hall (1973) demonstrated that, as well as an increase in heart weight, an increase in kidney weight was also apparent.

However, in recent years, one of the major areas of research into the differences between hypertensive and
normotensive animals has been the role of the sympathetic nervous system, which has been implicated in the development of hypertension in certain animal models (Finch & Leach, 1970; Grewal & Kaul, 1971; Iriuchijima et al., 1975; De Champlain & Van Ameringen, 1975).

The turnover of noradrenaline in peripheral organs has been shown to be increased in the hypertensive animal (Nakamura, Gerold & Thoenen, 1971). Also increased levels of plasma noradrenaline have been detected (Reid, Zivin & Kopin, 1975; De Champlain & Van Ameringen, 1975). These results imply increased sympathetic activity in the hypertensive animal.

As has already been discussed in Chapter 1, central adrenergic neurones have been implicated in both normal cardiovascular control and the development of hypertension. Differences in the levels and turnover of catecholamines, in particular noradrenaline, in the brain have been demonstrated to be associated with hypertension (Robertson et al., 1968; Chalmers, 1975; Petty & Reid, 1977). Experiments have shown that, in certain animal models with elevated blood pressure, the turnover of noradrenaline in the hypothalamic and medullary regions of the brain is depressed (Nakamura et al., 1971; Ito, Tanaka & Omae, 1975).

Whether these changes result from, or cause, the hypertension is again still open to discussion. However, Haeusler, Finch and Thoenen (1972) suggest that, in the DOCA hypertensive rat, this decrease in turnover may be associated with a "trigger" mechanism in the brain, dis-
turbance of which may result in a self-perpetuating hypertensive disease.

Although, in this thesis, principal interest was in the relationship between central catecholamines and blood pressure, it was thought that a comparison of organ weight in normotensive and hypertensive animals would be instructive.

3.2 Differences in Organ and Tissue Weight

In a series of experiments comparing normotensive and renal/DOCA hypertensive animals (see Chapter 2), blood pressure, body weight, heart weight and whole brain (minus cerebellum) weight were measured. Table 3.1 shows a comparison of the results obtained.

The hearts of hypertensive animals were substantially heavier ($P < 0.001$) than those of normotensive animals. This increase in weight could not be attributable to greater animal size, as the mean body weight of the hypertensive animals was, in fact, lower than the mean body weight of the normotensives ($P < 0.05$).

Comparison of whole brain weights showed that brains from hypertensive animals were heavier than those from corresponding normotensive animals ($P < 0.05$). The results for brain weight shown in Table 3.1 represent only those brains that were used for the determination of noradrenaline turnover and levels. As has been stated in Chapter 2 (section 3.4), a small percentage of the brains from hyper-
TABLE 3.1: A comparison between blood pressures, body weight, heart weight and whole brain (minus cerebellum) weight of normotensive and renal/DOCA hypertensive animals.
(Mean ± S.E.M.) (Figures in parenthesis are numbers of animals)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensives</th>
<th>Renal/DOCA Hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Blood Pressure</td>
<td>137.4 ± 2.3 mmHg (23)</td>
<td>219.8 ± 3.7 mmHg (25) ***</td>
</tr>
<tr>
<td>Heart Weight</td>
<td>0.942 ± 0.016g (25)</td>
<td>1.565 ± 0.007g (26) ***</td>
</tr>
<tr>
<td>Body Weight</td>
<td>297.5 ± 6.9g (20)</td>
<td>275.5 ± 35.8 g (21) *</td>
</tr>
<tr>
<td>Brain Weight (minus cerebellum)</td>
<td>1.674 ± 0.010g (30)</td>
<td>1.709 ± 0.016g (30) *</td>
</tr>
</tbody>
</table>

*** P < 0.001
* P < 0.05
tensive rats was significantly heavier than the mean of the brain weights expressed in Table 3.1 (see Chapter 2, Table 2). These brains were very obviously oedematous, having a very spongy texture with high fluid content which increased their weight by up to 40%. Consequently, these brains were excluded from all experiments.

However, examination of the weights of various regions of brains from normotensive and hypertensive rats, dissected using the method of Glowinski and Iversen (1965), showed no significant differences between the two groups of animals (Table 3.2). This discrepancy of increased whole brain weight, yet with no significant differences in the weights of brain regions, may be explained in a number of ways. The increase in weight of the hypertensive brain was only 35mg over the normotensive brain weight. As this increase is small, the difference may have been proportioned out among the dissected brain regions and the difference lost by weighing inaccuracies. Alternatively, it is possible that the increase in whole brain weight was due to tissue oedema. Dissection of the brain would lead to some fluid loss and, hence, result in no difference in the weights of brain regions. The whole brains contained the striata and the hippocampi. However, it does not seem likely that these specific regions would be any different in hypertensive rats when compared to normotensive animals.
TABLE 3.2: A comparison of the weights of various regions dissected from the brains of normotensive and renal/DOCA hypertensive rats (dissected using the method of Glowinski & Iversen (1966)).
(Mean ± S.E.M.)
(Figures in parenthesis are numbers of animals)

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Normotensive Weight of Tissue in g</th>
<th>Hypertensive Weight of Tissue in g</th>
<th>N/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medulla</td>
<td>0.197 ± 0.003 (26)</td>
<td>0.194 ± 0.004 (27)</td>
<td>N/S</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.094 ± 0.003 (26)</td>
<td>0.094 ± 0.003 (27)</td>
<td>N/S</td>
</tr>
<tr>
<td>Midbrain</td>
<td>0.217 ± 0.006 (20)</td>
<td>0.229 ± 0.006 (21)</td>
<td>N/S</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.946 ± 0.013 (20)</td>
<td>0.949 ± 0.015 (21)</td>
<td>N/S</td>
</tr>
</tbody>
</table>

N/S = Not Significant
3.3 Differences in Catecholamine Levels in Brain Regions

Comparisons of various brain regions from normotensive and renal/DOCA hypertensive animals generally showed no significant differences in the levels of noradrenaline or dopamine (Table 3.3). However, dopamine levels in the hypothalamus of hypertensive animals were shown to be significantly reduced when compared to levels in normotensive animals. Detailed examination of the noradrenaline results shows that, although not significant, the mean level of noradrenaline, in all brain regions except the hypothalamus, is higher than that of corresponding controls. It is perhaps possible that such a small increase is genuinely related to the hypertension and that the increase may be significant if larger groups of animals were to be examined.

3.4 Differences in Central Catecholamine Turnover

Turnover of noradrenaline and dopamine was estimated in brain tissue by calculating the rate of decline of the catecholamines after inhibition of synthesis by α-methyl-p-tyrosine, as described in Chapter 2, section 5.

The turnover of both noradrenaline and dopamine was found to be significantly decreased in the whole brains of renal/DOCA hypertensive rats when compared to age-matched
TABLE 3.3: Comparison of the levels of Noradrenaline and Dopamine in four areas of brains from normotensive and renal/DOCA hypertensive rats. (Mean ± S.E.M.) (Figures in parenthesis are numbers of animals)

<table>
<thead>
<tr>
<th>Region</th>
<th>Levels of Catecholamine ng/g Tissue</th>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Noradrenaline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medulla</td>
<td>530.7 ± 28.2 (14)</td>
<td>570.4 ± 15.7 (15)</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1177.6 ± 37.3 (13)</td>
<td>1152.9 ± 62.9 (15)</td>
<td></td>
</tr>
<tr>
<td>Midbrain</td>
<td>477.6 ± 19.6 (14)</td>
<td>505.4 ± 27.9 (15)</td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>270.5 ± 10.5 (14)</td>
<td>287.7 ± 15.4 (15)</td>
<td></td>
</tr>
<tr>
<td><strong>Dopamine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medulla</td>
<td>NOT DETECTABLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>404.4 ± 37.0 (9)</td>
<td>271.8 ± 18.2 ** (9)</td>
<td></td>
</tr>
<tr>
<td>Midbrain</td>
<td>138.4 ± 13.4 (9)</td>
<td>133.0 ± 12.1 (10)</td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>484.4 ± 58.8 (9)</td>
<td>465.9 ± 33.7 (10)</td>
<td></td>
</tr>
</tbody>
</table>

** P < 0.01
normotensives (Figure 3.1, Table 3.4). Noradrenaline turnover was found to be decreased by 30.1% of the control normotensive value, while dopamine was decreased by 29.6% of the normotensive control value, both changes being significant at the P < 0.01 level.

This phenomenon was further examined by estimating the turnover in various regions of the rat brain. The two areas chosen for examination were the hypothalamus and medulla, two areas which have been shown to be closely associated with the central control of blood pressure (see Chapter 1, Introduction).

After the administration of α-MT and the resultant inhibition of synthesis of catecholamines, it was found that, over the time period used to determine turnover rate (i.e. 4 hours), the concentrations of dopamine fell to levels that were below the level of sensitivity of the assay. Therefore, as the level of dopamine could not be accurately ascertained, results presented for turnover in hypothalamus and medulla are only for noradrenaline.

However, it was found that, in the hypertensive animal, the turnover of noradrenaline was decreased in both the brain regions studied when compared to values obtained from normotensive animals (Table 3.5). In the medulla, the turnover was found to be decreased by 24.2% of the control value, significant at the P < 0.001 level (Table 3.5, Figure 3.2). In the hypothalamus, however, although turnover appeared to be reduced by 20.6% of the control value, the change was found to be non-significant (Figure 3.3).
FIGURE 3.1: Comparison of the rate of decline of noradrenaline after synthesis inhibition by α-MT (250mg/Kg i.p.) in the whole brains of normotensive and hypertensive rats.

- Catecholamine Level ng/g Brain

- Time in hours after α-MT

- - - - Normotensive

- O---O Hypertensive

** P < 0.01
TABLE 3.4 : Rate of decline of catecholamine concentration in the brains of normotensive and renal/DOCA hypertensive rats after synthesis inhibition with a-MT (250mg/Kg i.p.).
(Rate of decline expressed as the slope of the graph of log concentration of catecholamine against time)

<table>
<thead>
<tr>
<th></th>
<th>No. of Animals</th>
<th>Mean Systolic Blood Pressure mmHg (+ S.E.M.)</th>
<th>Rate of Decline Noradrenaline</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive Rats</td>
<td>10</td>
<td>144 ± 2</td>
<td>0.07662</td>
<td>0.1357</td>
</tr>
<tr>
<td>Renal/DOCA Rats</td>
<td>10</td>
<td>219 ± 5***</td>
<td>0.05359</td>
<td>0.0952</td>
</tr>
</tbody>
</table>

% decrease of the control value

30.1%**   29.6%**

** $P<0.01$

*** $P<0.001$
TABLE 3.5 Differences in the rates of decline of Noradrenaline in the medulla and hypothalamus of normotensive and renal/DOCA hypertensive rats after synthesis inhibition with α-MT.
(Rates of decline expressed as the slope of the graph of log concentration of Noradrenaline against time)
(Number of animals in brackets)

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Rate of Decline</th>
<th>% Decrease from Control Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normotensive</td>
<td>Hypertensive</td>
</tr>
<tr>
<td>Medulla</td>
<td>0.07411 (15)</td>
<td>0.05621 (14)</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.05168 (16)</td>
<td>0.04102 (15)</td>
</tr>
</tbody>
</table>

*** P<0.001
FIGURE 3.2: Comparison of the rate of decline of Noradrenaline after synthesis inhibition by α-MT in the medullae of normotensive and renal/DOCA hypertensive rats.
FIGURE 3.3: Comparison of the rate of decline of Noradrenaline in the hypothalami of normotensive and renal/DOCA hypertensive rats after synthesis inhibition by α-MT.
3.5 Discussion

Although referred to as a "renal/DOCA" hypertensive rat, the model of hypertension used throughout this thesis resembles more closely the standard DOCA-saline model of hypertension used by many other workers (Finch & Leach, 1970; Nakamura, Gerold & Thoenen, 1971; Gavras et al., 1975; Reid, Zivin & Kopin, 1975). Consequently, the results presented in this Chapter would tend to confirm those presented by other workers who have examined this model of hypertension.

For example, in the hypertensive animal, heart weight has been shown to be greater than in normotensives, an observation in agreement with the results of De Champlain et al. (1967) and Nakamura, Gerold and Thoenen (1971).

However, comparison of the endogenous levels of noradrenaline and dopamine, in various regions of the brains from hypertensive and normotensive rats, showed no significant differences, with the exception of the dopamine content of the hypothalamus which was significantly reduced in the hypertensive animal.

Nakamura, Gerold and Thoenen (1971), reporting on experiments using the DOCA-saline hypertensive rat and matched controls, showed that, on measuring noradrenaline content of the hypothalamus, medulla and residual brain four weeks after the induction of hypertension, they were able to show a significant increase in the noradrenaline content of the medulla of the hypertensive animals.
Increases were also present in the hypothalamus and the rest of the brain, but were not statistically significant. This is a trend that is repeated in the results presented in this thesis, where levels appear to be higher in hypertensive medulla, midbrain and cortex but not significantly so. It is possible, as is discussed in section 3.3, that those increases could well be significant if larger groups of animals were to be examined. As the areas of brain examined were gross in comparison to the nuclei and pathways within them, which may be concerned with blood pressure regulation, it might not be unreasonable to suggest that a small change in detectable noradrenaline levels may represent a significant change in a discrete area concerned with blood pressure. The question may also be asked - what degree of change may be relevant? A small change in levels may well represent a major change in the status of the regulatory system.

It is interesting to note that similar observations by other workers on spontaneously hypertensive (SH) rats have resulted in conflicting results. Robertson et al (1968) indicated that, in certain areas of the brains of SH-rats, noradrenaline levels were increased when compared to values obtained from age-matched controls. Conversely, Yamori, Lovenberg and Sjoerdsma (1970) reported that they found in the SH-rat the concentration of noradrenaline in the lower brain stem and hypothalamus was significantly reduced when compared with control animals. Although these two groups of workers used different strains of SH-rats, Robertson et
al using the New Zealand strain while Yamori et al used the Japanese Okamoto strain, it is still interesting, however, that contrasting results have been presented.

However, later work by Yamabe, De Jong and Lovenberg (1973) has indicated that these previously reported differences in noradrenaline levels between SH-rats and control normotensives may not be attributable to the hypertension but may be solely attributable to genetic variation and the possibility that the control animals used may not, in fact, be suitable animals to compare with an inbred SH strain.

If one accepts, however, that the levels of noradrenaline in various brain regions are maintained as a result of a dynamic balance between synthesis, efflux and metabolism (Brodie et al, 1966), it might be expected that catecholamine levels would remain static. Hence, a measurement of "turnover" would probably be a better guide to the state of neural activity, the rate of turnover varying, increasing or decreasing as the demand for, or utilisation of, noradrenaline changed.

The results reported in this thesis are again in agreement with those reported by Nakamura, Gerold and Thoenen (1971). In the hypertensive animal, the turnover of noradrenaline and dopamine is decreased in the whole brain and also in the medullary and hypothalamic regions. It is not possible to decide from these results whether the decrease in noradrenaline turnover is a result of, or a causative factor in, the hypertension. It is clear,
however, that the decrease in turnover is in some way related to the hypertension and not to other factors.

Accepting the idea that in a large number of areas of the brain noradrenaline produces a predominantly inhibitory effect on blood pressure (see Chapter 1), then the decrease in noradrenaline turnover and hence, presumably, a decreased availability of noradrenaline at certain central synapses may imply a causative role in hypertension, or at least a role in the development of the hypertension.
CHAPTER 4

THE EFFECTS OF CLONIDINE ON BLOOD PRESSURE
AND ON THE LEVELS AND TURNOVER OF
CENTRAL CATECHOLAMINES
4.1 Introduction

In this Chapter, an attempt is made to correlate the blood pressure-lowering action of clonidine treatment with possible effects on the levels and turnover of central catecholamines.

In considering the central action of clonidine, the probability that hypertension is associated with altered catecholamine metabolism in the brain must also be taken into account (see Chapter 3 and, for example, Nakamura, Gerold & Thoenen, 1971; Chalmers, 1975; Ito et al, 1975). It might be expected that, under these circumstances, a drug such as clonidine which exerts its action by stimulating α-receptors (see Chapter 1, section 1.4) might, as a consequence, disturb or alter the metabolism of central catecholamines to produce its hypotensive action.

Available evidence indicates that clonidine treatment causes a decrease in the turnover of both noradrenaline and dopamine in certain brain structures (Laverty & Taylor, 1969; Andén et al, 1970; Braestrup, 1974; Rochette & Bralet, 1975; Scheel-Kruger, Braestrup & Nielsen, 1975). However, all these reports present results on the effects of single acute doses of clonidine, invariably administered to normotensive animals only.

The results presented in this thesis demonstrate the effects of more prolonged clonidine administration on blood pressure and central catecholamine levels and turnover in both normotensive and hypertensive rats.
4.2  **Effects on Blood Pressure**

4.2.1  **Method**

To examine the effects of prolonged clonidine administration on blood pressure, rats were dosed twice daily for a total of seven days. Drug was given at 09.00h and 17.00h each day and administered by the intraperitoneal route (i.p.). The drug was given as the hydrochloride dissolved in 0.9% saline. Control animals were included in all experiments and were given 0.9% saline, 1ml/Kg, instead of a drug-containing solution.

In the examination of the effects of clonidine in hypertensive animals, the control group always consisted of hypertensive animals from the same batch as the test group.

Using the tail cuff method, as described in Chapter 2, the blood pressure of each experimental animal was measured on the morning before the first dose of drug (given at 17.00h) and two hours after the last morning dose of the drug seven days later. The two series of blood pressure measurements were performed at the same time each day to reduce any possible difference due to circadian variation. The blood pressure of test and control animals were measured alternately to further reduce possible circadian effects and also to ensure an equal drug effect in relation to the time after injection.

The effects of this dosing schedule with clonidine was
examined in three animal models, normotensive controls, renal/DOCA hypertensives (see Chapter 2) and also in one strain of spontaneously hypertensive (SH) rat. The results obtained from the experiments using the SH-rat are presented in section 4.5.

4.2.2 Results

Clonidine, when administered according to the schedule outlined above, produced a dose-related decrease in the blood pressure of normotensive animals over the dose range 20ug/Kg to 100ug/Kg. Figure 4.1 is a dose response curve of clonidine against percentage decrease in blood pressure. As can be seen, over the dose range examined the response pattern is relatively linear.

The dose levels of 50ug/Kg and 100ug/Kg both produced significant falls in blood pressure and were therefore chosen as the doses upon which further investigations would be carried out.

Administration of these doses of clonidine to renal/DOCA hypertensive rats produced significant falls in blood pressure ($P < 0.01$ at both doses), 14.1% and 20.1% respectively for the low and high doses. In terms of percentage decrease of pre-treatment values, the falls in blood pressure can be seen to be comparable with those obtained from the treatment of normotensive animals (Figure 4.1).
FIGURE 4.1: A dose response curve for the effect of Clonidine on Blood Pressure.

% Decrease in Control Blood Pressure

Clonidine Dose ug/Kg

-▼-▼ Normotensive

-△-△ Renal/DOCA Hypertensive
4.2.3 **Discussion**

The results presented above indicate that clonidine caused a dose-related decrease in blood pressure in both normotensive and hypertensive animals. In terms of percentage decrease from pre-treatment and untreated control values, the drug appears to be equipotent in both animal models. However, in terms of blood pressure decrease expressed in mmHg, the result in hypertensive animals after clonidine treatment is one and a half to two times larger than that found in normotensives. Table 4.1 shows the actual blood pressure values obtained before and after treatment in both normotensive and hypertensive animals.

As the dose levels of 50ug/Kg and 100ug/Kg of clonidine produced significant effects on blood pressure, these doses were used in further investigations where the effects of clonidine on the levels and turnover of endogenous noradrenaline were examined.

4.3 **Effects on Endogenous Catecholamine Levels in Brain Regions**

4.3.1 **Introduction**

A number of drugs which produce their functional effects by interfering with central catecholaminergic systems have been shown to produce changes in the endogenous
TABLE 4.1: Comparison of the blood pressure falls induced by prolonged dosing with Clonidine between normotensive and hypertensive rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Mean Systolic Blood Pressure in mmHg</th>
<th>% Decrease</th>
<th>Decrease in mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control 146.7 ± 3.4 (15) 150.8 ± 2.4 (20)</td>
<td>Drug Treated 128.9 ± 2.6 (13) 120.3 ± 2.3 (20)</td>
<td></td>
</tr>
<tr>
<td>Normotensives</td>
<td>50ug/Kg</td>
<td>12.6% ***</td>
<td>17.8mmHg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100ug/Kg</td>
<td>20.2% ***</td>
<td>30.5mmHg</td>
<td></td>
</tr>
<tr>
<td>Hypertensives</td>
<td>50ug/Kg</td>
<td>14.1% **</td>
<td>32.4mmHg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100ug/Kg</td>
<td>20.1% **</td>
<td>47.9mmHg</td>
<td></td>
</tr>
</tbody>
</table>

** P<0.01
*** P<0.001

Numbers in brackets are number of samples.

Blood pressures are expressed as the mean ± S.E.M.
levels of the catecholamine. For example, levels of endogenous noradrenaline and dopamine are changed by dosing with (+)-amphetamine (Costà & Groppetti, 1970; Groppetti, Naimzada & Costa, 1971; Riffee & Gerald, 1976) while chronic dosing with the tricyclic antidepressant desmethylimipramine (DMI) produces decreases in noradrenaline but not dopamine concentration (Roffler-Tarlov, Schildkraut & Draskoczy, 1973).

Available reports on the effects of acute dosing of rats with clonidine indicates little or no effect on endogenous catecholamine levels when doses, similar to those producing blood pressure effects, are used. However, higher doses (i.e. 2.5mg/Kg) have been reported to produce some increases in endogenous catecholamines (cited by Zaimis in Conolly, 1970, p54; Laverty & Taylor, 1969; Rochette, Bralet & Bralet, 1974; Braestrup & Nielsen, 1976). A paper by Sugimoto, Hashida & Kasahara (1976) has indicated that chronic dosing of spontaneously hypertensive rats with clonidine may result in an increase in the endogenous levels of brain noradrenaline.

However, as the majority of previously presented results were produced using acute dosing with clonidine and as certain drugs affecting catecholaminergic systems, for example, DMI, produce different effects depending on whether acute or chronic dosing is used (Roffler-Tarlov, Schildkraut & Draskoczy, 1973), it was thought that an examination of the effects of more prolonged dosing with clonidine on catecholamine levels would possibly prove
instructive.

4.3.2 Method

Groups of ten animals were dosed with clonidine at either 50ug/Kg or 100ug/Kg i.p. using the schedule outlined in section 4.2.1. On the final day, the last dose of clonidine was administered at 08.30h and the animals killed two hours later. Age-matched control animals were included in all experiments and dosed with 0.9% saline.

Control and test animals were killed in alternating order to equalise the possible effects of circadian variation over the killing period and also to equalise the effect of the drug between these two groups. Brains were removed and dissected into regions using the method of Glowinski and Iversen (1966), previously described in Chapter 2. Catecholamines in the brain samples were extracted and assayed fluorimetrically using the methods of Anton and Sayre (1962) and Shellenburger and Gordon (1971), details of which are given in Chapter 2.

Four brain regions were examined, those designated cortex, midbrain, hypothalamus and medulla. Total brain levels were calculated from the sum of the above parts, and therefore did not include striatal, cerebellum or hippocampal regions.

Differences between the mean catecholamine levels for each region, comparing control and drug treated animals,
were examined for significance using the t-test.

4.3.3 Results

Examination of the results obtained from these experiments indicates that prolonged clonidine administration may have some effects on the endogenous levels of central catecholamines. However, the results do not appear consistent or dose-related.

In normotensive animals, clonidine at 50ug/Kg or 100ug/Kg i.p. produced no significant changes in the endogenous levels of noradrenaline, with the exception of levels in the medullary region of rats dosed with clonidine at 50ug/Kg where noradrenaline levels were significantly increased (Figure 4.2). Consideration of the dopamine results, however, showed an increase in dopamine levels in the cortex and whole brain in rats receiving clonidine at 50ug/Kg, while in rats receiving the higher dose, dopamine levels were only significantly increased in the midbrain and hypothalamic regions (Figure 4.3).

The lower dose administered to hypertensive animals produced increases in endogenous noradrenaline content of the cortex, midbrain, medulla and whole brain, while at the higher dose, the increase in noradrenaline was found to be significant only in the cortex and whole brain (Figure 4.4). Dopamine values at both dose levels were not significantly changed.
FIGURE 4.2: The effect of Clonidine treatment on the endogenous levels of Noradrenaline in various brain regions of normotensive rats. (Plain bars control values, hatched bars values from drug treated animals) (T-bars = S.E.)

50ug/Kg

100ug/Kg
FIGURE 4.3: The effect of Clonidine treatment on the endogenous levels of Dopamine in various brain regions of normotensive rats.

(Plain bars control values, hatched bars values from drug treated animals)
(T-bars = S.E.)

50ug/Kg

* = P < 0.05
ND = Not Detectable

100ug/Kg

* = P < 0.05
** = P < 0.01
ND = Not Detectable

Cortex Mid- Brain Hypothalamus Medulla Total Pons Brain
FIGURE 4.4: The effect of Clonidine treatment on the endogenous levels of Noradrenaline in various brain regions of hypertensive rats. (Plain bars control values, hatched bars values from drug treated animals) (T-bars = S.E.)

* = P < 0.05
** = P < 0.01
4.3.4 Discussion

On the whole, the results reported in these experiments agree with results from other sources (see section 4.3.1) that clonidine produces no change or, at least, a slight increase in the endogenous levels of catecholamines in the brain. The results obtained, however, are not consistent enough to enable any firm conclusions to be made.

Examination of the results does show a general trend of increased catecholamine levels after clonidine therapy, but the majority of the changes are not significant. It is possible that the examination of greater numbers of animals may show that these increases are significant even though they are small. However, the value of the results so obtained would probably not warrant the use of the number of animals needed.

It is perhaps possible that the measured small change in endogenous catecholamine levels represents:

i) a significant change in the status of central adrenergic activity (even although the change is small);

ii) a large change occurring in some more discrete part of the gross brain areas studied.

While the first possibility may be hard to assess, the second possibility could be checked by examining very small
areas of the brains by utilising methods discussed in Chapter 2, section 2.4.1.

However, the lack of effect of clonidine in raising endogenous catecholamine level does not exclude effects on other parts of the adrenergic systems, for example, effects on the turnover of the catecholamines. The following section examines this possibility.

4.4 Effects on Central Catecholamine Turnover

4.4.1 Introduction

Clonidine, when given in an acute dose to normotensive animals, has been shown to decrease the turnover of noradrenaline and, to a lesser extent, dopamine in both peripheral tissues (Bralet & Rochette, 1973) and in certain brain tissues (Andén et al, 1970; Rochette & Bralet, 1975; Rochette, Bralet & Bralet, 1974; Braestrup, 1974). Prolonged dosing with clonidine has also been demonstrated to produce a decrease in the activity of the enzyme tyrosine hydroxylase in the spinal cord (Reid, 1975).

In this thesis, the results of prolonged clonidine administration on the turnover of noradrenaline and dopamine were assessed in the whole brains of both normotensive and hypertensive rats and also in two specific brain regions implicated in blood pressure control and the site
of action of clonidine, the medulla and the hypothalamus.

4.4.2 Method

Animals were dosed twice daily with clonidine, as described in section 4.2.1. Blood pressures were measured before commencing the dosing and again two hours after the last morning dose of clonidine, given at 08.30h. Immediately after the measurement of blood pressure, the animals were dosed with the synthesis inhibitor α-methyl-p-tyrosine (α-MT) at 250mg/Kg i.p. and killed four hours later.

Untreated control animals were included in all experiments and, in all procedures, including blood pressure measurements, dosing with α-MT and killing, they were alternated with test animals to proportion equally any effects due to circadian variation.

On killing, the brains were removed, dissected where necessary and homogenised in 0.4N perchloric acid in preparation for extraction and assay of catecholamines by methods previously described.

Catecholamine concentrations at time zero and four hours after α-MT were estimated and regression analysis applied to the results. The resultant slopes were then compared by t-test and any change in turnover, induced by the drug in the test animal, was expressed as a percentage change of the control value.
4.4.3 Results

4.4.3.1 Effects on the Turnover of Catecholamines in Whole Brain

In normotensive animals, clonidine, at 50ug/Kg i.p., produced a 12.1% decrease in blood pressure ($P < 0.01$) but no significant change in the turnover of either noradrenaline or dopamine (Figure 4.5, Table 4.2). When the dose was increased to 100ug/Kg, a significant reduction in the turnover of noradrenaline, as well as a 20.6% decrease in blood pressure ($P < 0.01$), was found (Figure 4.6, Table 4.2). However, little effect was found on dopamine turnover.

Application of the same dose levels of clonidine to renal/DOCA hypertensive animals produced similar results. Both doses produced significant falls in blood pressure and also decreases in the turnover of both noradrenaline and dopamine. The only statistically significant result, however, was the decrease in noradrenaline turnover produced by the higher dose of clonidine, where the turnover was decreased by 40.5% of the control value (Figure 4.7, Table 4.2).

4.4.3.2 Effects on Catecholamine Turnover in Brain Regions

The two brain areas studied were the hypothalamus and
FIGURE 4.5: The effect of Clonidine 50ug/Kg i.p. on the turnover of catecholamines in the whole brains of normotensive rats.

- Control
- Clonidine treated

Noradrenaline ——— Dopamine
FIGURE 4.6: The effect of Clonidine 100μg/Kg i.p. on the turnover of catecholamines in the whole brains of normotensive rats.
FIGURE 4.7: The effect of Clonidine, 50ug/Kg and 100ug/Kg, on the turnover of Noradrenaline in the whole brains of hypertensive rats.

* P<0.05

- Control
- 50ug/Kg Clonidine
- 100ug/Kg Clonidine
### TABLE 4.2: Effect of Clonidine on blood pressure and turnover of Noradrenaline and Dopamine in the brains of normotensive and hypertensive rats.

<table>
<thead>
<tr>
<th></th>
<th>Blood Pressure Decrease</th>
<th>Noradrenaline Turnover Decrease</th>
<th>Dopamine Turnover Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normotensives</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clonidine 50ug/Kg</td>
<td>12.1% **</td>
<td>10.7%</td>
<td>5% +</td>
</tr>
<tr>
<td>Clonidine 100ug/Kg</td>
<td>20.6% **</td>
<td>48.7% **</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Hypertensives</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clonidine 50ug/Kg</td>
<td>15.1% **</td>
<td>15.9%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Clonidine 100ug/Kg</td>
<td>20.9% **</td>
<td>40.5% *</td>
<td>12.1%</td>
</tr>
</tbody>
</table>

* = P<0.05  ** = P<0.01  + = INCREASE
the medulla. Results are only presented for effects on noradrenaline turnover for reasons previously discussed (Chapter 3, section 3.4).

The effect of only one dose level of clonidine, that of 100μg/Kg, was examined. This dose was chosen as it had been shown to produce both significant falls in blood pressure and significant decreases in the turnover of noradrenaline in the whole brain of both normotensive and renal/DOCA hypertensive rats (Table 4.2).

In the normotensive animal, this dose of clonidine was found to reduce noradrenaline turnover in the medulla by 42.6% (P<0.05) of the control value, while in the hypothalamus, turnover was decreased by 24.9% of control values but was not significant (Table 4.3). In the renal/DOCA hypertensive rat, turnover was only reduced by 21.5% (P<0.05) of control values in the medulla, while in the hypothalamus, turnover appeared to be unaffected (Table 4.3).

4.4.4 Discussion

The results presented above tend to confirm the findings of others that the administration of clonidine results in a decrease in the turnover of central catecholamines (see section 4.4.1). A major difference between this and previously reported work is the dosing schedule and the animal models used. Previous work utilised acute dosing, invariably in normotensive animals, while here
<table>
<thead>
<tr>
<th>Group</th>
<th>Region</th>
<th>No. of Animals</th>
<th>Percentage decrease in the Turnover of Noradrenaline after Clonidine treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>Medulla</td>
<td>10</td>
<td>42.6% P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Hypothalamus</td>
<td>10</td>
<td>24.9% N/S</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>Medulla</td>
<td>19</td>
<td>21.5% P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Hypothalamus</td>
<td>19</td>
<td>1.0% N/S</td>
</tr>
</tbody>
</table>

N/S = NOT SIGNIFICANT
more prolonged dosing with clonidine, in both normotensive and hypertensive animal models, has been used.

In experiments examining the effects in whole brain, clonidine produced substantial decreases in the turnover of noradrenaline, both in the normotensive and hypertensive models, the turnover decrease being smaller in the hypertensive model. This was also reflected on the examination of turnover changes, after clonidine, in hypothalamus and medulla. Although decreases occurred in the two regions, in both animal models, the decrease was smaller in the hypertensives. In the light of results presented in Chapter 3, the smaller decrease in noradrenaline turnover in hypertensive animals might not be unexpected, as, when compared to age-matched normotensive controls, the turnover of noradrenaline in hypertensive brains is already depressed. This may reflect some basal limit to the possible reduction of turnover, lower than which cannot be reached by drug action.

However, if the hypothesis that hypertension is, in some way, associated with decreased turnover of central catecholamines, in particular noradrenaline (see Chapter 3), is accepted, then it might be expected that relief or reversal of the hypertension would be associated with a reversal of the turnover effect. In fact, drug treatment exacerbates the turnover effect yet still produces a decrease in blood pressure.

Assuming that both effects are produced by α-receptor stimulation by the drug, at least two possibilities could
exist:

i) that drug effects on turnover and blood pressure are not related;

ii) that clonidine has two sites of action centrally producing different effects.

A fuller discussion on the above will be presented in the final Chapter of this thesis.

4.5  Effects of Clonidine on Blood Pressure and Central Catecholamine Turnover in the Spontaneously Hypertensive Rat

4.5.1  Introduction

It has been suggested that the spontaneously hypertensive (SH) rat is the animal model that most closely resembles human "essential" hypertension (Grollman, 1972). Consequently, the importance and use of the SH-rat in cardiovascular research has increased and it has become the topic of an increasing number of publications (e.g. Okamoto, 1972; Hill, 1976; Roba, 1976).

Two major colony-types of SH-rat are presently commercially available and in use. One is a Japanese strain developed by selective inbreeding of Wistar rats at Kyoto
University, Japan, (Okamoto et al, 1972) while the second, although also derived from original Wistar stock, has been developed at Otago University in New Zealand (Phelan et al, 1972). Both strains have been developed by successive brother-sister mating of rats that displayed higher than normal blood pressures when compared to the mean blood pressure of the original stock. Inbreeding through over 21 generations has, by now, produced, from a biological viewpoint, "pure" inbred strains.

The SH-rats display a consistent and reproduceable elevated blood pressure and also other differences from control animals that make them a separate strain (Okamoto, 1972).

A small breeding colony of the New Zealand strain was kindly donated by Dr. M. Armstrong (Wellcome Research) for use in this project. Unfortunately, problems in breeding occurred resulting in the production of small litters and weak animals. As a result, animals sufficient for only one experiment were produced. However, the results obtained from this experiment were thought to be sufficiently interesting to warrant inclusion in this thesis.

4.5.2 Method and Results

Animals were dosed with clonidine, 100ug/Kg i.p., or saline, 1ml/Kg, using the schedule described previously (section 4.4.2).
The mean systolic blood pressure before treatment was $208 \pm 4\text{mmHg}$ (mean ± S.E.M., n = 19). Clonidine treatment produced a 26.0% fall in the mean systolic blood pressure ($P < 0.001$) and also significant decreases in the turnover of both noradrenaline and dopamine when comparing the decline of endogenous levels in whole brain after synthesis inhibition with α-MT (Figure 4.8).

The turnover of noradrenaline was decreased by 66.4% of the control value ($P < 0.001$) and dopamine turnover decreased by 19.8% of the control value ($P < 0.001$).

4.5.3 Discussion

A comparison of the results obtained from SH-rats with those obtained from normotensive and renal/DOCA hypertensives appears to indicate that the SH-rat is more susceptible to clonidine treatment than the other models, with larger decreases in both blood pressure and central noradrenaline occurring. These results would tend to agree with those of Ohkusu (1971) who came to a similar conclusion. However, it is not possible from these results to offer an explanation of this apparently greater susceptibility, except perhaps to say that it could be due to a strain difference in the animals.

It is interesting, however, that the effect of clonidine in lowering blood pressure and central catecholamine turnover is common with all three animal models examined.
FIGURE 4.8: The effect of Clonidine 100ug/Kg on catecholamine turnover in the whole brains of New Zealand strain spontaneously hypertensive rats.

---

*** P < 0.001

- Control

- Clonidine treated

Noradrenaline ——— Dopamine
4.6 **General Discussion**

The results presented in this Chapter certainly indicate that there appears to be associated with the blood pressure-lowering effect of clonidine a reduction in central catecholamine turnover. However, for reasons discussed in section 4.4.4, it is possible that the turnover effect is not causally related to the hypotensive action, but is either secondary to it or, indeed, totally unrelated.

A discussion on how the blood pressure-lowering action and the reduction in central catecholamine turnover may be achieved by clonidine will be presented in the final Chapter of this thesis.

It was thought, however, that an examination of the interaction said to occur between clonidine and the tricyclic antidepressant desmethylinipramine (DMI) would help to clarify the mechanisms by which clonidine produces its effects. Hence, the following Chapter reports on the effects of combined clonidine and DMI treatment with relation to the hypotensive action and reduction in central catecholamine turnover produced by clonidine.
CHAPTER 5

AN EXAMINATION OF THE INTERACTION BETWEEN CLONIDINE AND DESMETHYLIMIPRAMINE: EFFECTS ON BLOOD PRESSURE AND CENTRAL NORADRENALINE TURNOVER
5.1 Introduction

The simultaneous administration of a number of different drugs is all too common in modern therapy and the fact that interactions can occur between certain drugs has become a well recognised problem. These interactions can result in a potentiation of the effect of one of the drugs (i.e. warfarin potentiation by aspirin) or, alternatively, in a reduction in the efficiency of one of the drugs (i.e. simultaneous administration of phenobarbitones which induces microsomal enzyme activity).

An important group of compounds in which clinical interactions have been reported are the antihypertensive agents (see, for example, Stafford & Fann, 1977; Van Zwieten, 1975a). In particular, both the centrally acting antihypertensive drugs clonidine and $\alpha$-methyldopa have sedative properties that are potentially enhanced by other drugs such as anxiolytics, hypnotics, antiepileptics or anaesthetics, which also have sedative properties.

A more important interaction involving clonidine has been with the tricyclic antidepressant desmethylimipramine (DMI). Briant, Reid and Dollery (1973) reported that simultaneous administration of these two drugs clinically could result in an interaction causing a reduction in the hypotensive action of clonidine. Further work by these authors showed that this interaction could also be demonstrated in rabbits (Reid, Briant & Dollery, 1973). Other workers contested these results and implied that no
interaction occurred either in the rabbit (Hoefke & Warnke-Sachs, 1974) or the rat (Scholtysik & Salzmann, 1973). However, later work by a number of other workers has confirmed the findings of Briant et al and demonstrated that an interaction does occur, and in a variety of animal species including the rat (Finch et al, 1975; Kaul & Grewal, 1975) and also anaesthetised cats (Van Spanning & Van Zwieten, 1973; Van Zwieten, 1975a and b).

Similar interactions have been reported to occur between other centrally active antihypertensive drugs (i.e. α-methyldopa) and a variety of other drugs including other antidepressants and neuroleptic agents (Van Zwieten, 1975a and b, 1976, 1977).

Reid, Briant and Dollery (1973), working on the basis that DMI acts primarily by the blockade of pre-synaptic uptake systems of noradrenaline, suggested that the site of the clonidine/DMI interaction, and hence possibly the site of action of clonidine itself, was on the pre-synaptic neurone, implying that clonidine produced its effect by some action on endogenous noradrenaline. However, other evidence suggests that clonidine acts post-synaptically, as it has been demonstrated that the hypotensive action of clonidine still occurs even after depletion of central noradrenaline by reserpine and/or α-methyl-p-tyrosine treatment (Haeusler, 1974; Kobinger & Pichler, 1975, 1976).

Hence, it has been postulated that, if the interaction occurs at a central site, then it may be a result
of the α-adrenolytic properties of DMI in blocking the
α-receptor normally stimulated by clonidine or, altern­
avely, a peripheral effect of uptake blockade of noradrenaline increasing the concentration of noradrenaline
at peripheral receptors, resulting in a "physiological"
antagonism (Haeusler, 1974; Van Spanning & Van Zwieten,

Most of the previous work performed on the interaction
has involved the acute administration of the drugs by a
variety of routes with subsequent blood pressure recording.
In anaesthetised cats, administration was by the vertebral
arteries (see, for example, Van Zwieten, 1975a, 1976, 1977),
in conscious rabbits by a cannulated ear vein (Reid, Briant
& Dollery, 1973) and in conscious rats either intraperiton­
eally (Finch et al, 1975) or orally (Kaul & Grewal, 1975).

Little information is available on the interaction
with regards to effects on the central catecholaminergic
systems. Consequently, it was thought that an investi­
gation of possible effects on these systems during the
interaction would be instructive.

5.2 Method

Previous work on the clonidine/DMI interaction
generally involved pretreatment of the animals with the
antidepressant drug and subsequent examination of any
reduction in the hypotensive effect of clonidine
administration (for references see Introduction).
In fact, from a clinical point of view, the reverse situation is more important. If a hypertensive patient is well controlled on clonidine and also needs antidepressant therapy, administration of DMI concurrently with clonidine would result in a reversal of the hypotensive effect of clonidine and loss of control of the hypertension. It was therefore decided to use this type of situation experimentally.

The effects of clonidine were established by dosing according to the schedule described in Chapter 4 and then challenged with two doses of the antidepressant drug. A reappraisal was then made of any effects on blood pressure and central noradrenaline turnover.

Pilot experiments indicated that a dose of 3mg/Kg i.p. of DMI would produce an antagonism of the hypotensive effect of 100ug/Kg i.p. of clonidine in normotensive rats, so these two dose levels were incorporated into the dosing schedule described below.

Both drugs were administered i.p. as the hydrochloride dissolved in 0.9% saline solution. Control animals were included in all experiments and given saline (1ml/Kg) instead of drug, except in experiments involving the comparison of effects due to clonidine alone and the combination of clonidine and DMI therapy, where the "control" animals therefore received clonidine.

The dosing schedule for the administration of both drugs is described below:
Day 1: Blood pressures measured in the morning. First dose of clonidine or saline given at 17.00h.

Day 2-7: Animals dosed twice daily with either clonidine or saline at 09.00h and 17.00h.

Day 7: Concurrent with the 17.00h dose of clonidine (or saline), the first dose of DMI (or saline) was administered.

Day 8: Last doses of all drugs given at 08.30h and blood pressures measured 2 hours later. This was followed by the administration of α-MT 250mg/Kg i.p., after which the animals were killed and their brains removed 4 hours later.

On killing, the brains were removed, dissected where necessary and the tissue prepared for extraction and assay of catecholamines as previously described (see Chapter 2). Turnover rates were examined by comparing the slopes of the decline of noradrenaline, after synthesis inhibition, of control and drug treated animals.
5.3 Results

5.3.1 Effects on Blood Pressure

In normotensive animals, the administration of two doses of DMI 3mg/Kg i.p. (15.5h and 2h before blood pressure measurements) produced some effect on blood pressure, reducing untreated control blood pressures by 14.4% (P<0.001, Figure 5.1, Table 5.1). Clonidine 100ug/Kg i.p., when administered twice daily for seven days, also produced a fall in blood pressure, 18.0% of the control untreated value (P<0.001, Figure 5.1, Table 5.1). The combined administration of clonidine and DMI, given according to the schedule laid out in the previous section, also resulted in a significant fall in blood pressure, but only by 10.2% of the control untreated value (P<0.001, Figure 5.1, Table 5.1). However, on comparison with the fall in blood pressure produced by clonidine treatment alone, this fall was found to be significantly smaller. Calculation of the difference showed that the inclusion of DMI in the dosage regimen had reduced the clonidine response by 43.5% (P<0.001, Figure 5.1, Table 5.1).

A similar result was apparent when the experiments were repeated using hypertensive animals. DMI 3mg/Kg i.p. again produced a significant decrease in the control values (9.8%, P<0.005, Figure 5.2, Table 5.2), while clonidine administration produced a 21.6% decrease in
FIGURE 5.1: The effect of Clonidine, DMI and a combination of these two drugs on the blood pressure of normotensive rats.

Mean Control Systolic B.P. =

145.6 ± 1.5mmHg (n=23)

The combination of Clonidine and DMI reduced the Clonidine response by 43.5%***

*** P < 0.001
TABLE 5.1: The effect of Clonidine, DMI and a combination of these two drugs on the blood pressure of normotensive rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Mean Systolic Blood Pressure mmHg (± S.E.M.)</th>
<th>Decrease from Control Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-Saline</td>
<td>23</td>
<td>145.6 ± 1.5</td>
<td>-</td>
</tr>
<tr>
<td>DMI 3mg/Kg i.p. (2 doses)</td>
<td>13</td>
<td>124.4 ± 1.8</td>
<td>14.4%***</td>
</tr>
<tr>
<td>Clonidine 100ug/Kg i.p. (2 x daily for 7 days)</td>
<td>18</td>
<td>119.4 ± 1.2</td>
<td>18.0%***</td>
</tr>
<tr>
<td>Clonidine + DMI (combined therapy)</td>
<td>16</td>
<td>130.8 ± 1.6</td>
<td>10.2%***</td>
</tr>
</tbody>
</table>

The combination of Clonidine and DMI reduced the Clonidine response by 43.5%***.

*** P < 0.001
FIGURE 5.2: The effect of Clonidine, DMI and a combination of these two drugs on the blood pressure of renal/DOCA hypertensive rats.

Mean Control Systolic B.P. =

\[ 231 \pm 4.7\text{mmHg} \ (n=24) \]

The combination of Clonidine and DMI reduced the Clonidine response by 16% (N/S).

**  \( P < 0.005 \)

***  \( P < 0.001 \)
TABLE 5.2: The effect of Clonidine, DMI and a combination of these two drugs on the blood pressure of renal/DOCA hypertensive rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Mean Systolic Blood Pressure (± S.E.M.)</th>
<th>Decrease from Control Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-Saline</td>
<td>24</td>
<td>231 ± 4.7</td>
<td>-</td>
</tr>
<tr>
<td>DMI 3mg/Kg i.p. (2 doses)</td>
<td>10</td>
<td>208.3 ± 5.6</td>
<td>9.8% **</td>
</tr>
<tr>
<td>Clonidine 100ug/Kg i.p. (2 x daily for 7 days)</td>
<td>9</td>
<td>181.1 ± 7.3</td>
<td>21.6% ***</td>
</tr>
<tr>
<td>Clonidine + DMI (combined therapy)</td>
<td>8</td>
<td>189.1 ± 3.1</td>
<td>18.0% ***</td>
</tr>
</tbody>
</table>

The combination of Clonidine and DMI reduced the Clonidine only response by 16% (N/S).

** P < 0.005

*** P < 0.001
control blood pressures (P < 0.001, Figure 5.2, Table 5.2).
The combination of clonidine and DMI treatment again
resulted in a smaller decrease in blood pressure (18.0%,
P < 0.001) than that produced by clonidine alone and was
found to be a 16% reduction of the response due to
clonidine (Figure 5.2, Table 5.2). This was not found to
be statistically significant.

5.3.2 Effects on the Turnover of Noradrenaline
in Whole Brain

The effect of the various dosage regimen on central
noradrenaline turnover was, at first, examined in whole
brains of normotensive animals (Table 5.3). DMI treatment
produced very little change in the turnover of noradrenaline in the whole brain, while clonidine, as previously
shown, produced a significant decrease (P < 0.01) when
compared to saline controls. The decrease was 48.7% of
the control value.

When the combination of clonidine and DMI therapy
was compared to that of clonidine alone, it was found that
the combination therapy produced a potentiation of the
turnover decrease caused by clonidine treatment alone.
Although this decrease was 19.5%, it was found to be non-
significant. However, this is a 19.5% decrease on a turn-
over rate already depressed by 48.7%.

The above result would indicate that, regarding turn-
over effects, the combination of DMI and clonidine results
TABLE 5.3: The effect of Clonidine, DMI and a combination of these two drugs on the turnover of Noradrenaline in the whole brains of normotensive rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Slope</th>
<th>% Change in Turnover</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Saline vs. DMI 3mg/Kg i.p.</td>
<td>10</td>
<td>0.065569</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2) Saline vs. Clonidine 100ug/Kg i.p.</td>
<td>10</td>
<td>0.063465</td>
<td>2.6%</td>
<td></td>
</tr>
<tr>
<td>(3) Clonidine vs. Clonidine + DMI</td>
<td>10</td>
<td>0.039784</td>
<td>48.7%</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19.5%</td>
<td></td>
</tr>
</tbody>
</table>
in a potentiation of the effect of clonidine to decrease turnover, even though there is an antagonism of the hypotensive action.

Whole brain contains a large number of catecholaminergic neurones and pathways, many of which are not related to blood pressure control. As a result of this, changes in turnover, in more discrete areas of the brain concerned with blood pressure regulation, may well be masked. Therefore, an examination of changes in more discrete areas would certainly be of great value.

The above experiments were repeated and an examination for effects on turnover was carried out, concentrating on two areas of the brain which have been implicated in blood pressure control and the site of action of clonidine. These two areas were the medulla and the hypothalamus.

5.3.3 **Effects on Noradrenaline Turnover in Medulla and Hypothalamus**

The effect of the various treatments with clonidine, DMI and a combination of the two drugs on noradrenaline turnover in medulla and hypothalamus, was examined in both normotensive and hypertensive animals.

In the normotensive animal (Table 5.4), DMI 3mg/Kg i.p. produced falls in the turnover of noradrenaline in both the medulla and the hypothalamus but the decreases were non-significant. Clonidine produced a significant decrease in noradrenaline turnover of 42.6% ($P < 0.05$) in the medulla.
TABLE 5.4: The effect of Clonidine, DMI and a combination of these two drugs on the turnover of Noradrenaline in the medullary and hypothalamic regions of the brains of normotensive rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>MEDULLA</th>
<th>HYPOTHALAMUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Slope</td>
<td>% Change in</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Turnover</td>
</tr>
<tr>
<td>(1) Saline vs. DMI 3mg/Kg i.p.</td>
<td>10</td>
<td>0.071699</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.064443</td>
<td>10.1%</td>
</tr>
<tr>
<td>(2) Saline vs. Clonidine 100ug/Kg i.p.</td>
<td>10</td>
<td>0.064854</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.037189</td>
<td>42.6%</td>
</tr>
<tr>
<td>(3) Clonidine vs. Clonidine + DMI</td>
<td>10</td>
<td>0.064228</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.032567</td>
<td>49.3%</td>
</tr>
</tbody>
</table>

* P < 0.05
and a 24.9% (non-significant) decrease in the hypothalamus. When clonidine and DMI were given as a combined treatment and compared to effects produced by clonidine treatment alone, it was found that the combined therapy produced a 49.3% decrease in noradrenaline turnover in the medulla (P<0.05) but no change in the turnover in the hypothalamus. This decrease of 49.3% was a further decrease on noradrenaline turnover already depressed by 42.6% due to clonidine and represents a turnover decrease, therefore, of around more than 60% from saline treated control animals.

When the experiments were repeated on hypertensive animals, a similar profile of results was obtained (Table 5.5). DMI produced a significant decrease in noradrenaline turnover in the medulla of 33.6% (P<0.01) but little change in the hypothalamus. Clonidine produced only a 21.5% (P<0.05) decrease in noradrenaline turnover in the medulla but, again, little change in the hypothalamus. The small decrease in medulla noradrenaline turnover is not entirely unexpected in the light of the results presented in Chapter 3, which show that, in the hypertensive animal, turnover is already significantly reduced when compared to normotensive controls. However, when the result of combined treatment with clonidine and DMI was compared with that obtained from clonidine treatment alone, it was found that, in the medulla, turnover was further decreased by 56.6% (P<0.01). This represents a decrease from saline treated hypertensive control values
TABLE 5.5: The effect of Clonidine, DMI and a combination of these two drugs on the turnover of Noradrenaline in the medullary and hypothalamic regions of the brains of renal/DOCA hypertensive rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>MEDULLA</th>
<th>HYPOTHALAMUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Slope</td>
<td>% Change in</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Turnover</td>
</tr>
<tr>
<td>(1) Saline vs. DMI 3mg/Kg i.p.</td>
<td>10</td>
<td>0.07561</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.05014</td>
<td>33.6% **</td>
</tr>
<tr>
<td>(2) Saline vs. Clonidine 100ug/Kg i.p.</td>
<td>19</td>
<td>0.061018</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>0.047842</td>
<td>21.5% *</td>
</tr>
<tr>
<td>(3) Clonidine 100ug/KG i.p. vs. Clonidine + DMI</td>
<td>10</td>
<td>0.06066</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.02631</td>
<td>56.6% *</td>
</tr>
</tbody>
</table>

** P < 0.01

* P < 0.05
of around 60%. Effectively no change was found in the hypothalamus.

5.4 Discussion

The results presented in this Chapter confirm the findings of other workers (see Introduction) that an interaction does occur between clonidine and the tricyclic antidepressant desmethylimipramine, which results in a reduction of the hypotensive effect of clonidine. However, with respect to effects on central noradrenaline turnover, DMI treatment appears to potentiate the effect produced by clonidine.

If gross changes are compared, then, in general, the results obtained from experiments involving normotensive animals are similar to those from hypertensives.

For ease of comparison, the results from this Chapter are presented as a summary table (Table 5.6).

Clonidine 100ug/Kg i.p., when given twice daily for seven days, produced effects on blood pressure and also on central noradrenaline turnover. Treatment with DMI alone, two doses of 3mg/Kg i.p., also produced decreases in blood pressure and decreases in central noradrenaline turnover, but to a lesser extent than the effect produced by clonidine treatment. When the two drugs are administered in combination, an interaction occurs which manifests as opposite effects on blood pressure and central
<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Effect on Noradrenaline Turnover</th>
<th>Effect on Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLONIDINE</td>
<td>![Arrows]</td>
<td>![Arrows]</td>
</tr>
<tr>
<td>DMI</td>
<td></td>
<td>![Arrows]</td>
</tr>
<tr>
<td>CLONIDINE + DMI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
noradrenaline turnover. The combination results in a reduction of the hypotensive effect of clonidine but, conversely, causes a potentiation of the decrease in noradrenaline turnover resulting from clonidine-only administration.

Examination of the results in detail shows that there are anomalies not explained by this simplified version. The turnover changes resulting from clonidine treatment appear to be substantially larger in the normotensive animal than in the hypertensive animal. This is probably due to the already reduced noradrenaline turnover found in the hypertensive animal as previously discussed. Also, the interaction resulting in a decrease in the hypotensive effect of clonidine appears again more pronounced in the normotensive animal than in the hypertensives. This may possibly be due to the wider variation in recorded blood pressure for the hypertensives, resulting in a greater variation of effect.

It is interesting to note that nearly all the significant turnover effects, both the reductions produced by clonidine and DMI when administered alone and also the potentiation of the clonidine effect by DMI when the two drugs are administered concurrently, appear to be confined to the medullary area. This may well be taken as an indication that the site of action of clonidine is located within the medulla.

If the idea advanced in the previous Chapter is accepted, that the turnover decrease produced by clonidine
is unrelated to its hypotensive effect, then the potentiation of the turnover effect by DMI may also be unrelated to the antagonistic effects of DMI on the hypotensive response to clonidine.

A discussion on the possible explanations which may be put forward to explain these two effects and also on the various sites and mechanisms possibly involved in the interaction will be presented in the final Chapter of this thesis.

However, in an attempt to gain more information on drug effects on turnover and also to provide more information on the DMI interaction, the following Chapter presents results of an examination into the effects of other drugs, including other antihypertensive agents, on central noradrenaline turnover.
CHAPTER 6

THE EFFECTS OF OTHER DRUGS,
BOTH ALONE AND IN COMBINATION,
ON BLOOD PRESSURE AND CENTRAL NORADRENALINE TURNOVER
6.1 Introduction

In the preceding Chapter, results of an examination into the interaction occurring between clonidine and DMI were presented. It was demonstrated that effects on blood pressure and central noradrenaline turnover were associated with the interaction.

This Chapter presents results obtained from an examination of a variety of miscellaneous drugs. The drugs were chosen as they produce effects either on blood pressure or on central noradrenaline turnover and it was thought that the results obtained from their examination would be of some value in attempting to clarify the mechanism of action of clonidine.

In the first instance, the effects of two other antihypertensive agents were examined. The two drugs were α-methyldopa, said to work by a central mechanism (see Chapter 1 for references), and guanethidine, an adrenergic neurone blocker said to be devoid of central hypotensive activity (Wilhelm & De Stevens, 1976; Baum, Shropshire & Varner, 1972).

It has been reported that these two compounds also display an interaction with the tricyclic antidepressants, especially DMI (Kaumann, Basso & Aramendia, 1965; Cocco & Agué, 1977). As this interaction occurs with both centrally acting antihypertensives (i.e. clonidine and α-methyldopa) and peripherally acting compounds (i.e. guanethidine), it was thought that an examination of the
interaction occurring between DMI and the latter two compounds would provide additional information towards the determination of the possible site and mechanism of the interaction occurring between DMI and clonidine.

As clonidine is reported to exert its effects by \(\alpha\)-receptor stimulation (see, for example, Van Zwieten, 1973, 1975a), it was thought that an examination of the effects of an \(\alpha\)-blocking agent on central noradrenaline turnover would also be a useful comparison.

Finally, results are presented of an examination of an interesting interaction reported to occur clinically between clonidine and the \(\beta\)-blocking antihypertensive drug, sotalol (Saarimaa, 1976).

6.2 Guanethidine and \(\alpha\)-methyldopa

6.2.1 Guanethidine

6.2.1.1 Introduction

Guanethidine is one of a large group of compounds which exert a hypotensive action by adrenergic neurone blockade (see, for example, Laverty, 1973; Wilhelm & De Stevens, 1976). Its action depends on the uptake of the drug into the adrenergic neurone where it prevents the release of noradrenaline normally resulting from stimulation of the nerve. This is achieved partially by inter-
ference with transmission of the nerve impulse and partially by displacement of noradrenaline from storage sites within the neurone (Wilhelm & De Stevens, 1976).

The antihypertensive action appears to be a result solely of a peripheral effect with no involvement of a central component (Baum, Shropshire & Varner, 1972).

Guanethidine is known to interact with the tricyclic antidepressants resulting in a reduction in the efficacy of the hypotensive drug (Kaumann, Basso & Aramendia, 1965; Stafford & Fann, 1977; Cocco & Agué, 1977).

The purpose, therefore, of including an investigation into the effects of guanethidine in this study was two-fold:

1. to see if a peripherally acting antihypertensive would produce any effects on central noradrenaline turnover which could be collated with its hypotensive action;

2. if it could be shown that guanethidine had no central component to its action, yet still displayed an interaction with DMI, this might be evidence for a peripheral mechanism for the interaction. If so, it may be possible to suggest that the clonidine/DMI interaction may be mediated, partially at least, by a peripheral component.
Guanethidine was, therefore, examined using a similar dosage schedule to that used in the investigation into the effects of clonidine and the clonidine/DMI interaction (see Chapter 5). The effects of the drug on blood pressure and central noradrenaline turnover was assessed.

6.2.1.2 Method

To determine the effects of guanethidine treatment on blood pressure and central noradrenaline turnover, guanethidine monosulphate (Ismelin) was administered at a dose of 3mg/Kg i.p. twice daily for seven days to groups of normotensive rats. The drug was dissolved in 0.9% saline. Consequently, controls received saline only at a dose of 1ml/Kg i.p. Blood pressure was measured before commencement of dosing and two hours after the last morning dose of drug seven days later.

In experiments examining the interaction between guanethidine and DMI, guanethidine was administered twice daily for six days and blood pressure measured two hours after the last morning dose on the sixth day. DMI, 3mg/Kg i.p., was then included in a similar way to that described for clonidine/DMI experiments (see Chapter 5, section 5.2). Final blood pressure measurements were taken two hours after the last combined morning doses of guanethidine and DMI which were given at 08.30h on the seventh day.

Estimations of noradrenaline turnover were performed
by injecting the animals with α-MT 250mg/Kg i.p. and killing them four hours later. Brain regions were dissected out and levels of noradrenaline estimated by methods described previously (Chapter 2).

6.2.1.3 Results

6.2.1.3.1 Effects on Blood Pressure (Figure 6.1)

Guanethidine, when given at 3mg/Kg i.p. for seven days, produced a 21.6% decrease in blood pressure (P<0.001). As previously demonstrated (see Chapter 5), DMI 3mg/Kg i.p. (two doses) also displayed some effects on blood pressure, producing a 14.4% decrease (P<0.001).

In the series of experiments examining the interaction between guanethidine and DMI, after seven days of dosing guanethidine produced an 18.5% decrease in blood pressure (P<0.001). However, the combination of guanethidine and DMI resulted in a 10.3% fall only (P<0.001) when compared to untreated controls, indicating an interaction between guanethidine and DMI. This represented a reduction of the guanethidine response of 44% (P<0.01).

6.2.1.3.2 Effects on Central Noradrenaline Turnover (Table 6.1)

Guanethidine, although said to have no central effects and thought not to cross the blood brain barrier, produced
FIGURE 6.1: The effect of Guanethidine and DMI, administered alone and in combination, on the blood pressure of normotensive rats.

The combination of guanethidine and DMI reduced the guanethidine response by 44% (P < 0.01).

*** P < 0.001
TABLE 6.1: The effects of Guanethidine and DMI, alone and in combination, on the turnover of noradrenaline in the medulla and hypothalamus of normotensive rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>% Decrease of Noradrenaline Turnover from Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Medulla</td>
</tr>
<tr>
<td>Saline vs. DMI 3mg/Kg i.p.</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Saline vs. Guanethidine 3mg/Kg i.p.</td>
<td>20</td>
<td>14.0%</td>
</tr>
<tr>
<td>Guanethidine vs. Guanethidine/DMI</td>
<td>10</td>
<td>13.0%</td>
</tr>
</tbody>
</table>

+ = significantly different from saline controls only P < 0.01
some effects on central noradrenaline turnover.

A significant reduction in turnover was found in the hypothalamus (30.8%, P < 0.02) but little effect was noted in the medulla. DMI treatment, as previously demonstrated (see Chapter 5), also appeared to produce some effect on noradrenaline turnover in the medulla and hypothalamus. However, these decreases were not found to be statistically significant.

The combination of guanethidine and DMI, when compared to a control group consisting of animals receiving only guanethidine, appeared to result in a potentiation of the turnover decreases apparently resulting from guanethidine treatment. This seemed to occur both in the medulla and the hypothalamus. The greater effect was apparent in the hypothalamus, but statistical evaluation of the results showed no significant difference from guanethidine-treated controls (Table 6.1).

6.2.1.4 Discussion

The results presented above confirm the findings of others (see section 6.2.1.1) that an interaction occurs between guanethidine and DMI, resulting in a reduction of the hypotensive action of guanethidine. They also show that the interaction can be demonstrated in the normotensive rat.

As DMI is said to exert its antidepressant effect by a central mechanism (see, for example, Byck, 1975),
the first thought is that its interaction with guanethidine may be a result of a central effect.

Early work on the mechanism of action of guanethidine indicated that intracisternal administration of the drug to dogs resulted in a central inhibition of vasomotor tone and, hence, a reduction in blood pressure (Kaneko, McCubbin & Page, 1962). These results led the authors to suggest that, on chronic guanethidine administration, small amounts of the drug may cross the blood brain barrier and hence contribute with a central effect to the hypotensive action of the drug.

A more recent paper by Ito, Tanaka and Omae (1975), who injected guanethidine intracisternally into rats, presents results which appear to be at variance with the work by Kaneko et al. Ito et al show that, after intracisternal administration in rats, guanethidine produced not a decrease in blood pressure but an increase. Perhaps species difference may account for this apparent conflict of results.

However, Baum, Shropshire and Varner (1972), also using rats, concluded from their experiments, administering guanethidine systemically (via oral dosing), that there was no significant central component to the hypotensive action of guanethidine.

It would therefore seem that, at least in the rat, the hypotensive effect of guanethidine is mediated by a peripheral action.

Early work on the possible effects of guanethidine
on central endogenous noradrenaline indicated that systemic administration of the drug might produce some decreases in the endogenous levels of noradrenaline in certain brain areas. Sanan and Vogt (1962) showed some effects but their results were very variable, which led them to suggest that the effects were a result of a reflex action. Dagirmanjian (1963) showed that prolonged dosing with guanethidine produced a significant decrease in the level of noradrenaline in the hypothalamus and suggested that either this was a direct drug effect, implying that guanethidine crossed the blood brain barrier, or, if not, then the decrease was a reflex action in sympathetic centres in response to the hypotension caused by the drug.

It is interesting that in the experiments reported in this Chapter, guanethidine caused a significant decrease in noradrenaline turnover in the hypothalamus but not in the medulla. This is the reverse of that produced by clonidine, a drug known to cross the blood brain barrier and exert its effect by a central mechanism. On this basis, it may be possible to argue that the decrease in noradrenaline turnover associated with guanethidine treatment may be of reflex origin. However, this does not exclude the possibility of a direct action of guanethidine in certain brain areas, producing a reduction in blood pressure by reducing noradrenaline turnover at some pressor site in the brain.

The results presented by Ito et al (1975) on the
effects of intracisternal administration of guanethidine show that this treatment produces a significant decrease in turnover in the hypothalamic-medullary brain area, yet an elevation in blood pressure. Although this may be evidence for the hypothesis that decreased central norepinephrine turnover is associated with hypertension, it does not clarify how systemic guanethidine administration causes a significant decrease in hypothalamic norepinephrine turnover. If the drug is not able to cross the blood brain barrier, then it may be reasonable to suggest that the effect is, in some way, reflex originated, as, on the basis of Ito et al.'s results, if the drug did pass the blood brain barrier, one might expect an increase in blood pressure.

Administration of DMI along with guanethidine results in a potentiation of the decrease in norepinephrine turnover associated with guanethidine treatment while producing an antagonism of the hypotensive action. This result does not lend itself to easy explanation.

If the apparent central effects of guanethidine are reflex in origin, it is perhaps possible that the potentiation by DMI reflects a summation of the effects of the reflex and the DMI-induced effects. This would imply, perhaps, that the antagonism of guanethidine by DMI is of peripheral origin, an action that certainly occurs (e.g. Koe & Constantine, 1972). However, if the antagonism occurs at a peripheral site, preventing the effect of guanethidine to lower blood pressure, it might be expected
that the central effects of guanethidine on noradrenaline turnover, if reflex in origin, would also be reversed, but as is seen, they are potentiated.

It is obvious that more work needs to be performed on this subject before a satisfactory explanation of all the results can be made.

6.2.2 \textit{\alpha-}methyl\textit{dopa}

6.2.2.1 \textit{Introduction}

Alpha-methyl\textit{dopa} is an antihypertensive drug which exerts its action in a similar way to \textit{clonidine}, by the stimulation of \textit{\alpha}-receptors (see, for example, Van Zwieten, 1973, 1975a). It has also been reported to display a similar interaction with the tricyclic antidepressants (see Kale & Satoskar, 1970; Van Zwieten, 1973, 1975a & b).

6.2.2.2 \textit{Method}

The dosing schedule used was the same as that used for the \textit{clonidine/DMI} investigation (Chapter 5, section 5.2) with the substitution of \textit{\alpha-methyl\textit{dopa}} 250mg/Kg for \textit{clonidine}. The drug was administered orally as the base suspended in 5% acacia solution in distilled water. Control animals were given 5% acacia 1ml/Kg instead of the drug-containing solution.
Blood pressure measurements were taken and brain samples obtained for turnover studies using methods described previously.

6.2.2.3 Results

6.2.2.3.1 Effects on Blood Pressure (Figure 6.2)

The administration of α-methyldopa, 250mg/Kg p.o., twice daily for seven days produced a 15.8% decrease (P < 0.001) in blood pressure compared to control animals receiving vehicle only.

When α-methyldopa and DMI were given on a combined schedule, after seven days α-methyldopa alone produced a 15% fall in blood pressure while the groups of animals receiving the combined therapy displayed only a 7.4% fall in blood pressure when compared to untreated control blood pressure values. Both falls were significantly different from the control values (Figure 6.2). The response resulting from the combined α-methyldopa/DMI treatment represents a reduction in the α-methyldopa response of 50.7% (P < 0.001).

6.2.2.3.2 Effects on Noradrenaline Turnover

When the effect of α-methyldopa on noradrenaline turnover was assessed, it was found that the levels of endogenous noradrenaline appeared to be substantially
FIGURE 6.2: The effects of α-methyldopa and DMI, administered alone and in combination, on the blood pressures of normotensive rats.

The combination of α-methyldopa and DMI reduced the α-methyldopa response by 50.7% (P < 0.01).

*** P < 0.001

** P < 0.01
decreased. Comparison with the values obtained from control animals showed an 80% decrease in medullary noradrenaline and an 82.6% decrease in hypothalamic noradrenaline (both significant at $P < 0.001$).

The administration of $\alpha$-MT did not produce any change in this effect, either at time zero or four hours after the administration of the synthesis inhibitor.

Similar results were obtained when the combination of $\alpha$-methyldopa/DMI was examined. As a result, it was not possible to assess the effects of the above treatments on central noradrenaline turnover.

6.2.2.4 Discussion

Regarding the interaction between $\alpha$-methyldopa and DMI, the above results of effects on blood pressure would appear to confirm the findings of others that such an interaction can be demonstrated experimentally (see section 6.2.2.1).

However, no effects on noradrenaline turnover were measured due to the apparent depletion of central noradrenaline by the drug. An explanation of this effect may be offered considering that in vivo $\alpha$-methyldopa is converted to its active metabolite $\alpha$-methylnoradrenaline (see Chapter 1, section 1.4.3.1). This then displaces and replaces the endogenous noradrenaline in the storage sites in the neurone. Stimulation of the neurone results in the release of $\alpha$-methylnoradrenaline to act on the same
receptors stimulated by endogenous noradrenaline or clonidine to decrease blood pressure.

Hence, the apparent depletion in endogenous noradrenaline would seem to reflect the degree of displacement by α-methylnoradrenaline.

The assay method used for detecting catecholamines in this thesis did not allow the estimation of α-methylnoradrenaline to be carried out simultaneously. However, it was demonstrated that α-methylnoradrenaline did not interfere with the noradrenaline determination. A simple experiment compared standard solutions of both noradrenaline and α-methylnoradrenaline, alone and in mixed solutions. Table 6.2 shows that there is apparently no significant interference between the two compounds at the assay wavelengths used for noradrenaline.

It can also be seen from the Table that, although α-methylnoradrenaline appears to display some fluorescence at the noradrenaline wavelengths, it is less than one-fifteenth that of the noradrenaline fluorescence. This is a figure comparable to that found by Anton and Sayre (1962).

It is interesting to note that the DMI treatment did not alter the degree of apparent noradrenaline depletion by α-methyldopa. This would perhaps indicate that, if the depletion is a function of α-methyldopa entering the neurone to be converted to α-methylnoradrenaline, then it would appear that the DMI does not affect the uptake of α-methyldopa into the neurone. Therefore, if this is so, it might be said that the antagonism of the hypotensive
A comparison of the fluorescence of standard solutions of Noradrenaline and α-methylnoradrenaline at the wavelengths used for the fluorimetric assay of Noradrenaline.
(i.e. excitation 380mu, emission 495 mu)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration in Original 1ml Aliquot</th>
<th>RF Value (mean of 3 samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline</td>
<td>50ng</td>
<td>30.2</td>
</tr>
<tr>
<td></td>
<td>100ng</td>
<td>62.25</td>
</tr>
<tr>
<td>α-methylnoradrenaline</td>
<td>50ng</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>100ng</td>
<td>3.5</td>
</tr>
<tr>
<td>Mixture</td>
<td>50ng each</td>
<td>31.2</td>
</tr>
<tr>
<td></td>
<td>100ng each</td>
<td>61.3</td>
</tr>
<tr>
<td>Reagent blanks</td>
<td></td>
<td>1.7</td>
</tr>
</tbody>
</table>
action of α-methyldopa by DMI is not due to a blockade of pre-synaptic uptake. In this case, it might be reasonable to suggest that the antagonism is due either to the post-synaptic α-adrenolytic property of DMI blocking the actions of α-methylnoradrenaline released from the central neurone, or an effect on the release of α-methylnoradrenaline from the neurone, or a peripheral effect, that of peripheral uptake blockade of noradrenaline.

6.2.3 Discussion

It is interesting that the hypotensive actions of clonidine, α-methyldopa and guanethidine are all antagonised by DMI treatment. As the antagonism occurs with both the centrally acting and the peripherally acting drugs, it is possible to propose a number of situations for the interaction.

Firstly, as the interaction occurs with guanethidine, which apparently has no central component to its anti-hypertensive activity, it is tempting to suggest that the interaction between DMI and the centrally acting compounds would also be a result of a peripheral effect, in that DMI is causing a potentiation of peripheral noradrenaline.

Secondly, if the above hypothesis is wrong, then the mechanism of the interaction may involve different sites for each of the drugs involved. These may vary from α-block centrally to a general potentiation of central pressor mechanisms overcoming the hypotensive action of
the drugs (c.f. Discussion, final Chapter).

A further discussion on these possibilities will be attempted in the final Chapter of this thesis.

6.3 Phenoxybenzamine, an α-blocking Agent: Effects on Central Noradrenaline Turnover

6.3.1 Introduction

If clonidine, as an α-receptor-stimulating agent, causes a decrease in the turnover of central noradrenaline (see Chapter 4), then it might be expected that the reverse would result from the administration of an α-blocking agent. This has been demonstrated in the whole brain by Andén et al (1970).

6.3.2 Method

Phenoxybenzamine was administered to normotensive rats at a dose of 20mg/Kg i.p. as the hydrochloride dissolved in 0.9% saline. This dose was chosen as it had been shown by previous workers to produce a significant effect on central noradrenaline turnover in the rat (Andén et al, 1970). In these present experiments, two doses of the drug were administered, two hours and 15 hours before the administration of α-MT for turnover studies.

Estimates of noradrenaline turnover in the medulla
and hypothalamus were obtained using methods described previously.

6.3.3 Results

A comparison of the slopes of the decline of noradrenaline after α-MT between control animals receiving saline and test animals receiving phenoxybenzamine indicates that the α-blocking drug caused substantial and significant increases in the turnover of noradrenaline in both the medulla and the hypothalamus (Table 6.3).

The increase in the medulla was 67.3% ($P < 0.02$), while in the hypothalamus the increase was greater, 80.8% ($P < 0.002$).

6.3.4 Discussion

The above results, when compared to those obtained for clonidine (see Chapter 4), demonstrate that α-agonists (represented by clonidine) and α-blockers (represented by phenoxybenzamine) produce opposite effects on central noradrenaline turnover. This result verifies the findings of Andén et al (1970) who showed a similar result in whole brain.

It is interesting also that the α-blocker shows a greater effect in the hypothalamus than the medulla, while the α-agonist shows the reverse of this, with a greater effect in the medulla than in the hypothalamus.
TABLE 6.3: The effect of Phenoxybenzamine on the turnover of Noradrenaline in the medulla and hypothalamus of normotensive rats.

Comparison of slopes of the rate of decline of Noradrenaline after synthesis inhibition with α-MT.

<table>
<thead>
<tr>
<th></th>
<th>Saline Controls</th>
<th>Phenoxybenzamine 20mg/Kg i.p.</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>10</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Slope - Medulla</td>
<td>0.05278</td>
<td>0.08839</td>
<td>67.3% **</td>
</tr>
<tr>
<td>Slope - Hypothalamus</td>
<td>0.05086</td>
<td>0.09208</td>
<td>80.8% ***</td>
</tr>
</tbody>
</table>

** $P < 0.02$

*** $P < 0.002$
An attempt will be made in the final Chapter of this thesis to offer an explanation of the possible mechanisms by which \( \alpha \)-agonists and \( \alpha \)-blockers produce their effects on central noradrenaline turnover.

6.4 An Interaction between Clonidine and the \( \beta \)-blocking Antihypertensive Drug Sotalol

6.4.1 Introduction

Sotalol is a derivative of the phenyl-ethanolamine type of \( \beta \)-blockers, of which the first was pronethalol (Figure 6.3). Both these and other \( \beta \)-blockers have been used successfully in the treatment of hypertension (Wilhelm & De Stevens, 1976; Clarkson, 1976).

Recently, a report has appeared in the literature that indicates an apparent interaction between sotalol and the centrally acting antihypertensive clonidine when both drugs are administered concurrently (Saarimaa, 1976). This interaction results in a reduction in the hypotensive effects of both drugs. In the majority of patients in which the interaction occurred, blood pressure approached pre-treatment levels (i.e. the level before any therapy whatsoever). Removal of one of the drugs resulted in blood pressure returning to the level produced by single drug therapy.

With the recent evidence that certain \( \beta \)-blocking
FIGURE 6.3: Structural formulae of Sotalol and Pronethalol

SOTALOL
MJ. 1999

PRONETHALOL
drugs may have a central component to their hypotensive action (see, for example, Day & Roach, 1975; Lewis, 1975; Day, Peters & Roach, 1976; Klevans, Kovacs & Kelly, 1976), the interaction between sotalol and clonidine was thought to be of sufficient interest to merit an examination in this thesis, especially as a large part of the work presented here was concerned with the interaction of clonidine with another drug (DMI), also resulting in a reduction of the hypotensive action of clonidine.

A study was made of the sotalol/clonidine interaction, therefore, in a similar way to that applied to the clonidine/DMI interaction.

6.4.2 Method

In order to find out if sotalol alone produced any correlatable effects on blood pressure and central noradrenaline turnover, the drug was administered to normotensive rats at a dose of 10mg/Kg i.p. twice daily for seven days. The drug was given as the hydrochloride (obtained from BDH Chemicals Ltd.) dissolved in 0.9% saline. Control animals received saline only at a dose of 1ml/Kg i.p.

Blood pressure was measured before the start of dosing and again two hours after the last morning dose of the drug. Noradrenaline turnover was estimated as previously described.

To examine for an interaction between sotalol and
clonidine, two groups of normotensive rats were dosed with clonidine at a dose of 100ug/Kg i.p. twice daily for seven days. Blood pressure was measured before the dosing started and, as usual, again on the seventh day. Both groups of animals continued to receive clonidine for a further seven days with the concurrent administration to one group of sotalol 10mg/Kg i.p. At the end of this second seven day dosing period, the blood pressures of all animals were again measured two hours after the last morning doses of the two drugs. Estimation of the turnover of noradrenaline was carried out on the medulla and hypothalamus of both groups of animals.

6.4.3 Results

6.4.3.1 Effects on Blood Pressure

(Figure 6.4, Table 6.4)

Sotalol, 10mg/Kg i.p. administered twice daily for seven days, produced a 20.5% decrease (P<0.001) in control blood pressure values. In the examination of the interaction between clonidine and sotalol, clonidine, 100ug/Kg i.p. given for seven days, produced a 21% decrease (P<0.001) in control blood pressure values. However, when sotalol was administered concurrently with clonidine, the result was to produce a reversal of the hypotensive effects of both clonidine and sotalol, producing no significant difference between the blood pressure of this group
FIGURE 6.4: The effects of Clonidine and Sotalol and combined administration of the two drugs on blood pressure.

The combination of clonidine and sotalol reduced the clonidine response by 83.9% (P < 0.001).

*** P < 0.001
N/S Not significant
TABLE 6.4: The effect of Sotalol, Clonidine and combined administration of the two drugs on blood pressure.

<table>
<thead>
<tr>
<th>Treatment (Controls vs. Drug)</th>
<th>No. of Animals</th>
<th>% Decrease in Blood Pressure from Control Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline vs. Sotalol 10mg/Kg</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>20.5%</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>Saline vs. Clonidine 100ug/Kg</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>21.0%</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>Untreated Controls vs. Clonidine + Sotalol</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>3.5%</td>
<td>N/S</td>
</tr>
<tr>
<td>Clonidine vs. Clonidine + Sotalol</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>83.9% (reversal of Clonidine response)</td>
<td>$P &lt; 0.001$</td>
</tr>
</tbody>
</table>
and the control untreated value. This represented an 83.9% reversal of the hypotensive effect of clonidine and also a similar degree of reversal of the hypotensive effect of sotalol.

6.4.3.2 Effects on Noradrenaline Turnover

Table 6.5 shows the effects of the above treatments on the turnover of noradrenaline in the medulla and hypothalamus.

The Table shows that clonidine produced a significant decrease of 42.6% in the turnover of noradrenaline in the medulla and a small, but non-significant, change in the hypothalamus. Sotalol treatment apparently produced a small decrease in turnover in the medulla (not significant) but had little effect on the turnover in the hypothalamus.

Comparison of the effects of combined clonidine and sotalol treatment with a control group consisting of animals receiving only clonidine showed a result similar to that obtained with combined clonidine/DMI treatment. The combined treatment caused a significant potentiation of the turnover decrease in the medulla associated with clonidine (37.6%, P < 0.05), while, in the hypothalamus, although there was also an apparent potentiation, this was statistically insignificant.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Decrease in Noradrenaline Turnover</th>
<th>Medulla</th>
<th>Hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Controls vs. Drug)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline vs. Clonidine 100ug/Kg</td>
<td>42.6% (20)</td>
<td>24.9% (19)</td>
<td></td>
</tr>
<tr>
<td>Saline vs. Sotalol 10mg/Kg</td>
<td>19.9% (20)</td>
<td>N/C (18)</td>
<td></td>
</tr>
<tr>
<td>Clonidine vs. Clonidine + Sotalol</td>
<td>37.6% (19)</td>
<td>12.7% (19)</td>
<td></td>
</tr>
<tr>
<td>N/C</td>
<td>no change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*</td>
<td>P &lt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6.4.4 Discussion

The results presented above show that the interaction between clonidine and sotalol reported to occur clinically (Saarimaa, 1976) can be demonstrated in the normotensive rat.

Both clonidine and sotalol produce a similar hypotensive effect in the doses examined when administered separately. Yet, on combined administration, they appear to reduce the hypotensive effects of each other. However, the order of drug administration dictated by the dosing schedule used in the above experiments implies that sotalol is antagonising the clonidine.

The result that combined application of the drugs produces an antagonism of the blood pressure effect of clonidine, yet an apparent potentiation of the turnover effects does not allow a simple explanation of the interaction.

With recent evidence that a number of β-blockers may have a central component to their hypotensive action (for references see section 6.4.1), it is tempting to speculate that the interaction between clonidine and sotalol may occur in the central nervous system.

However, available evidence suggests that sotalol is one β-blocking drug which does not have a central component to its hypotensive action (i.e. Garvey & Ram, 1975; Klevans, Kovacs & Kelly; 1976). These results do appear to be at variance with other reports which indicate a
central action (e.g. Day, Peters & Roach, 1976). It is, perhaps, possible that this disagreement of results may be attributable to either strain differences of the cats used in the experiments or possible differences in experimental procedures. For example, Garvey and Ram and Klevans et al used anaesthetised cats, while Day et al used conscious cats.

Fuxe et al (1976) have demonstrated that the β-blocker propranolol (said to have a central component to its hypotensive action, see Day, Peters & Roach, 1976), when given intraperitoneally, produces decreases in the turnover of noradrenaline in various regions of the brain.

If it is accepted, therefore, that drugs acting by a central mechanism (i.e. clonidine, α-methyldopa, propranolol, etc.) may directly or indirectly produce effects on central catecholamine metabolism, then it would seem, from the results presented in this thesis, that, as sotalol produces very little effect on noradrenaline turnover, it might be considered to have no central effect.

However, this idea may be too simplistic as the combination of clonidine and sotalol results in a significant potentiation of the clonidine effect on turnover. This would indicate some central effect of sotalol but it is possible that the effect is unrelated to the hypotensive action of the drug.

Fuxe et al (1976) suggest that the central effects of β-blockers may well be mediated by effects on
adrenaline receptors. This type of possibility cannot be excluded with regard to the results in this thesis as only effects on noradrenergic systems have been estimated.

It is also possible that effects on transmitter systems other than those mentioned above may result in, or contribute to, the interaction. Certainly, evidence is available showing that pharmacological manipulation of central cholinergic and serotonergic transmitter systems will also produce effects on blood pressure (e.g. Laubie, 1976; Bhargava, 1976).

The possibility of central effects occurring as a result of reflex activity also cannot be excluded.

Further comments on the clonidine/sotalol interaction are made in the next Chapter.
7.1 **Introduction**

The primary aim of this thesis was to examine the effects of clonidine on catecholamine metabolism in the brain. An attempt has been made to correlate changes produced with the hypotensive action of the drug, in the hope that the results so obtained would indicate the mechanism of action of clonidine. In pursuing this objective, two animal models, normotensive and renal/DOCA hypertensive rats, have been used.

The results of this study indicated that prolonged, as opposed to acute, treatment with clonidine (e.g. Andén *et al.*, 1970), as well as producing significant falls in blood pressure, also produces significant changes in the turnover of catecholamines, in particular noradrenaline. This effect occurs in both normotensive and hypertensive rats.

The results presented in Chapter 4, on the effects of clonidine on central catecholamines, and also the results presented in Chapter 5, on the interaction between clonidine and the tricyclic antidepressant DMI, suggest that the effects of clonidine on central noradrenaline turnover (to be referred to as the biochemical effects) are not directly related to what might be described as the functional effect, namely, the hypotensive action of the drug.

A discussion on the possible mechanisms by which the above effects occur is presented in this Discussion chapter.
The interaction between clonidine and DMI originally led to the proposal that clonidine produced its hypotensive action by an effect on pre-synaptic noradrenergic neurones located in the central nervous system (Reid, Briant & Dollery, 1973). However, subsequent work by other workers (e.g. Haeusler, 1974; Kobinger & Pichler, 1975, 1976) together with the results presented in this thesis, suggest that the hypotensive action of clonidine is mediated by stimulation of post-synaptic α-receptors.

Although it has been proposed that the α-receptor blocking properties of DMI account for the interaction between clonidine and DMI (see Van Spanning & Van Zwieten, 1973; Van Zwieten, 1975a), it is possible that the interaction is not solely mediated by a central mechanism. This may be substantiated by the fact that a similar interaction occurs between DMI and other antihypertensive and cardioactive drugs, some of which have no central activity (Kaumann, Basso & Aramendia, 1965; Van Zwieten, 1975a, 1976; Stafford & Fann, 1977; Cocco & Agüé, 1977).

This situation has been examined by the inclusion in this thesis of a study of the interaction between DMI and both α-methyldopa and guanethidine. A discussion of the possible sites and mechanisms involved in the above interactions is also presented here.

An attempt is also made to relate the observed effects of clonidine on blood pressure and central catecholamine metabolism in relation to the clinical use of clonidine as an antihypertensive agent. In the light of
the above results, suggestions are made which may explain
the occurrence of particular effects during clonidine
therapy.

Finally, suggestions are made for further work
indicating areas of possible interest, both directly and
indirectly related to the problem studied and arising
from the current work.

However, firstly, the involvement of central catecholamines in blood pressure control and hypertension will be discussed.

7.2 Central Catecholamines and Blood Pressure Regulation

It is now well established that central catecholaminergic neurones and the peripheral sympathetic nervous system play a very important part in the regulation of blood pressure and also that the central mechanisms are heavily implicated in the development of hypertension (see Chapters 1 and 2 and, for example, Chalmers, 1975; De Champlain & Van Ameringen, 1975; Fuxe et al, 1975; Haeusler, 1976).

The discovery of drugs like α-methyldopa and clonidine, which exert their antihypertensive action apparently by central noradrenergic mechanisms, has tended to confirm the role of central catecholaminergic neurones in blood pressure control (Van Zwieten, 1973,
Available evidence indicates that these central noradrenergic neurones, implicated in blood pressure control, display a predominantly inhibitory role (e.g. Gagnon & Melville, 1968; De Jong, 1974; Struyker-Boudier et al, 1975; Elliott & Clark, 1977). For instance, apart from the results presented by the workers mentioned above involving the application of exogenous noradrenaline to various areas of the brain resulting in depressor effects (see Chapter 1), it has been observed that, in certain models of hypertension, there is a decrease in the turnover of noradrenaline in areas of the brain which are associated with central regulation of blood pressure. Also, concomitant with this is an increased turnover of noradrenaline in peripheral organs (Yamori et al, 1970; Nakamura, Gerold & Thoenen, 1971; Chalmers & Reid, 1972; Haeusler, Finch & Thoenen, 1972). The implication of these results is that this decrease in central turnover represents a decrease in the availability of noradrenaline at a central receptor, the stimulation of which normally produces a controlling inhibition of sympathetic vasomotor outflow. This results in an increase in sympathetic outflow (reflected by an increase in peripheral noradrenaline turnover) and, consequently, an elevation in blood pressure.

It is difficult, however, to ascertain from the above results whether the alteration in central noradrenaline turnover is causal in the development of the
hypertension or somehow represents a reflex response to the increased blood pressure.

If these changes do occur at some inhibitory synapse and the changes are a result of a reflex effect to increase this inhibitory influence, then one might expect that turnover would increase (reflecting increased activity of the inhibitory neurone). My results do not support this idea as they show a decrease in turnover. This implies either an aberration of the inhibitory system or, if a reflex, a decrease in some noradrenergic pressor system. Haeusler, Finch and Thoenen (1972) suggest that the decrease in turnover found in hypertensive animals is a disturbance in the control of a central "trigger" mechanism which finally results in a self-perpetuating hypertensive disease.

Ito, Tanaka and Omae (1975) showed that the intracisternal administration of guanethidine resulted in a decrease in turnover of noradrenaline in the hypothalamic-medullary area. Associated with this was an elevation in blood pressure. This result supports the idea of decreased central noradrenaline turnover being causal in hypertension and also that noradrenaline plays a predominantly inhibitory role in central blood pressure regulation.

The results presented in Chapter 3 of this thesis confirm the findings of others (see above) that associated with hypertension in the DOCA-hypertensive rat is a decrease in central noradrenaline turnover in brain areas which are said to be important in blood pressure control.
Although there are reports that changes in endogenous levels of central noradrenaline can be correlated with elevated blood pressure and hypertension (Robertson et al., 1968; Nakamura, Gerold & Thoenen, 1971; Petty & Reid, 1977), the results presented in Chapter 3 of this thesis, examining levels of noradrenaline in a variety of brain regions, do not, on the whole, show any significant differences between normotensive and hypertensive animals.

### 7.3 Comments on the Measurement of Noradrenaline in Gross Regions of the Brain

The measurement of endogenous levels of noradrenaline, in both whole brain and relatively gross regions such as medulla, hypothalamus, cortex, etc., may not accurately reflect central catecholaminergic neurone activity in a particular pathway. Definite conclusions cannot really be drawn from the study of such gross areas as they may contain nuclei and neurone pathways which in some cases may, in no way, be associated with the particular functional parameter measured (in the case of the present work, blood pressure). It is perhaps also possible that some of the neurones may be producing opposite changes and, hence, the measurement of endogenous levels may, in fact, result in either an exaggeration or a much reduced estimate of the status of any particular neurone system.
If one accepts the idea that levels of endogenous catecholamines in neurones are maintained by a dynamic balance between, on the one hand, synthesis and, on the other, release and metabolism (Brodie et al., 1965), then the measurement of the "turnover" of noradrenaline may well be considered a better guide to neurone activity than static levels.

However, the argument put forward above concerning the validity of the measurement of endogenous levels of catecholamines applies equally well to turnover estimations. Any measured change in catecholamine turnover may reflect the final result of an equation involving both increases and decreases in turnover occurring in a variety of neurones and pathways, some of which may not be related to either the parameter studied or, if drug effects are being studied, the direct action of the drug at its "target" synapse.

It is clear, therefore, that one must examine much more discrete areas of the brain to allow definite correlation between a specific parameter measured (i.e. blood pressure) and any central effect that might occur at a neurone or synapse involved in the central control of the parameter. This may be particularly relevant in the examination of drug effects, as the drug could well produce opposing effects within a certain brain region.

To this end, techniques of microdissection of specific brain nuclei (e.g. Palkovits, 1973) may be of use in providing clearer information on any such changes mentioned
However, at the start of this project, such methods were only just becoming available (see Chapter 2) and, due to reasons discussed in Chapter 2, they were not employed in this thesis. Even considering the above comments, however, the results presented in this thesis, on changes in gross brain regions, may be of some value in determining the mechanism of action of clonidine.

As a working hypothesis to offer an explanation of the effects of various drugs on the central noradrenergic systems in relation to blood pressure control, the hypothesis that blood pressure regulation is mediated by a central mechanism in which noradrenaline plays a predominantly inhibitory role will be advanced. It will also be assumed that the measured turnover decrease described in Chapter 3 is associated with the hypertension (indicating an aberration of the inhibitory controlling mechanism of blood pressure) and that the drug-induced changes in turnover (cf. Chapter 4) occur at the site of this controlling mechanism.

7.4 The Mechanism of Action of Clonidine

In this thesis, results have been presented on the effects of prolonged clonidine administration on the levels and turnover of central catecholamines and an attempt made to correlate such changes with the hypotensive action of
the drug.

The effects of clonidine on the levels of endogenous noradrenaline in various regions from the brains of both normotensive and hypertensive animals are neither consistent nor conclusive (see Chapter 4).

However, examination of the results presented in Chapter 4 (Figures 4.2, 4.3 and 4.4) does appear to indicate a general trend of increased endogenous noradrenaline concentration after clonidine treatment. It can be said that, if an increase is not statistically significant, then it occurs merely by chance. This may be so, but the results mentioned above seem to show too consistently a trend of increased concentration after clonidine for this apparent effect to be discarded completely.

It has been reported by others that clonidine does produce significant increases in brain noradrenaline levels but, in these experiments, the drug has either been administered in high doses or for a long time (see, for example, Laverty & Taylor, 1969; Conolly, 1970, cited by Zaimis, p54; Rochette, Bralet & Bralet, 1974; Braestrup & Nielsen, 1976; Sugimoto, Hashida & Kasahara, 1976).

It is, perhaps, possible that these increases occur because clonidine decreases the release of noradrenaline (see later). However, as the increases are small and, in most cases, not statistically significant, their possible relevance is difficult to ascertain.

It may be that the use of greater numbers of animals may provide a clearer indication of a possible connection
between these increased levels and the action of clonidine. However, it must be considered whether the value of any result so obtained would outweigh and justify the expenditure of both time and animals, as the value of the measurement of levels of endogenous noradrenaline may be limited (as discussed in section 7.3).

If the hypothesis that decreased central noradrenaline turnover is associated with hypertension is correct, then it might not be unreasonable to expect that an antihypertensive drug producing its effects by a central mechanism might exert its action by producing, in some way, a reversal of this turnover effect, or, in the case of the normotensive animal, cause an increase in noradrenaline turnover at the inhibitory synapses.

In fact, as demonstrated by others using acute dosing with clonidine (e.g. Andén et al, 1970) and the results, presented in Chapter 4 of this thesis, from the use of prolonged dosing, although clonidine produces a hypotensive effect, it causes a decrease in central noradrenaline turnover. This effect occurs in both the medulla and the hypothalamus and in both normotensive and hypertensive animals. The effect was, however, more pronounced in the medulla, the area in which clonidine is thought to have its predominant site of action (i.e. Kobinger, 1975, 1976; Van Zwieten, 1975a; Haeusler, 1975).

Questions already become evident from these results. If decreased turnover of noradrenaline is associated with increased blood pressure (see earlier) and if clonidine
further decreases noradrenaline turnover, is this drug-induced decrease related to the drug's hypotensive action?

The answer to this question would appear to be that the effects of clonidine on noradrenaline turnover and blood pressure are not related, a conclusion supported by the results of Andén et al (1976). However, these workers used not blood pressure as the functional parameter affected by clonidine, but hindlimb flexor reflex activity in rats and the potentiation of apomorphine-induced hypermotility in mice. It may be that the effects of clonidine on blood pressure may not be comparable to these other parameters.

However, the idea that there is no relation between the turnover effects and the blood pressure lowering effects of clonidine appears to be further substantiated by the results obtained from the interaction between clonidine and DMI (Chapter 5). This line of argument will be considered again later in this section and also in the next section in the light of the clonidine/DMI results.

If it is to be assumed there is no relation between these two effects, then some explanation must be found for this phenomenon. There seems little reason to doubt that clonidine produces its hypotensive action by the stimulation of α-receptors which appear to be located predominantly in the medullary axis of the brain (Kobinger, 1975, 1976; Van Zwieten, 1973, 1975a; Haeusler, 1973, 1975). However, there is still controversy as to where these
α-receptors are situated and whether they are on a pre- or post-synaptic neurone.

In the periphery, clonidine stimulates post-synaptic α-receptors, resulting in a vasoconstriction, an action which is said to be responsible for the initial hypertensive effect of clonidine on intravenous administration (Zaimis, 1970; Van Zwieten, 1975a). However, there is also considerable evidence indicating that, in the periphery, clonidine also stimulates pre-synaptic α-receptors to prevent the release of noradrenaline from the post-ganglionic nerve ending (Armstrong & Boura, 1973; Starke & Altman, 1973; Doxey, 1977; Scriabine & Stavorski, 1977). Evidence is also available to indicate that clonidine displays a greater affinity for pre-synaptic α-receptors than post-synaptic α-receptors, at least in the periphery (Starke et al., 1974; Starke, Endo & Taube, 1975).

As a result, it has been suggested that the central hypotensive action of clonidine is also mediated by pre-synaptic α-receptors (Starke & Montel, 1973; Starke, Montel & Altman, 1973). An attempt to explain the interaction between clonidine and DMI has also led to a similar conclusion that clonidine's action is mediated by an effect on a pre-synaptic neurone site (see Reid, Briant & Dollery, 1973; and Chapter 5).

If the conclusions from these experiments are correct, it might be expected that clonidine acts by releasing endogenous noradrenaline from central neurones. However,
a number of workers have shown that even after almost total depletion of central noradrenaline with a combination of reserpine and α-MT treatment, the central hypotensive action of clonidine could still be demonstrated (Haeusler, 1974; Kobinger & Pichler, 1975, 1976). This would therefore argue in favour of a post-synaptic site for the hypotensive action of clonidine, but does not explain how the effects on turnover are mediated.

The simplest explanation is that clonidine produces both effects by α-receptor stimulation (see Andén et al., 1970, 1976), but that, apart from their common cause, the two effects are not directly related. The turnover effect might be regarded therefore as a side effect of the drug treatment.

If both the effects are apparently mediated by α-receptor stimulation, yet are unrelated to each other, then it is not unreasonable to assume that the α-receptors themselves may differ (see Andén et al., 1976). If we assume that both α-receptors producing the different effects are positioned close to one another (i.e. within the same synapse at which stimulation by clonidine produces a fall in blood pressure), then the following explanation may be advanced.

Figure 7.1 is a diagrammatic representation of a hypothetical synapse at which clonidine is supposed to act. It might be supposed that, in the normal state, noradrenaline is released in response to impulses from baroreceptors to stimulate the post-synaptic α-receptor on a neurone
FIGURE 7.1: Diagrammatic representation of a hypothetical inhibition synapse controlling blood pressure.

(a) Impulse → α-receptors → NA → NA

Stimulation decreases blood pressure

(b) α-receptors → NA → Clonidine

Feed back loop
which inhibits sympathetic outflow and hence results in controlling decreases in blood pressure (Figure 7.1(a)). The presence of clonidine in the synapse stimulates the post-synaptic α-receptor to decrease blood pressure. The decrease in noradrenaline turnover may then result either from the presence of a feed-back inhibition loop from the post- to the pre-synaptic neurone, decreasing the release of noradrenaline and hence indirectly decreasing turnover, or from the stimulation by clonidine of pre-synaptic α-receptors direct to inhibit the release of noradrenaline from the neurone. This too would result in decreased turnover by product inhibition of synthesis by noradrenaline (Figure 7.1(b)).

As has been stated, the presence of pre-synaptic α-receptors in the periphery is well documented (see earlier and also, for example, Langer et al, 1975, 1977; Starke, Taube & Borowsk, 1977) as is the effect on these receptors of clonidine (Armstrong & Boura, 1973; Starke & Altman, 1973; Doxey, 1977; Scriabine & Stavorski, 1977). It has also been shown that clonidine will reduce the release of noradrenaline from field stimulated brain slices (Starke & Montel, 1973; Farnebo & Hamberger, 1971) so that the postulated effects on the hypothetical synapse described above are well within the bounds of possibility. Therefore, if it is accepted that clonidine produces its effects by stimulation of post-synaptic α-receptors, the turnover effect certainly appears to be incidental to this main action.
Andén et al (1976) suggest that small doses of clonidine may produce a hypotensive effect by a pre-synaptic action, inferring that the hypotensive effect of clonidine may be related to the biochemical effect. This is an observation basically at variance with the ideas developed in this thesis.

However, for clonidine to act via a pre-synaptic mechanism to decrease blood pressure, in the hypothetical synapse detailed earlier, the drug would have to function as an α-receptor antagonist. This might be expected to increase the release, and hence increase the turnover, of noradrenaline (cf. results from phenoxybenzamine, Chapter 6.3). This does not occur. Therefore, even on this basis, it would appear that the hypotensive action of clonidine is mediated by the stimulation of central post-synaptic receptors.

This does not, however, preclude the possibility of a pre-synaptic agonist effect, decreasing the release of noradrenaline at some other noradrenergic synapse, possibly situated on a pressor pathway (for example, possibly in the hypothalamus) or in the periphery (Starke et al, 1974).

The line of argument developed in this section, that the biochemical (turnover) effects of clonidine appear unrelated to the functional (hypotensive) effect, would seem to be further substantiated when the results of the clonidine/DMI interaction are examined. These results are discussed in the next section.
The tricyclic antidepressants, including DMI, display some cardiovascular effects in that they produce small decreases in blood pressure (Kaumann, Basso & Aramendia, 1965; Van Spanning & Van Zwieten, 1973; Bobkiewicz et al, 1975). Their effect may be explained by the action of the drugs in blocking the uptake of noradrenaline into the pre-synaptic neurone. This increases the availability of noradrenaline at the post-synaptic receptor and, if such an effect occurs at a synapse which has an inhibitory effect on blood pressure (see earlier), then a fall in blood pressure would result. DMI also appears to have an effect on central noradrenaline turnover and, although, in this thesis, the effect was not statistically significant, this is a comparable result to that obtained by Leonard and Kafoe (1976) after chronic dosing with DMI.

As has been previously stated, clonidine causes a significant decrease in blood pressure and also a significant decrease in noradrenaline turnover, particularly in the medullary brain area.

The combined administration of clonidine and DMI, following the dosing schedule detailed in Chapter 5.2, results in an antagonism of the hypotensive effects of clonidine, yet an apparent potentiation of the turnover effects.

Table 7.1 summaries the general results obtained.
<table>
<thead>
<tr>
<th>Drugs Administered</th>
<th>Blood Pressure Response</th>
<th>Turnover Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonidine</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>DMI</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Clonidine + DMI</td>
<td>↓</td>
<td>↓↓↓</td>
</tr>
</tbody>
</table>
If the hypothesis of decreased central noradrenaline turnover being associated with hypertension (or elevated blood pressure) is sound, then the fact that DMI causes a potentiation of the turnover decreases resulting from clonidine treatment would seem to be further evidence that drug-induced biochemical effects (at least in relation to clonidine) are unrelated to their functional effects.

How can we therefore explain both the antagonism of the hypotensive effects of clonidine by DMI and the potentiation of the turnover effects?

If we consider that the interaction occurs at the central synapse at which clonidine acts, which will again be represented by the hypothetical model proposed in the previous section, then the following explanation may be advanced.

As has been stated earlier, clonidine stimulates the post-synaptic receptor to bring about a fall in blood pressure and produces its turnover effects by either a feed-back loop or, more likely, stimulation of pre-synaptic α-receptors to decrease release and, therefore, turnover of noradrenaline (Figure 7.2(a)).

The effects of DMI could probably occur in much the same way, with the uptake blockade effect of DMI increasing noradrenaline concentration in the synaptic cleft, which might be expected to exert both an increase in post-synaptic α-receptor stimulation (hence a decrease in blood pressure) and also an increase in pre-synaptic receptor stimulation.
to decrease turnover (Figure 7.2(b)).

When clonidine and DMI are given concurrently, it might be expected that, as DMI potentiates the effect of central noradrenaline (e.g. see Nakamura & Fukushima, 1976), it may result in an apparent potentiation of clonidine. In fact, it antagonises the hypotensive action of clonidine yet potentiates the turnover effects. This effect of DMI to potentiate the effects of noradrenaline may be considered in an explanation of the potentiation of the turnover effects of clonidine. It might be that the apparent potentiation of the clonidine effect is an addition of the effects produced by clonidine and the increased noradrenaline concentration in the synaptic cleft due to uptake blockade by DMI, resulting in increased pre-synaptic stimulation (Figure 7.2(c)).

But why does not a similar effect occur at the post-synaptic receptor, producing a potentiation of the hypotensive response also?

One explanation may lie in the α-blocking properties of DMI (McCulloch & Story, 1972). It has been suggested that the antagonism of the hypotensive action of clonidine by DMI is a result of α-receptor blockade of the post-synaptic receptors stimulated by clonidine (Van Spanning & Van Zwieten, 1973; Van Zwieten, 1975a).

It has been suggested earlier that the decrease in noradrenaline turnover produced by clonidine is caused either by feed-back inhibition or by stimulation of pre-synaptic α-receptors. If either of these mechanisms are
FIGURE 7.2: Diagrammatical summary of the mechanisms by which Clonidine, DMI and combined administration of the two drugs produce their effects.

(a) CLONIDINE

(b) DMI

(c) CLONIDINE + DMI

<table>
<thead>
<tr>
<th>Noradrenaline Turnover Decrease</th>
<th>Blood Pressure Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>
involved (both initiated by α-receptors), it is difficult to see how the observed potentiation of the turnover effects, yet antagonism of the hypotensive effect, could occur unless pre- and post-synaptic receptors are different. In this case, if the turnover decrease was associated with the stimulation of the post-synaptic receptor, it might be expected that α-block of the clonidine effect would antagonise this feed-back turnover effect. This does not occur and would imply a pre-synaptic mechanism for the turnover effects.

It has been shown by others that some α-blockers appear to display a certain degree of specificity towards pre- or post-synaptic α-receptors, demonstrated by the α-blockers affecting either the turnover or functional effects of clonidine and other drugs (Andén et al, 1970; Andén, Grabowska & Strömbom, 1976). It is perhaps possible, therefore, that DMI shows a greater affinity for central post-synaptic α-receptors than pre-synaptic receptors with respect to its α-blocking properties. This would result in the antagonism of the hypotensive effect but not the turnover decreasing effects if these are mediated by pre-synaptic α-receptor stimulation. However, without more results any further comment would be speculative.

If, however, the idea of α-receptor antagonism by DMI is not correct and the drug does not antagonise preferentially the post-synaptic action of clonidine, there must be other mechanisms involved in the interaction.

An obvious site must be in the periphery with DMI.
acting as an uptake blocker and, as a result, potentiating the peripheral effects of noradrenaline. This might result in vasoconstriction and hence an increase in blood pressure. The antagonism might then be considered a physiological antagonism rather than a true pharmacological action.

As it has been shown that DMI potentiates the effects of noradrenaline at certain central sites (Nakamura & Fukushima, 1976), it is possible that this could also occur at some noradrenergic synapse in the brain which is located on a pressor pathway and results in an effect which has more significance than the inhibitory effect of clonidine.

It is obvious that more exact experiments need to be carried out before any definite conclusion as to the mechanism by which this interaction occurs can be drawn.

However, the results from the examination of the interaction reported in this thesis, on the basis of the arguments presented above, would appear to verify the hypothesis that the decrease in central noradrenaline turnover, resulting from clonidine administration, is unrelated to the hypotensive effect of the drug.

The results may also be interpreted as further evidence for a post-synaptic action of clonidine.
7.6 Effects of other Drugs

Guanethidine & α-methyldopa

Interaction with DMI is not confined to clonidine but is a property shared by a number of other drugs which affect the cardiovascular system (Cocco & Agüé, 1977).

In this thesis, it has been demonstrated that α-methyldopa and the peripherally acting antihypertensive guanethidine interact with DMI, confirming the reports of others (Kaumann, Basso & Aramendia, 1965; Stafford & Fann, 1977; Van Zwieten, 1975a, 1976).

The effects produced by guanethidine will be considered first.

Guanethidine is not thought to have a central component to its hypotensive action nor be able to cross the blood brain barrier (Wilhelm & De Stevens, 1976; Baum, Shropshire & Varner, 1972). Yet, in the experiments in this thesis, it produced, in addition to a reduction in blood pressure, a significant reduction in the turnover of noradrenaline in the hypothalamus. There was little change in the turnover of noradrenaline in the medulla.

Although only a single dose study was performed, this result is in apparent conflict to the results obtained after clonidine treatment, where the apparently greater effect on noradrenaline turnover occurred in the medulla. These differing effects may imply that the drugs, if both acting by a central mechanism, act at different
sites. Or, if it is accepted that guanethidine does not enter the brain, then the effect related to guanethidine treatment may be of reflex origin, the decrease in turnover representing a decreased activity of an inhibitory pathway normally activated by, for example, baroreceptors and terminating in the hypothalamus (cf. Chapter 1, Figure 1.3; Calaresu & Thomas, 1975).

If, however, this is a reflex effect, one might expect that on administration of DMI, which antagonises the hypotensive effect of guanethidine by a peripheral mechanism, preventing the uptake of the drug into the neurone (Koe & Constantine, 1972; Stafford & Fann, 1977), then the reflex decrease in hypothalamic noradrenaline turnover would be reversed. This would occur as a result of the blood pressure rising, hence increasing baroreceptor activity, and thus restoring the activity of the inhibitory pathway proposed above. As can be seen from the results in Chapter 6, this does not occur and, in fact, the decrease in noradrenaline turnover is apparently potentiated.

Ito et al (1975) report that intracisternal administration of guanethidine results in decreases in noradrenaline turnover in areas concerned with blood pressure control (i.e. hypothalamus and medulla), but, at the same time, produces an elevation in blood pressure.

It is perhaps possible that, on long term administration of guanethidine, small quantities of the drug enter the brain in sufficient amounts to produce an effect
on noradrenaline turnover, similar to that resulting from intracisternal administration. This could result in an increased sympathetic outflow and, consequently, an elevation in blood pressure. However, one would expect this to be more than compensated for by the profound peripheral adrenergic neurone blockade produced by the drug, a situation possible when considering that postural hypotension occurs with guanethidine treatment, indicating some block of centrally mediated reflex control of blood pressure.

If, therefore, the DMI interaction is due to a peripheral blockade of the uptake of guanethidine into the neurone, this would result in a less profound neurone blockade, which might allow some emergence of the central pressor effects. Equally, by the same argument, one might expect that a similar blockade of any central effects of guanethidine would occur when, in fact, as shown by the results in Chapter 6, the turnover effects produced by guanethidine are potentiated by DMI. The explanation of the DMI antagonism and the effects of guanethidine becomes therefore complicated.

The results obtained from the examination of \(\alpha\)-methyldopa and DMI confirm that an interaction occurs between the two drugs. However, because \(\alpha\)-methyldopa causes a depletion of noradrenaline (see Chapter 6, section 2.2.4), no results on turnover were obtained.

The interaction between \(\alpha\)-methyldopa and DMI does not involve the uptake blockade properties of DMI. This has
been demonstrated by Van Zwieten (1976) in experiments where cocaine (which blocks the uptake mechanism of the neurone) failed to abolish the hypotensive actions of α-methyldopa (and clonidine) and where Ipindole, a tricyclic antidepressant with no uptake blockade properties, still produced an antagonism of the α-methyldopa (and clonidine) response.

The inference from these experiments is that, if the interaction between centrally acting antihypertensives and the tricyclic antidepressants is a result of a central mechanism, it would seem probable that it is a result of effects on post-synaptic α-receptors by the antidepressant drugs (i.e. α-block).

This does not, however, exclude other sites for the interaction, sites which may be as, or more, important as the mechanisms proposed above, or may at least contribute to the central mechanism. Therefore, with particular reference to the drugs examined in this thesis, the following may be proposed sites of the interactions with DMI which may work separately or in conjunction to result in the interactions

1. central α-blockade of post-synaptic α-receptors;

2. potentiation of peripheral noradrenaline;

3. a general potentiation of adrenergic/
noradrenergic pressor mechanisms in the central nervous system;

and, particularly relevant to guanethidine and other peripherally acting compounds,

4. uptake blockade of the antihypertensive drug into the neurone.

It is perhaps possible that no unified hypothesis can be forwarded to explain all the interactions and that each interaction is a result of a different mechanism (i.e. for clonidine, central α-block, while for guanethidine, peripheral uptake blockade of the drug).

Phenoxybenzamine

The increase in noradrenaline turnover produced by treatment with phenoxybenzamine is not altogether surprising. It might be expected that, if an α-agonist (i.e. clonidine) produces decreases in turnover, then by the same mechanism, an α-blocker might have the opposite effect.

If the explanation for the effects produced by clonidine, detailed in section 7.4, are correct, then it would follow that an α-blocker would prevent the stimulation by noradrenaline of pre-synaptic α-receptors. This would result in an increase in the release of noradrenaline
and hence an increase in turnover, which does occur (see Chapter 6 and, for example, Andén et al., 1970).

The effect of the α-blocker is more pronounced in the hypothalamus than the medulla. It is difficult to offer any explanation for this. As phenoxybenzamine causes hypotension by a peripheral α-blocking effect, it might be that central turnover changes may reflect a reflex action. This, however, is difficult to accept if the line of argument discussed earlier with respect to guanethidine is correct, as there it was implied that peripherally induced hypotension may reflexly result in a decreased central noradrenaline turnover.

It is more probable that the turnover effects of phenoxybenzamine are due to a direct drug effect, on central noradrenergic neurones, but not necessarily those involved in blood pressure control.

Clonidine and Sotalol

The interaction between clonidine and the β-blocking drug sotalol is a surprising phenomenon. One might expect that, as the two drugs work by different mechanisms to decrease blood pressure, there would, at the least, be no interaction or, alternatively, an additive or synergistic effect.

As demonstrated from the report by Saarimaa (1976) and also by the results of the animal study presented in Chapter 6 of this thesis, this does not occur. In fact,
the reverse occurs with a nearly complete antagonism of the hypotensive effects of both drugs.

Another interesting report on the effects between clonidine and β-blockers details a case history of a patient who abruptly ceased taking clonidine after inclusion of the β-blocker Timolol in his dosage regimen (Bailey & Neale, 1976). The result was the manifestation of a severe hypertensive crisis and the display of classic clonidine withdrawal symptoms (see Hansson et al, 1973). The crisis was controlled and reduced by the reintroduction of clonidine therapy. The authors suggest that the rebound blood pressure effect was exacerbated by the presence of the β-blocker due to its ability to increase peripheral vascular resistance. However, it is not possible to ascertain from the paper whether, in fact, the β-blocker had any part in the hypertensive crisis, as one is not able to say whether such a rebound hypertensive crisis would not have occurred anyway on cessation of clonidine therapy (see section 7.8).

In the experiments on the clonidine/sotalol interaction reported in Chapter 6 of this thesis, as well as producing a marked antagonism of the hypotensive action of clonidine, sotalol also produced an apparent significant potentiation of the decreased noradrenaline turnover which results from clonidine treatment. This occurred only in the medullary region, in which sotalol itself had no significant effect.

If, as has been discussed in Chapter 6, section 6.4.4,
sotalol has no central component to its hypotensive action and does not cross the blood brain barrier, then the antagonism of the hypotensive action of clonidine, coupled with a potentiation of the turnover response, becomes difficult to reconcile.

The antagonism of the blood pressure response may be a result of one of the peripheral β-blocking actions of sotalol resulting in a blockade of β-vasodilatory mechanisms. This might result in more pronounced effects from α-vasoconstrictor mechanisms. If this were so, the peripheral effect of clonidine (i.e. vasoconstriction) may well be potentiated and become significant.

However, the central effects allow no easy explanation to be made for their occurrence without more information from experiments investigating the central effects of β-blockers in general and sotalol in particular. It would certainly be of value to ascertain if this interaction can be shown to occur with other β-blockers, in particular those which are reputed to have a central component to their mechanism such as propranolol (e.g. Day et al, 1976) and which also produce changes in central catecholamine turnover (see Chapter 6, section 6.4.4, and Fuxe et al, 1976).

As discussed in Chapter 6, section 6.4.4, the possibility of effects on other central mechanisms cannot be fully excluded so that adrenergic neurone systems (Fuxe et al, 1976) or cholinergic and serotonergic systems (e.g. Laubie, 1976; Bhargava, 1976) may also be involved.
However, to ascertain this, much work would need to be done on the topic.

Possibly crucial in this respect would be definite evidence as to whether sotalol has a central action or not. Again, this is an area in which more detailed work requires to be carried out.

Therefore, although an explanation has been offered for a peripheral mechanism of the antagonism of clonidine by sotalol, on the available evidence, it is not possible to offer a satisfactory explanation of the central effects.

7.7 The Localisation of the Central Effects of Clonidine

If the hypothetical synapse proposed in section 7.4 is accepted as that at which clonidine produces its effects, then where might this synapse be located?

Ample evidence is available to confirm that the predominant hypotensive action of clonidine is mediated by an effect in the medulla (for references see section 7.4).

Early work by Schmitt (1971) and Haeusler (1973b) led to the proposal that the baroreceptor reflex arc and the Nucleus Tractus Solitarii (NTS) were important in the hypotensive action of clonidine, stimulation by clonidine of α-receptors located in the NTS being responsible for the
blood pressure lowering action. There have, however, been some conflicting results from more recent work on this area of the medulla. Laubie et al (1976) demonstrated that, after the NTS in the dog has been lesioned, the bradycardiac, but not the hypotensive, effect of clonidine is decreased. This observation has also been made in the cat by Antonaccio and Halley (1977). However, Lipski et al (1977) have shown that, after NTS lesioning in the rat, the hypotensive effect of clonidine is decreased. They propose species difference and incomplete lesioning of the NTS in larger animals (such as cats and dogs) to account for this conflicting result.

Therefore, if the NTS in the rat is the predominant site for the hypotensive action of clonidine, then it may not be unreasonable to suggest that the hypothetical synapse proposed earlier is located in this area. Hence the effect of clonidine to decrease noradrenaline turnover in the medulla may be explained by the discussion presented in section 7.4.

The effects of clonidine on noradrenaline turnover in the hypothalamus is not, however, so easily explained.

If, as proposed by Calaresu and Thomas (1975), there is an inhibitory connection between the NTS and the hypothalamus (Figure 1.3, Chapter 1) and this synapse at the hypothalamic end is noradrenergically innervated, if the neurone is stimulated by clonidine in the NTS, it might be expected that an increase in turnover would result in the hypothalamus. This does not apparently occur. Therefore,
either the synapse, if it exists, is not noradrenergically innervated or the hypothalamic turnover decreases reflect an effect on other noradrenergic neurone systems.

Clonidine has been shown to produce depressor effects when injected stereo-taxically into the anterior and posterior hypothalamus (Struyker-Boudier & Van Rossum, 1972; Struyker-Boudier et al, 1974). This indicates the presence of α-receptors and, hence, possibly noradrenergic neurones in these areas, which may be affected in a similar way to that proposed for noradrenergic nerve endings in the NTS. Clonidine has also been shown to reduce the pressor effect of electrical stimulation of the hypothalamus (Philippu, 1976), presumably by decreasing the release of noradrenaline initiated by electrical stimulation. This too might be expected to result in a decrease in noradrenaline turnover.

It is perhaps possible, therefore, that the hypothalamic turnover decrease reflects the effect of the inhibitory pathway activated by clonidine on a noradrenergic pressor system in the hypothalamus, as it has also been demonstrated that clonidine applied to the NTS will decrease the pressor response from electrical stimulation of the hypothalamus (Philippu, 1976).

The effects of clonidine on noradrenaline turnover in the hypothalamus is, however, less than in the medulla and is of secondary importance to the drug's hypotensive action in the medulla (Van Zwieten, 1975a).

Regarding the potentiation of the turnover effects of
clonidine by DMI, the discussion presented in section 7.5 may well be applied to the specific areas discussed above.

It may be that the interaction between DMI and clonidine involves two actions of DMI at separate sites in the brain. For example, α-block of the effects of clonidine in the NTS, yet a potentiation of the noradrenergic pressor system in the hypothalamus. Philippu (1976) indicates that β-blockers will reduce the pressor response from electrical stimulation of the hypothalamus, implying that these pressor responses may be mediated by β-receptors and, hence, be unaffected by α-block from the DMI. This suggestion is feasible considering that some β-blockers are thought to exert their hypotensive actions by a central effect (e.g. Day, Peters & Roach, 1976).

However, the effects produced by guanethidine and phenoxybenzamine on noradrenaline turnover are not easy to explain within the context of the discussion presented above. Until it is made clear whether the effects produced by these drugs are due to a reflex effect or a direct drug action, it is not possible to offer an acceptable explanation.

7.8 Clinical Implications of the Effects of Clonidine reported in this Thesis

Clonidine is an antihypertensive drug widely used in
the treatment of hypertension and, although it has some side effects, mainly sedation and the production of dry-mouth, it produces an effective control of blood pressure (Callingham, 1971; Bostock, 1975; Fragachan et al, 1975; Kosman, 1975; Conolly, 1975; Wilhelm & De Stevens, 1976; Dollery, 1976).

There are two major factors associated with clonidine treatment which are causes for concern.

The first is the interaction of clonidine with other drugs resulting in a loss of the hypotensive effects of clonidine. This is particularly relevant with the tricyclic antidepressants which have been shown to antagonise the action of clonidine clinically (Briant, Reid & Dollery, 1973) and also in experimental animals (Van Zwieten, 1975a; Chapter 5 of this thesis). Also, as has been previously discussed, it appears that certain β-blockers may also produce an antagonism of the clonidine response (see section 7.6).

Animal experiments have also indicated that other psychotropic drugs may produce adverse actions when administered with clonidine (Van Zwieten, 1976, 1977).

The second major factor important in clonidine therapy and probably one of the more important factors as it is more likely to occur due to "patient error" is the "rebound" hypertensive crisis that is reported to occur on abrupt cessation of clonidine treatment (Hansson et al, 1973; Hunyor et al, 1973; Bailey, 1975; Goldberg et al, 1976; Bailey & Neale, 1976; Pettinger, 1975). It is to
this phenomenon that the findings of this thesis may be relevant.

The withdrawal phenomenon and blood pressure crisis have been shown to be associated in man with an increased sympathetic activity (Hansson et al, 1973) and have also been demonstrated in animal experiments (Cavero et al, 1977; Dix & Johnson, 1977; Grimes, 1974, unpublished observations; Petty, 1975, unpublished observations).

Assuming that in man blood pressure is controlled by a noradrenergic mechanism which has an inhibitory controlling function on blood pressure and that clonidine acts and produces its effects on this mechanism in much the same way as in animals (see sections 7.2 and 7.4), then it is possible that the decrease in central noradrenaline turnover resulting from clonidine treatment could also occur in man.

It has been said that the pre-synaptic effects of clonidine persist longer than the post-synaptic effects after removal of clonidine (cited by Sleight, 1976). If so, then the hypotensive effect of clonidine ceases soon after ceasing therapy, but the decreased central noradrenaline turnover persists (accepting the argument set forth that this is a result of a pre-synaptic effect) and at a lower level than existed before clonidine treatment was initiated. Hence, the normal function of the inhibitory controlling pathway is impaired, which results in increased sympathetic outflow from the brain and, therefore, increased sympathetic activity resulting in vaso-
constriction and raised blood pressure. The idea of increased sympathetic activity seems to be substantiated by the fact that the "overshoot" crisis can be controlled by return to clonidine therapy or by combined administration of α- and β-blocker therapy.

7.9 Conclusions

The major conclusion to be drawn from this thesis is that prolonged dosing with clonidine results both in a fall in blood pressure and a decrease in central norepinephrine turnover. It would appear that the effect on turnover, however, is not directly related to the hypotensive action of the drug, but seems to be an incidental effect mediated by the stimulation of pre-synaptic α-receptors by clonidine.

The results of the examination of the clonidine/DMI interaction appear to confirm this idea and also indicate that the interaction itself (antagonism of the hypotensive action of clonidine) is not related to the effect of DMI to block central pre-synaptic uptake mechanisms. Various other mechanisms have been suggested which may solely or partially be responsible for this interaction.

On the basis of the results obtained, the thesis argues for a post-synaptic site for the α-receptors stimulated by clonidine to decrease blood pressure.
7.10 Suggestions for Further Work

Apart from continuing the research into the causes of hypertension and the relationship of central regulatory control mechanisms to elevated blood pressure, there are three major suggestions for further work arising from this thesis:

1. As suggested in section 7.3, the examination of gross brain areas may not provide a clear indication of the true state of neurone activity in the central nervous system. The estimation of turnover in much more discrete areas, such as individual brain nuclei (i.e. NTS or hypothalamic nuclei), using methods outlined in Chapter 2, section 2, and correlation of any such changes with blood pressure may be of greater value in determining the function of central noradrenergic neurones in blood pressure control and also the effect of drugs on these systems.

These types of experiments could be performed in conjunction with stereo-taxic application of drugs to certain brain areas (i.e. clonidine on to the NTS) with concurrent measurement of noradrenaline turnover in other brain areas.

The peripheral manipulation of blood pressure by peripherally acting drugs or mechanical means, inducing reflex effects, could also be performed and
correlated with changes in turnover in specific brain nuclei.

Experiments of this type might shed light on the cause of the apparent central effects produced by compounds such as guanethidine and sotalol, which are not thought to enter the brain (see earlier discussion). The results of such experiments may also aid in a greater understanding of the central pathways involved in blood pressure control.

2. Further study of the interaction between clonidine and, for example, the tricyclic antidepressants. Experiments involving the concurrent recordings of central sympathetic outflow, blood pressure and possibly peripheral nerve activity (i.e. by turnover estimations or measurement of labelled transmitter release) may help determination of whether the interactions are a result of a central or a peripheral effect. Again, the results of such experiments may also provide explanations towards determining the true site of action of centrally acting antihypertensives.

3. A more detailed study of the clonidine/sotalol interaction to attempt to define the true cause of this interaction.

It also would certainly be of value to see if the interaction could be demonstrated with other β-blockers.
Examination of \( \beta \)-blockers in general in a similar way to that outlined in Suggestion 1 may provide information both on the effects and site of action of \( \beta \)-blockers in the brain and also possible central sites for the interaction with clonidine.
REFERENCES


BAILEY, N.T.J. (1959), "Statistical Methods in Biology", ENGLISH UNIVERSITIES PRESS, LONDON.


GEROLD, M. & H. TSCHIRKY (1968), Measurement of blood pressure in unanaesthetised rats and mice, Arzneimittel Forschung, 18, 1285-1287.


GOLDBERG, A.D., P.R. WILKINSON & E.B. RAFTERY (1976), The overshoot phenomenon on withdrawal of clonidine therapy, Post Grad. Med. J., 52(Suppl. 7), 128-134.


HAEUSLER, G. (1974), Clonidine induced inhibition of sympathetic nerve activity: no indication for a central presynaptic or an indirect sympathomimetic mode of action, Naunyn-Schmiedebergs Arch. Pharmacol., 285, 1-.


HAEUSLER, G., L. FINCH & H. THOENEN (1972), Central adrenergic neurones and the initiation and development of experimental hypertension, Experienta, 28, 1200-1203.


HANSSON, L., S.N. HUNYOR, S. JULIUS & S.W. HOOBLER (1973), Blood pressure crisis following withdrawal of Clonidine, with special relevance to arterial and urinary catecholamine levels, and suggestions for acute management, Amer. Heart J., 85, 605-610.


KAUMANN, A., N. BASSO & P. ARAMENDIA (1965), The cardiovascular effects of N-(gamma-methyl-aminopropyl-imino-dibenzyl)-HCL (DMI) and Guanethidine, J. Pharmacol. Exp. Therap., 147, 54-64.


KOPIN, I.J. (1968), Biosynthesis and metabolism of catecholamines, Anaesthesiology, 29, 654-660.


LANGER, S.Z., W. ADLER-GRASCHINSKY & O. GIORGI (1977),
Physiological significance of alpha-adrenoreceptor-
mediated negative feedback mechanism regulating
noradrenaline release during nerve stimulation,

LAUBIE, M. (1976), Pharmacological evidence for interactions
of cholinergic and noradrenergic mechanisms in central
cardiovascular control, in: "Proceedings of the Sixth
International Congress of Pharmacology", vol. 4, Ed.

LAUBIE, M., H. SCHMITT & M. DROUILLAT (1976), Action of
Clonidine on the baroreceptor pathway and medullary
sites mediating vagal bradycardia, Eur. J. Pharmacol.,
38, 293-303.

LAVERTY, R. (1973), The mechanism of action of some anti-

LAVERTY, R. & K.M. TAYLOR (1969), Behavioural and bio-
chemical effects of 2-(2,6-dichlorophenylamino)-2-
imidazoline HCl (ST155) on the central nervous system,

LEAKE, C.D. (1962), The historical development of cardio-
vascular physiology, in: "Handbook of Physiology: Circulation", vol. 1, Ed. W.F. Hamilton & P. Dow,

system in essential hypertension and mineralocorticoid excess, Lancet, 2, 308-309.

LEDINGHAM, J.M. (1971), The Etiology of Hypertension,
The Practitioner, 207, 5-19.

LEDINGHAM, J.M. (1975), Experimental Renal Hypertension,

LEONARD, B.E. & W.F. KAFOE (1976), A comparison of the acute
and chronic effects of four antidepressant drugs on the
turnover of Serotonin, Dopamine and Noradrenaline in
the rat brain, Biochem. Pharmacol., 25, 1939-1942.

LEW, G.M. (1976), Circadian rhythms in blood pressure and
norepinephrine in genetically hypertensive and

LEWIS, P.J. (1975), Propranolol - an antihypertensive drug
J.L. Reid, Pitman, Tonbridge Wells, p206-214.

LIPSKI, J., J. PRZYBYLSKI & E. SOLNICKA (1976), Reduced hypo-


Okamoto, K., Ed. (1972), "Spontaneous Hypertension", IGAKU SHOIN LTD., TOKYO.

ONESTI, G. et al, Ed. (1973), "Hypertension, Mechanisms and Management", GRUNE & STRATTON, NEW YORK.

ONESTI, G., M. FERNANDES & K.E. KIM, Ed. (1976), "Regulation of Blood Pressure by the Central Nervous System", GRUNE & STRATTON, NEW YORK.


PICKERING, G. (1974), "Hypertension: Causes, Consequences and Management", CHURCHILL LIVINGSTONE, LONDON.


REID, J.L. & C.T. DOLLLERY (1976), Central and peripheral catecholamine mechanisms in circulatory control, Cardiology, 61(Suppl. 1), 113-124.


SCHOLTYSIK, G. & R. SALZMANN (1973), Interaction between antidepressant and centrally acting antihypertensives, Naunyn-Schmiedebergs Arch. Pharmacol., 279, R33.

SCHOLTYSIK, G. et al (1975), Pharmacological actions of the antihypertensive agent N-amidino-2-(2,6-Dichlorophenyl) acetamide Hydrochloride (BS 100-141), Arzneimittel Forschung, 25, 1483-1491.


STAFFORD, J.R. & W.E. FANN (1977), Drug interactions with guanidinium antihypertensives, Drugs, 13, 57-64.


STARKE, K. & H. MONTEL (1973), Involvement of α-receptors in Clonidine induced inhibition of transmitter release from central monoamine neurones, Neuropharmacol., 12, 1073-1080.


Van ZWIETEN, P.A. (1977), The interaction between Clonidine and various neuroleptic agents and some benzodiazepine tranquillizers, J. Pharm. Pharmacol., 29, 229-234.


WEEKS, J.R. (1976), Cardiovascular techniques using unanaesthetised and freely moving rats, Upjohn Booklet, UPJOHN, KALAMAZOO.


PUBLICATIONS
The effect of chronic clonidine administration on catecholamine metabolism in the rat brain

A.J. DRAPER, D. GRIMES* and P.H. REDFERN (School of Pharmacy and Pharmacology, University of Bath)

The antihypertensive drug clonidine is thought to have a central action [2], although there is conflicting evidence of its effect on amine metabolism in the brain [1].

In the work reported here we have examined the effects of chronic clonidine on the levels and turnover of noradrenaline (NA) and dopamine (DA) in discrete brain regions of normotensive and hypertensive rats. Clonidine (50 μg/kg or 100 μg/kg i.p.) twice daily for 7 days, in normotensive rats produced significant falls in blood pressure, but no significant changes in DA or NA levels of the cortex, midbrain, hypothalamus or medulla.

In renal hypertensive rats, the blood pressure falls were greater, and were accompanied by significant increases in whole brain levels of NA (P < 0.01). Increase of NA in discrete brain regions was apparently confined to the cortex, the increase being greater after the lower dose of clonidine. Little change in DA level was observed in any region of the brain. In spontaneously hypertensive rats after 7 days treatment with 100 μg/kg clonidine i.p. twice daily there was a significant decrease (P < 0.001) in both NA and DA turnover estimated in whole brain. The relation of these results to the antihypertensive action of clonidine will be discussed.

THE INTERACTION BETWEEN CLONIDINE AND DESMETHYLIMIPRAMINE: EFFECTS ON BLOOD PRESSURE AND CENTRAL CATECHOLAMINE METABOLISM

A.J. Draper, D. Grimes, P.M. Redfern, Dept. of Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY.

Evidence has recently been presented which suggests an interaction between the centrally acting antihypertensive clonidine and the tricyclic antidepressant drug desmethyliimipramine (DMI) which could lead to a reduction in the clinical efficacy of the hypotensive agent. (Briant & Reid 1972; Van Zwieten 1975).

We have examined the effect of these two drugs, alone and in combination, on blood pressure and noradrenaline (NA) metabolism in normotensive rats. DMI 3mg/kg i.p. given 20h and 6h before killing produced a 12.0% fall in blood pressure (p<0.001). As has previously been shown, (Draper & others 1976), clonidine also produced a significant fall in blood pressure of 20% when given in a dose of 100 μg/kg i.p. daily for 7 days. However, when DMI was given at the end of the period of clonidine treatment, the fall in blood pressure was only 12.4%, significantly less (p<0.001) than the fall recorded after clonidine alone.

This result lends weight to the belief that the antihypertensive effect of clonidine may be antagonised by DMI. We have also investigated the possibility that interaction between the two drugs involves the metabolism of NA at central synapses. Turnover of NA was assessed in medulla and hypothalamus of normotensive rats by measuring the rate of decline of NA levels after administration of 3-methyl-p-tyrosine. We have previously shown that treatment with clonidine for 7 days produces a significant decrease in whole brain turnover in both normotensive and hypertensive animals. (Draper & others 1976).

In the present experiments clonidine alone produced significant decreases in the Medulla and Hypothalamus. Treatment with DMI alone also produced decreases in the turnover of NA in Medulla and Hypothalamus but less than that attributable to Clonidine. Combination of DMI and clonidine resulted in a further decreased rate of turnover in both medulla and hypothalamus, when compared to values from Clonidine treated animals. The reason for this effect is not clear, although it might be suggested that pretreatment with clonidine results in a changed sensitivity of central pre- or post-synaptic receptors, such that the accumulated NA resulting from DMI-blockade of re-uptake is capable of producing a secondary inhibition of NA synthesis.

It can however be concluded that the reported antagonism of clonidines' hypotensive action by DMI is not easily explained by any simple interaction at central adrenergic synapses.


The effect of prolonged clonidine administration on catecholamine metabolism in the rat brain

ANTHONY J. DRAPER, DAVID GRIMES, PETER H. REDFERN*, Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, Bath, Avon, U.K.

Acute doses of the antihypertensive drug clonidine cause a decrease in the turnover of central catecholamines (Laverty & Taylor, 1969, Andén, Corrodi & others, 1970), although the extent of the decrease appears widely variable. We have examined the effects of more prolonged clonidine administration on both blood pressure and central catecholamine turnover, in normotensive and two types of hypertensive rat, namely renal clip/DOCA rats, and New Zealand spontaneously hypertensive rats (SH-rats).

Male rats of the CFY strain were used, normotensive animals at 200-250 g, while hypertensive animals were used 4 to 5 weeks after surgery on 70-90 g animals, made hypertensive by a modification of the method of Finch & Leach (1970), in which the left renal artery was occluded with a silver clip, and a 25 mg pellet of deoxycorticosterone acetate was implanted subcutaneously. These animals had free access to 1% saline; control animals (144 ± 2 mmHg, n = 20) resulted. SH-rats were from a colony of the New Zealand strain raised in our own laboratories, with blood pressures of 208 ± 4 mmHg (n = 19). All blood pressures were measured in the conscious restrained animal by the tail cuff method before dosing and on the sixth day. Clonidine hydrochloride in 0.9% saline was given intraperitoneally twice daily at 9.00 and 17.00 h for 7 days, controls were given saline. Statistical significances were determined using Student's t-test.

To estimate the effect of clonidine on the concentration of noradrenaline and dopamine in the brain, animals were killed 2 h after the last morning dose of clonidine, the brains dissected into regions (Glowinski & Iversen, 1966) and the catecholamines extracted according to Anton & Sayre (1962) and then assayed fluorimetrically (Shellenberger & Gordon, 1971).

The turnover of the two amines was estimated in whole brains by measuring the rate of decline of the amines concentrations after synthesis inhibition with α-methyl-p-tyrosine methyl ester hydrochloride (α-MT) (250 mg kg⁻¹, i.p.) (Brodie, Costa & others, 1966). Concentrations of the amines were measured at time 0 and 4 h after α-MT, previous experiments having shown that the decline was linear over this period. Turnover rates were compared by log-linear regression analysis of the results and comparison of the resultant slopes by t-test. In clonidine-treated animals, α-MT was administered 2 h after the last morning dose, and the animals killed 4 h later.

Drugs used were: clonidine hydrochloride (Boehringer-Ingelheim), α-methyl-p-tyrosine methyl ester hydrochloride, (Sigma) and deoxycorticosterone acetate (Organon).

In normotensive animals clonidine (50 or 100 µg kg⁻¹, i.p.) produced significant falls in blood pressure (12.1 and 20.6 %, P < 0.001 for both), but produced no significant changes in the concentration of the amines in brain regions (cortex, midbrain, hypothalamus, and medulla-pons) or whole brain. However in the renal/DOCA hypertensive animal the same doses of clonidine not only produced similar significant changes in blood pressure (15.1 and 20.9 %, for the low and high doses of clonidine, P < 0.001) but also significant increases in the concentrations of noradrenaline (Fig. 1), in certain brain areas no consistent changes were found in dopamine concentration.

The rate of decline of noradrenaline concentration after synthesis inhibition with α-MT was measured in whole brains of normotensive and renal/DOCA hypertensive rats. In the latter, the rate of decline was significantly decreased by 30.1 % of the control value (Table 1). Similar results were obtained for the rate of decline of dopamine concentrations (Table 1).

When these experiments were repeated using clonidine-treated animals it was found that in all animals clonidine produced significant and dose related decreases in the turnover of both amines (as well as significant falls in blood pressure (Table 2).

![Fig. 1. The effect of clonidine 100 µg kg⁻¹ (i.p.) twice daily for 7 days on the concentrations of noradrenaline in rat brain (ng g⁻¹ tissue). Open columns represent control values, hatched columns represent clonidine treated values. Values are the mean of 10 samples (±s.e.m.) * P < 0.05, ** P < 0.01. a-Cortex, b-midbrain, c-hypothalamus, d-medulla pons, e—total brain.](image-url)
Table 1. Rates of decline of catecholamine concentrations in the brains of normotensive and renal/DOCA hypertensive rats after synthesis inhibition. Blood pressures were measured, and concentrations of noradrenaline (NA) and dopamine (DA) estimated after synthesis inhibition by α-methyl-p-tyrosine (250 mg kg⁻¹, i.p.). Rates were compared by comparison of slopes of graphs.

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Mean blood pressure mm Hg (± s.e.m.)</th>
<th>Rates of decline</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive rats</td>
<td>10</td>
<td>144 ± 2</td>
<td>0-07662</td>
</tr>
<tr>
<td>Renal/DOCA hypertensive rats</td>
<td>10</td>
<td>219 ± 5</td>
<td>0-05359</td>
</tr>
</tbody>
</table>

The rate of decline of amine concentration after synthesis inhibition is presumably related to the rate at which the amines are released from their binding sites within the nerve endings, and subsequently destroyed and/or removed from the brain.

We have demonstrated that this 'turnover' rate of noradrenaline is significantly less in hypertensive than in normotensive animals, confirming the results of Nakamura, Gerold & Thoenen, (1971) and Ito, Tanaka & Omac, (1975). While noradrenaline can be shown to be either depressor or pressor depending on the area of the brain to which it is applied, (Gagnon & Melville, 1968; Phillipu, Przuntek & others, 1971), it is now believed to have a predominantly inhibitory role of noradrenaline, it must be assumed that clonidine is acting presynaptically as an antagonist to noradrenaline at α-adrenoceptors, and allowing more noradrenaline to be released in response to stimulation of the presynaptic neuron. In other words the fall in blood pressure produced by clonidine would be associated with an increased turnover of noradrenaline. But we have found that the rate of decline of noradrenaline following synthesis inhibition to be significantly slowed by clonidine given over seven days at 100 μg kg⁻¹ (a decrease also occurred at 50 μg kg⁻¹ but was not statistically significant). This occurred in normotensive rats, and in hypertensive rats in which noradrenaline turnover was already significantly reduced.

Using another functional model, Andén, Grabowska & Strombom (1976) have suggested that the metabolic and functional effects of clonidine are unrelated, being mediated by pre- and post-synaptic α-receptors respectively. While we cannot rule out the possibility that a similar separation occurs in the areas of the brain responsible for blood pressure control, there seems no reason to suppose that bombardment of post-synaptic α-receptors should not be responsible for both the recorded fall in blood pressure and the decreased turnover of noradrenaline, by a feedback homeostatic mechanism.

Thus our results using prolonged clonidine administration are compatible with the hypothesis that the antihypertensive effect of clonidine is brought about by an agonist action on post-synaptic receptors.

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REFERENCES


