THE EFFECT OF THYROIDECTOMY
ON THE RESPONSIVENESS OF RAT ATRIA
TO ADRENERGIC AMINES

by

WILLIAM WILSON SIMPSON
B.Sc., University of Ottawa, 1973
M.Sc., University of British Columbia, 1976

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
in
THE FACULTY OF GRADUATE STUDIES
in
THE FACULTY OF PHARMACEUTICAL SCIENCES
Division of Pharmacology and Toxicology

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
July 1980
© William Wilson Simpson, 1980
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Division of Pharmacology and Toxicology
Faculty of Pharmaceutical Sciences

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date July 30/88
ABSTRACT

To examine effects of thyroid state on cardiac adrenoceptor sensitivity, left and right atria isolated from male euthyroid and hypothyroid rats were exposed to the agonists methoxamine, isoproterenol and phenylephrine and to the antagonists \(10^{-6}\text{M}\) phenoxybenzamine, phentolamine and propranolol. Isoproterenol and phenylephrine increased rate and force of both euthyroid and hypothyroid atria. Methoxamine also increased force of euthyroid and hypothyroid atria, but increased the rate of hypothyroid atria only. In all cases in which a response was observed, the hypothyroid state increased the potency of methoxamine and of phenylephrine, and decreased the potency of isoproterenol. The hypothyroid state also increased the inotropic and chronotropic effectiveness of methoxamine, but did not alter the maximum responses to phenylephrine or isoproterenol. Phenoxybenzamine abolished all responses to methoxamine, but only partially inhibited the effects of phenylephrine. Propranolol had no effect on the responses to methoxamine, blocked the chronotropic response to phenylephrine, and blocked the inotropic and chronotropic responses to isoproterenol. In the presence of the cholinergic agonist carbachol, the basal rate and force of euthyroid right and left atria, respectively, were decreased to the basal level observed in the hypothyroid controls. Methoxamine was found to increase both rate and force in the treated euthyroid atria similar to that observed in the hypothyroid controls. Conversely, in the presence of the adrenergic agonist isoproterenol, the basal rate and force of hypothyroid right and left atria, respectively, were increased to the basal level observed in the euthyroid controls. The hypothyroid atria then responded to methoxamine as was observed in the euthyroid right and left atria. Methoxamine was found to have no effect on cyclic AMP production in either the euthyroid or hypothyroid left or right atria, even though a greater increase in both rate and force was
observed in the hypothyroid right and left atria, respectively, as compared to the euthyroid atria. While the hypothyroid state decreased the potency of isoproterenol on the force of contraction and atrial rate, it did not affect the cyclic AMP response to this beta adrenoceptor agonist. Isoproterenol increased cyclic AMP production, rate and force to the same extent in both the euthyroid and hypothyroid right and left atria. The data of the present study do not support the hypothesis that rat heart adrenoceptors undergo a conversion from beta to alpha in the hypothyroid state, in terms of cyclic AMP production, atrial rate and force development. The data do, however, support the hypothesis that there is an increased alpha adrenoceptor responsiveness in the hypothyroid state as compared to the euthyroid state.
# TABLE OF CONTENTS

Responsiveness of Hypothyroid Rat Atria to Adrenergic Amines

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>ix</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xi</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>xiv</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>9</td>
</tr>
<tr>
<td>I. Animal Preparation</td>
<td>9</td>
</tr>
<tr>
<td>II. Thyroxine Concentration Determination</td>
<td>9</td>
</tr>
<tr>
<td>III. Tissue Preparation</td>
<td>10</td>
</tr>
<tr>
<td>IV. Apparatus</td>
<td>12</td>
</tr>
<tr>
<td>V. Chemical Agents</td>
<td>12</td>
</tr>
<tr>
<td>1. Agonists</td>
<td>12</td>
</tr>
<tr>
<td>2. Antagonists</td>
<td>12</td>
</tr>
<tr>
<td>VI. Experimental Procedure</td>
<td>12</td>
</tr>
<tr>
<td>VII. Cyclic Nucleotide Determination</td>
<td>13</td>
</tr>
<tr>
<td>1. Tissue Extraction</td>
<td>13</td>
</tr>
<tr>
<td>2. Cyclic AMP Determination</td>
<td>13</td>
</tr>
<tr>
<td>VIII. Data Presentation and Statistical Analysis</td>
<td>14</td>
</tr>
<tr>
<td>Results</td>
<td>16</td>
</tr>
<tr>
<td>I. Action of Phenylephrine on Atrial Rate and Contractile Force</td>
<td>16</td>
</tr>
<tr>
<td>1. Euthyroid State</td>
<td>16</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>2. Hypothyroid State</td>
<td>21</td>
</tr>
<tr>
<td>II. Effect of Increasing the Concentration of Phenoxybenzamine and Propranolol on the Responses to Phenylephrine in the Euthyroid State</td>
<td>22</td>
</tr>
<tr>
<td>III. Effect of Labetalol on the Responses to Phenylephrine in the Euthyroid State</td>
<td>25</td>
</tr>
<tr>
<td>IV. Action of Methoxamine on Atrial Rate and Contractile Force</td>
<td>28</td>
</tr>
<tr>
<td>1. Euthyroid State</td>
<td>28</td>
</tr>
<tr>
<td>2. Hypothyroid State</td>
<td>31</td>
</tr>
<tr>
<td>V. Action of Isoproterenol on Atrial Rate and Contractile Force</td>
<td>36</td>
</tr>
<tr>
<td>1. Euthyroid State</td>
<td>36</td>
</tr>
<tr>
<td>2. Hypothyroid State</td>
<td>36</td>
</tr>
<tr>
<td>VI. Effectiveness of Isoproterenol and Methoxamine in Euthyroid and Hypothyroid States</td>
<td>45</td>
</tr>
<tr>
<td>VII. Presentation of the Data as Percent Change in Tension and Rate of Isoproterenol Maximum Change in Tension and Rate for the Agonists</td>
<td>49</td>
</tr>
<tr>
<td>1. Phenylephrine</td>
<td>49</td>
</tr>
<tr>
<td>2. Methoxamine</td>
<td>49</td>
</tr>
<tr>
<td>VIII. Presentation of the Data for Isoproterenol in Absolute Terms</td>
<td>54</td>
</tr>
<tr>
<td>1. Tension Development</td>
<td>54</td>
</tr>
<tr>
<td>2. Rate Development</td>
<td>54</td>
</tr>
<tr>
<td>IX. Presentation of the Data for Methoxamine in Absolute Terms</td>
<td>54</td>
</tr>
<tr>
<td>1. Tension Development</td>
<td>54</td>
</tr>
</tbody>
</table>
2. Rate Development

X. Effect of Baseline on the Responses to Methoxamine in Euthyroid and Hypothyroid States

1. Tension Development
2. Rate Development

XI. Action of Calcium on Atrial Contractile Force and Rate in Euthyroid and Hypothyroid States

1. Tension Development
2. Rate Development

XII. Effect of Altering Baseline with Calcium Concentration on the Responses to Methoxamine in Euthyroid and Hypothyroid States

1. Tension Development
2. Rate Development

XIII. Effect of Altering the Temperature of the Bathing Medium on the Responses to Methoxamine

1. Euthyroid State - Rate Development
2. Hypothyroid State - Tension Development

XIV. Effect of Altering the Temperature of the Bathing Medium on the Rate Response to Isoproterenol in the Euthyroid State

XV. Effect of Carbachol Pretreatment on the Responsiveness of Euthyroid Rat Atria to

1. Methoxamine
   (i) Rate Development
   (ii) Tension Development
2. Isoproterenol
   (i) Rate Development
(ii) Tension Development

XVI. Effect of Carbachol Pretreatment, Low Calcium and Low Temperature on the Responsiveness of Euthyroid Atria to Phenylephrine

1. Tension Development

2. Rate Development

XVII. Effect of Carbachol Pretreatment, Low Calcium and Low Temperature on the Responsiveness of Euthyroid Atria to Isoproterenol

1. Tension Development

2. Rate Development

XVIII. Effect of Carbachol Pretreatment, Low Calcium and Low Temperature on the Responsiveness of Euthyroid Atria to Methoxamine

1. Tension Development

2. Rate Development

XIX. Effect of Phenoxybenzamine on the Force and Rate Responses to Phenylephrine in Euthyroid, Hypothyroid and Carbachol-Pretreated Euthyroid Rat Left and Right Atria

XX. Effect of Propranolol on the Force and Rate Responses to Isoproterenol in Euthyroid, Hypothyroid and Carbachol-Pretreated Euthyroid Left and Right Atria

XXI. The Influence of Methoxamine and Isoproterenol on Cyclic AMP Production

XXII. The Effect of Paired-Pacing on Euthyroid and Hypothyroid Left Atria

Discussion
<table>
<thead>
<tr>
<th>Summary</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>156</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>DESCRIPTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Chenoweth-Koelle Solution Composition</td>
<td>11</td>
</tr>
<tr>
<td>II</td>
<td>Methoxamine Maximum Force Response Relative to Isoproterenol Maximum Force Response</td>
<td>32</td>
</tr>
<tr>
<td>III</td>
<td>Methoxamine Maximum Rate Response Relative to Isoproterenol Maximum Rate Response</td>
<td>33</td>
</tr>
<tr>
<td>IV</td>
<td>ED50 Values of Isoproterenol, alone and in the presence of Propranolol, on Left and Right Atria from Euthyroid and Hypothyroid Rats</td>
<td>46</td>
</tr>
<tr>
<td>V</td>
<td>ED50 Values of Methoxamine on Left and Right Atria from Euthyroid and Hypothyroid Rats after Different Pretreatments</td>
<td>78</td>
</tr>
<tr>
<td>VI</td>
<td>ED50 Values of Isoproterenol on Left and Right Atria from Euthyroid and Hypothyroid Rats</td>
<td>104</td>
</tr>
<tr>
<td>VII</td>
<td>ED50 Values of Phenylephrine on Left and Right Atria from Euthyroid and Hypothyroid Rats</td>
<td>110</td>
</tr>
<tr>
<td>VIII</td>
<td>ED50 Values of Methoxamine on Left and Right Atria from Euthyroid and Hypothyroid Rats</td>
<td>115</td>
</tr>
<tr>
<td>IX</td>
<td>ED50 Values of Phenylephrine on Left and Right Atria from Euthyroid, Hypothyroid and Carbachol-Pretreated Euthyroid Rats</td>
<td>117</td>
</tr>
<tr>
<td>X</td>
<td>ED50 Values of Isoproterenol on Left and Right Atria from Euthyroid, Hypothyroid and Carbachol-Pretreated Euthyroid Rats</td>
<td>120</td>
</tr>
<tr>
<td>XI</td>
<td>Cyclic AMP Values in Response to Isoproterenol in Euthyroid and Hypothyroid Left Atria</td>
<td>123</td>
</tr>
<tr>
<td>XII</td>
<td>Cyclic AMP Values in Response to</td>
<td>124</td>
</tr>
<tr>
<td>TABLE</td>
<td>DESCRIPTION</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isoproterenol in Euthyroid and Hypothyroid Right Atria</td>
<td></td>
</tr>
</tbody>
</table>
# List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of Phenylephrine on Rate and Tension of Euthyroid and Hypothyroid Rat Atria</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>Potencies of Phenylephrine in the presence of Phenoxybenzamine and Propranolol in the Euthyroid and Hypothyroid States on Rate and Tension</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Effect of Increasing the Concentration of Phenoxybenzamine and Propranolol on the Responses to Phenylephrine in the Euthyroid State</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>Effect of Labetalol on the Responses to Phenylephrine in the Euthyroid State</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>Effect of Methoxamine on Rate and Tension of Euthyroid and Hypothyroid States</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>Potencies of Isoproterenol and Methoxamine in Euthyroid and Hypothyroid States</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>Effect of Isoproterenol on Rate of Euthyroid Rat Atria</td>
<td>37</td>
</tr>
<tr>
<td>8</td>
<td>Effect of Isoproterenol on Tension of Euthyroid Rat Atria</td>
<td>39</td>
</tr>
<tr>
<td>9</td>
<td>Effect of Isoproterenol on Rate of Hypothyroid Rat Atria</td>
<td>41</td>
</tr>
<tr>
<td>10</td>
<td>Effect of Isoproterenol on Tension of Hypothyroid Rat Atria</td>
<td>43</td>
</tr>
<tr>
<td>11</td>
<td>Effectiveness of Isoproterenol and Methoxamine in Euthyroid and Hypothyroid States</td>
<td>47</td>
</tr>
<tr>
<td>12</td>
<td>Effect of Phenylephrine on Rate and Tension of Euthyroid and Hypothyroid Rat Atria Presented as Percent Change of Isoproterenol Maximum Change</td>
<td>50</td>
</tr>
<tr>
<td>13</td>
<td>Effect of Methoxamine on Rate and Tension of Euthyroid and Hypothyroid Rat Atria Presented as Percent Change of Isoproterenol</td>
<td>52</td>
</tr>
<tr>
<td>FIGURE</td>
<td>DESCRIPTION</td>
<td>PAGE</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>14</td>
<td>Maximum Change</td>
<td>55</td>
</tr>
<tr>
<td>15</td>
<td>Effect of Isoproterenol on Absolute Tension Development in Euthyroid and Hypothyroid Atria</td>
<td>57</td>
</tr>
<tr>
<td>16</td>
<td>Effect of Isoproterenol on Absolute Rate Development in Euthyroid and Hypothyroid Atria</td>
<td>59</td>
</tr>
<tr>
<td>17</td>
<td>Effect of Methoxamine on Absolute Tension Development in Euthyroid and Hypothyroid Atria</td>
<td>62</td>
</tr>
<tr>
<td>18</td>
<td>Effect of Methoxamine on Absolute Rate Development in Euthyroid and Hypothyroid Atria</td>
<td>64</td>
</tr>
<tr>
<td>19</td>
<td>Effect of Basal Tension on the Responses of Methoxamine in Euthyroid and Hypothyroid States</td>
<td>67</td>
</tr>
<tr>
<td>20</td>
<td>Effect of Basal Rate on the Responses of Methoxamine in Euthyroid and Hypothyroid States</td>
<td>70</td>
</tr>
<tr>
<td>21</td>
<td>Effect of Increasing the External Calcium Concentration on Absolute Tension Development in Euthyroid and Hypothyroid States</td>
<td>73</td>
</tr>
<tr>
<td>22</td>
<td>Effect of Increasing the External Calcium Concentration on Absolute Rate Development in Euthyroid and Hypothyroid States</td>
<td>76</td>
</tr>
<tr>
<td>23</td>
<td>Effect of Altering Basal Tension with External Calcium Concentration on the Responses to Methoxamine in Euthyroid and Hypothyroid Atria</td>
<td>80</td>
</tr>
<tr>
<td>24</td>
<td>Effect of Altering Basal Rate with External Calcium Concentration on the Responses to Methoxamine in Euthyroid Atria</td>
<td>83</td>
</tr>
<tr>
<td>25</td>
<td>Effect of Altering Basal Rate with Temperature on the Responses to Methoxamine in Euthyroid Atria</td>
<td>85</td>
</tr>
<tr>
<td>FIGURE</td>
<td>DESCRIPTION</td>
<td>PAGE</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>26</td>
<td>Effect of Altering Basal Rate with Temperature on the Responses to Isoproterenol in Euthyroid Right Atria</td>
<td>88</td>
</tr>
<tr>
<td>27</td>
<td>Effect of Carbachol Pretreatment on the Responsiveness of Euthyroid Right Atria to Methoxamine</td>
<td>91</td>
</tr>
<tr>
<td>28</td>
<td>Effect of Carbachol Pretreatment on the Responsiveness of Euthyroid Left Atria to Methoxamine</td>
<td>94</td>
</tr>
<tr>
<td>29</td>
<td>Effect of Carbachol Pretreatment on the Responsiveness of Euthyroid Right Atria to Isoproterenol</td>
<td>96</td>
</tr>
<tr>
<td>30</td>
<td>Effect of Carbachol Pretreatment on the Responsiveness of Euthyroid Left Atria to Isoproterenol</td>
<td>99</td>
</tr>
<tr>
<td>31</td>
<td>Effect of Carbachol Pretreatment, Low Calcium and Low Temperature on the Responsiveness of Euthyroid Left and Right Atria to Phenylephrine</td>
<td>102</td>
</tr>
<tr>
<td>32</td>
<td>Effect of Carbachol Pretreatment, Low Calcium and Low Temperature on the Responsiveness of Euthyroid Left and Right Atria to Isoproterenol</td>
<td>107</td>
</tr>
<tr>
<td>33</td>
<td>Effect of Carbachol Pretreatment, Low Calcium and Low Temperature on the Responsiveness of Euthyroid Left and Right Atria to Methoxamine</td>
<td>112</td>
</tr>
<tr>
<td>34</td>
<td>Effect of Methoxamine on Cyclic AMP Production in Euthyroid and Hypothyroid States</td>
<td>121</td>
</tr>
<tr>
<td>35</td>
<td>Effect of Paired-Pacing on Euthyroid and Hypothyroid Left Atria</td>
<td>127</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

"IT IS EASIER TO ACQUIRE FACTS, THAN TO JUDGE WHAT THEY PROVE, AND HOW, THROUGH THE FACTS WE KNOW, TO GET TO THOSE WHICH WE WANT TO KNOW."

John Stuart Mill
Inaugural Address
at St. Andrews, 1867

Work was supported by grants from the Medical Research Council of Canada and the British Columbia Heart Foundation.

Preliminary data have appeared in abstract and paper form in the following:

The Pharmacologist 21 (3): 257 Abs. #591 (1979)
Advances in Myocardiology I: 417-437 (1980)

I would like to thank Dr. John McNeill for suggesting the topic to me and guiding me through the project. I would also like to thank Drs. Tenner, Rodgers and Siegl for their interest and help through the course of this work.

I would also like to thank the members of my committee: Drs. Bellward, Godin, Katz and Sinclair for their guidance and suggestions.
INTRODUCTION

The classification of adrenoceptors proposed by Ahlquist (1948), identifying two types of adrenergic receptors, alpha and beta, is now well known. This classification was originally derived from the observations regarding relative potencies of a series of sympathomimetic amines on the responses of a number of different tissues. The ongoing discovery of compounds demonstrating relatively specific antagonism toward the responses associated with each receptor type has afforded an opportunity to test the validity of such a 'two receptor' concept. This has resulted in numerous investigations designed to classify the receptors in various organs (Govier et al., 1966).

Classically, the adrenoceptors mediating both inotropic and chronotropic responses in the heart were characterized as belonging to the beta, but not alpha, adrenoceptor type, since the responses of the myocardium to sympathomimetic agents could only be antagonized by beta adrenoceptor blocking agents (Black and Stephenson, 1962; Wagner et al., 1974; Martinez and McNeill, 1977; Endoh et al., 1978). The lack of contribution of alpha adrenoceptors to the inotropic and chronotropic responses of adrenergic stimulation has also been reported (Nickerson and Chan, 1961; Moran and Perkins, 1961), but more recent evidence suggests that alpha adrenoceptors can mediate these responses (Wenzel and Su, 1966; Govier, 1967; 1968; Nakashima and Hagino, 1972; Nakashima et al., 1973; Verma and McNeill, 1976). Wenzel and Su (1966) first reported the existence of alpha adrenoceptors in rat myocardial strips. In addition, Govier (1968) found that alpha adrenoceptors were present in guinea-pig atrium, while Benfey and Varma (1967) confirmed their existence in rabbit left atrium. Wagner and Reinhardt (1974), however, were unable to confirm the presence of alpha adrenoceptors in the atria of guinea-pigs and rabbits. On the other hand, other investigators have shown the existence of ventricular alpha adrenoceptors.
Investigations on the papillary muscle of rabbit right ventricle (Schümann et al., 1974); on the cat ventricle (Rodrigues-Pereira and Wagner, 1975); and in isolated perfused rabbit heart (Wagner et al., 1974) have clearly shown the presence of myocardial \textit{alpha} adrenoceptors. Schümann et al. (1978) reported the presence of \textit{alpha} adrenoceptors in human atria which were able to mediate positive inotropic effects of adrenergic agonists.

Historically, alterations in cardiac responsiveness to adrenergic amines and to specific adrenoceptor blocking agents have been observed with changes in ambient temperature as well as changes in thyroid state. Kunos et al. (1968; 1973) have demonstrated that the characteristics of the adrenoceptors mediating the inotropic effect of adrenaline on isolated frog myocardium transformed from \textit{beta} to the \textit{alpha} adrenoceptor type as the temperature was lowered. These workers later reported similar phenomena in mammalian cardiac preparations, induced by either lowering the temperature (Kunos and Nickerson, 1977) or by decreasing circulating thyroid hormone levels (Kunos et al., 1974). The apparent temperature-induced interconversion of \textit{alpha} and \textit{beta} adrenoceptor characteristics in mammalian heart has not been observed by other investigators (Benfey, 1977; Martinez and McNeill, 1977).

The physiological relationship between thyroid hormone and the catecholamines has been investigated for many years (Harrison, 1964; Brodie et al., 1966). Some physiological changes that occur in hypothyroidism are depressed basal metabolic rate and oxygen consumption of tissues (Barker and Klitgaard, 1952), lowered cardiac output (Straufer and Schulze, 1976) and decreased myocardial contractility (Buccino et al., 1967). Various hypotheses have been formulated to explain these observations. One possibility is a change in the effector-tissue responsiveness to adrenergic agents.
Kunos et al. (1974) showed that hypothyroidism produced changes in adrenoceptor properties which were similar to the changes produced by altering ambient temperature. The data indicated that the alpha adrenoceptors present in the hypothyroid rat atria were either absent, or were unaffected by phenoxybenzamine, in euthyroid rat atria. Kunos et al. (1974) were able to reverse the response of the hypothyroid state to that of the euthyroid state by administration of thyroid hormone to hypothyroid rats. Nakashima and Hagino (1972) found that alpha adrenoceptors mediated a significant positive chronotropic effect in hypothyroid rat atria. In addition, the alpha adrenoceptors became more sensitive in this altered thyroid state. On the other hand, in the euthyroid state, the positive chronotropic effect was mediated predominantly through beta adrenoceptors. Buckley and Jordan (1970) interpreted the observed shift in the balance of alpha and beta adrenoceptors as being due to the availability of two independent pools of receptors. Conversely, Kunos et al. (1974) attributed this shift to a single type of adrenoceptor which changes in allosteric configuration with altered thyroid state.

In the euthyroid state, the activation of the beta adrenoceptors in the heart has been reported to increase cyclic adenosine 3',5'-monophosphate (cyclic AMP) levels (Robison et al., 1965), while the stimulation of alpha adrenoceptors in the heart had no effect on these levels (Rabinowitz et al., 1975). However, Kunos et al. (1976) reported that in the hypothyroid state, activation of alpha adrenoceptors by the dual adrenergic agonist phenylephrine effected an increase in cyclic AMP levels which could be blocked by the alpha blocking agent phenoxybenzamine. This effect could be reversed by the administration of thyroid hormone to hypothyroid rats. They concluded that the difference in the adrenoceptor-mediated production of cyclic AMP between the
two thyroid states could also be attributed to an interconversion of \( \text{alpha} \) and \( \text{beta} \) adrenoceptors. In contrast, others have found that the increased inotropic and chronotropic responses to \( \text{alpha} \) adrenergic agonists in the hypothyroid state are not accompanied by increased levels of cyclic AMP (Hagino and Shigei, 1976; Osnes, 1976).

Cyclic AMP was initially discovered as the intracellular mediator of the glycogenolytic effect of epinephrine and glucagon in the liver (Sutherland and Rall, 1960), but it has since come to be recognized as an important metabolic regulator and as a second messenger mediating a variety of hormonal effects (Sutherland et al., 1965; Robison et al., 1968). In considering the evidence linking cyclic AMP to hormonal regulation, Sutherland et al. (1965) formulated the second messenger hypothesis. According to this hypothesis, the first messenger (the hormone) interacts with plasma membrane tissue-specific sites and stimulates the membrane-bound enzyme adenylate cyclase. Thereafter, the increased level of cyclic AMP production influences a variety of cell processes to elicit a certain physiological response. Some hormonal effects may not be mediated by cyclic AMP (Schümann et al., 1975; Rabinowitz et al., 1975; Endoh et al., 1976) but by parallel sequences of events, which may or may not interact with those processes influenced by cyclic AMP. However, the second messenger hypothesis has been useful in conceptualizing the mechanism of action of many chemical agents.

It is well established that catecholamines increase intracellular cyclic AMP levels in the heart (Martinez and McNeill, 1977). Evidence exists supporting the hypothesis that the increased cyclic AMP levels are associated, at least in part, with the positive inotropic effect of certain adrenergic amines (Robison et al., 1965; Sutherland and Robison, 1966; Wastilla et al., 1972;
Following the administration of catecholamines, myocardial cyclic AMP levels rise before or simultaneously with the rise in contractile force (Wasti et al., 1972). Furthermore, catecholamines have the same order of potency in stimulating adenylate cyclase and in increasing contractile force of the heart (Robison et al., 1965; Drummond et al., 1966; Benfey et al., 1974). Similarly, other agents mediating positive inotropic effects, such as glucagon and histamine, are also known to stimulate myocardial adenylate cyclase (Farah and Tuttle, 1960; Murad and Vaughn, 1969; Klein and Levey, 1971).

While the effects of specific beta adrenoceptor agonists, such as isoproterenol, on the contractile force of cardiac muscle are well documented, the effects of the dual adrenergic agonist phenylephrine are controversial. With respect to contractile force, phenylephrine has normally been considered to stimulate alpha adrenoceptors but have little effect on beta adrenergic receptors (Ahlquist and Levy, 1959). Govier (1967) also reported that phenylephrine effectively produced responses in the heart through stimulation of alpha adrenoceptors. However, other reports have indicated that phenylephrine could effectively produce responses in the heart through stimulation of beta adrenoceptors. For example, phenylephrine was shown to produce a relaxation in isolated guinea-pig trachea which was competitively antagonized by the beta adrenoceptor antagonist propranolol (Chahl and O'Donnell, 1969). Furthermore, in isolated heart preparations, phenylephrine was shown to produce positive inotropic and chronotropic effects which also were antagonized by beta adrenoceptor antagonists (Leon and Benfey, 1968; McNeill et al., 1972; Wagner and Reinhardt, 1974; Martinez and McNeill, 1977).

The effects of phenylephrine on cyclic AMP production, thought to be due
to stimulation of \textit{beta} adrenoceptors, are also controversial. Phenylephrine has been reported to increase levels of cyclic AMP in perfused guinea-pig heart (McNeill and Verma, 1973) and in rat atria (Kunos \textit{et al.}, 1976). However, the inotropic response of phenylephrine has also been reported in the absence of an increase in cyclic AMP in cat papillary muscles (Brückner \textit{et al.}, 1978) and cat atria (Rabinowitz \textit{et al.}, 1975). In addition, several studies have indicated that phenylephrine failed to produce increased cyclic AMP levels in rabbit heart slices (Benfey, 1971; Benfey and Carolin, 1971) and in a guinea-pig cardiac particle preparation (McNeill \textit{et al.}, 1972). Furthermore, Benfey \textit{et al.} (1974) reported a dissociation between cardiac-inotropic and adenylate cyclase-activating adrenoceptors and concluded that the adrenoceptors mediating cardiac inotropic responses are distinct from those mediating the production of cyclic AMP. In support of this conclusion, other workers have also proposed that the inotropic effects of adrenergic amines are not mediated by cyclic AMP (Schümann \textit{et al.}, 1975; Endoh \textit{et al.}, 1976; Watanabe \textit{et al.}, 1977). Therfore, the apparent dissociation between the inotropic response and cyclic AMP 'casts' doubt on the role of the second messenger hypothesis in positive inotropy.

In summary, it is generally accepted that there are at least two types of adrenoceptors, \textit{alpha} and \textit{beta}, which mediate myocardial inotropic and chronotropic responses (Govier, 1967; 1968; Martinez and McNeill, 1977). There is evidence that phenylephrine is a dual agonist effecting inotropic and chronotropic responses mediated by both types of adrenoceptors. This may partially explain the controversy among various studies arising from the use of phenylephrine, and emphasizes the importance of using specific \textit{alpha} and \textit{beta} adrenergic agonists to study cardiac responsiveness to adrenergic
amines. Further, an increased alpha adrenergic responsiveness and a decreased beta adrenergic responsiveness has been reported in the hypothyroid state (Nakashima et al., 1971; 1973). This has been interpreted as being due to an interconversion of myocardial alpha and beta adrenoceptors, and it has been suggested that the myocardial adrenoceptor forms a single unit whose properties are controlled by the metabolic environment such as changes in ambient temperature or thyroid state (Kunos and Szentivanyi, 1968; Kunos et al., 1974; Kunos and Nickerson, 1976; 1977; Kunos, 1977). The interconversion hypothesis has also been used to explain the apparent alpha adrenoceptor blockade of the phenylephrine-induced increase in cyclic AMP production in the hypothyroid state versus the apparent beta adrenoceptor blockade in the euthyroid state (Kunos et al., 1976). These particular controversies in the literature formed the basis from which the specific aims of the present study were formulated.

The specific aims of the present study were as follows:

1. to investigate the hypothesis that hypothyroidism produces an alteration of cardiac responsiveness to adrenergic amines, by examining the mechanical effects of specific alpha (methoxamine) and beta (isoproterenol) adrenergic agonists on isolated right and left rat atria;

2. to re-examine the proposal (Kunos et al., 1974; Kunos, 1977) that the alteration in responsiveness is due to an interconversion of alpha and beta adrenoceptors, by investigating the effects of alpha (phenoxybenzamine and phentolamine) and beta (propranolol) antagonists on the responses induced by specific alpha and beta adrenergic agonists and the dual adrenergic agonist phenylephrine;

3. further, to explore the possibility that the altered responsiveness is due
to the characteristic difference between the euthyroid and hypothyroid basal rates and forces of the right and left atria, respectively, by adjusting the basal levels of one thyroid state to that of the other, using ambient temperature, external calcium concentration, the muscarinic agonist carbachol, or the \textit{beta} adrenoceptor agonist isoproterenol;

4. to determine if the increased sensitivity of cardiac \textit{alpha} adrenoceptors in the hypothyroid state (as reported by Nakashima \textit{et al.}, 1971; Nakashima and Hagino, 1972; Kunos, 1977) is associated with an increased activity of the adenylate cyclase system, utilizing the specific \textit{alpha} adrenergic agonist methoxamine; and

5. conversely, to determine, whether or not, the decreased sensitivity of \textit{beta} adrenoceptors in the hypothyroid state is associated with a decreased activity of the adenylate cyclase system, utilizing the specific \textit{beta} adrenoceptor agonist isoproterenol.
MATERIALS AND METHODS

1. Animal Preparation

All experiments were carried out using 200-300 gram male Wistar rats, some of which were made hypothyroid by thyroidectomy (Canadian Breeding Farms, Montreal). Experiments involving hypothyroid rats were carried out 10-12 weeks post-surgery. To verify the extent of hypothyroidism, blood samples were analyzed for thyroxine levels utilizing a specific radioimmunoassay kit (Abbott Laboratories). Euthyroid and hypothyroid rats were found to have blood thyroxine levels of 59 ± 2 (n=25) and 21 ± 1 (n=110) ng/ml, respectively. Animals with thyroxine values > 30 ng/ml were rejected as being hypothyroid.

II. Thyroxine Concentration Determination

The T4 RIA (PEG) assay kit (Abbott Laboratories) provides a method for the quantitative measurement of the total circulating serum thyroxine. Since thyroxine is present in serum in two forms, free and protein bound, the test system is designed to measure total thyroxine, that is the sum of the free and protein bound forms. Radioimmunoassay (RIA) has been applied in various forms to the determination of total serum thyroxine (Chopra, 1972; Mitsuma et al., 1972; Beckers et al., 1973; Dunn and Foster, 1973). The major differences among them are in the methods of extraction of thyroxine from serum binding proteins and in techniques of separating the free or unbound thyroxine from the thyroxine bound to the antibody, during the incubation period. The Abbott Laboratories' test system for thyroxine utilizes 8-anilino-1-naphthalene-sulfonic acid to extract thyroxine (T4) from its binding proteins and to inhibit further binding of T4 by these proteins. The T4-antibody complex is precipitated by the addition of polyethylene glycol (PEG) and separated from the free thyroxine (unbound) by centrifugation.

Thyroxine, whether radiolabelled or not, will bind to a specific anti-
serum. Both radiolabelled thyroxine ($^{125}$I-T4) and unlabelled thyroxine will bind to a limited amount of antiserum in proportion to the concentration of each type of thyroxine in the mixture. When thyroxine from a serum sample or a standard solution is equilibrated with $^{125}$I-T4 and thyroxine antiserum, the amount of radiolabelled thyroxine that is bound to the antiserum will be inversely proportional to the amount of nonradioactive thyroxine present. After a two hour incubation period, an aliquot of PEG solution was added to the sample which was then centrifuged at room temperature (at 1000 xg) for 10 minutes. The antiserum complex was separated from unbound thyroxine and the radioactivity of $^{125}$I-T4 bound in the complex was measured over a two minute time period using a Searle Gamma Counter (Series #1185). The standard concentrations of the thyroxine added were plotted versus percent bound of labelled T4, and the standard curve so prepared was then used to determine the amount of thyroxine in the unbound samples.

III. Tissue Preparation

All rats were sacrificed by a blow to the head and their hearts were quickly excised and placed in Chenoweth-Koelle physiological salt solution (Chenoweth and Koelle, 1946) of the following composition: NaCl, 120.0 mM; Glucose, 10.0 mM; KCl, 5.6 mM; CaCl$_2$, 2.2 mM; MgCl$_2$, 2.1 mM (Table 1). The working Chenoweth-Koelle physiological salt solution was made by diluting 200 ml of the stock solution (Table 1) to 2000 ml with distilled water. The pH of the working solution was adjusted to a pH of 7.4 with NaHCO$_3$ (3.22 g/2000 ml; 19.2 mM). Right and left atria were isolated from the rat hearts and suspended under 1 gram tension in isolated tissue baths containing Chenoweth-Koelle solution, aerated with 95% $O_2$ - 5% $CO_2$ at 37°C. Right atria were allowed to beat spontaneously, while left atria were stimulated at a frequency
Table 1

CHENOWETH-KOELLE SOLUTION COMPOSITION

<table>
<thead>
<tr>
<th>Buffer Constituent</th>
<th>M.W.</th>
<th>Amount in Stock (g/2L)</th>
<th>Working Solution Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>58.5</td>
<td>140.0</td>
<td>120.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>180.2</td>
<td>36.0</td>
<td>10.0</td>
</tr>
<tr>
<td>KCl</td>
<td>74.5</td>
<td>8.4</td>
<td>5.6</td>
</tr>
<tr>
<td>CaCl$_2$.2H$_2$O</td>
<td>147.0</td>
<td>6.4</td>
<td>2.2</td>
</tr>
<tr>
<td>MgCl$_2$.6H$_2$O</td>
<td>203.0</td>
<td>8.6</td>
<td>2.1</td>
</tr>
</tbody>
</table>
of 1 Hz, with pulses of 5 msec duration at a voltage slightly above threshold using a Grass Stimulator (Model #SC6).

IV. Apparatus

The developed rate and tension of the right and left atria, respectively, were measured using Grass force-displacement transducers (Model #FT 03C) connected to the muscle by a threaded-clip and recorded by means of a Grass Polygraph (Model 79D).

V. Chemical Agents

1. Agonists:

   The atria were exposed to the agonists 1-phenylephrine hydrochloride (Sigma Chemical Co.), methoxamine hydrochloride (Burroughs, Wellcome and Co.), \( d, l \)-isoproterenol hydrochloride (Sigma Chemical Co.) and carbamylcholine chloride (carbachol - Sigma Chemical Co.).

2. Antagonists:

   The atria were exposed to the antagonists phentolamine hydrochloride (Ciba Pharmaceutical Co.), phenoxybenzamine hydrochloride (Smith, Kline and French), \( d, l \)-propranolol hydrochloride (Sigma Chemical Co.) and labetalol hydrochloride (Allen and Hanburys).

VI. Experimental Procedure

   The atria were allowed to equilibrate for a period of 30 minutes before the effects of drugs, alone or in the presence of antagonists, were tested. Blocking agents were added to the bath 30 minutes prior to the agonist administration. Cumulative dose-response curves were carried out on each preparation in a dose range of agonist from \( 10^{-10} \) to \( 10^{-3} \) M. From these data, the potency and effectiveness of the agonists in each thyroid state were determined.
Only one dose-response curve was obtained from each atrium.

VII. Cyclic Nucleotide Determination

The right and left atria were exposed to a single concentration of the agonist methoxamine (10^{-5} M on right atria and 10^{-4} M on left atria) or isoproterenol (10^{-6} M on both right and left atria). At various times after addition of either drug, the baths were rapidly lowered and the atria were frozen by means of tongs previously chilled in a mixture of dry ice and 2-methylbutane. The tension and rate achieved by the left and right atria, respectively, at the time points were recorded. The frozen samples were stored at -80°C until analyzed for cyclic nucleotide concentration.

1. Tissue Extraction:

Adenosine 3',5'-monophosphate (cyclic AMP) was analyzed by first extracting with 5% trichloroacetic acid (TCA) according to a method described by Gilman (1970). Frozen tissue samples (20-50 mg) were rapidly homogenized in 2 ml of 5% TCA. The samples were then centrifuged at 4°C using a bench top centrifuge (Damon/lec, IEC HN-SII centrifuge) at 2000 rpm for 20-30 minutes, which removed all the insoluble proteins. The pellet thus formed was then discarded. The remaining supernatant was extracted 6 times, each time using a 10 ml aliquot of water-saturated ethyl-ether, to remove the TCA which might otherwise interfere with the cyclic nucleotide assay. An appropriate control, containing 5% TCA in the absence of a tissue sample, was used to establish that the ether extraction procedure had removed all of the TCA. To remove any residual ether, the carrying gas, nitrogen, was bubbled into the sample extract for 10-15 minutes. An aliquot of the sample extract was then removed for cyclic AMP determination.
2. **Cyclic AMP Determination:**

Cyclic AMP determinations were carried out on right and left atrial tissue sample extracts of both euthyroid and hypothyroid hearts using a cyclic AMP assay kit from Amersham (Code TRK 432). The kit is a commercial adaptation of a competitive protein binding assay for plasma cyclic AMP, previously described by Latner and Prudhoe (1973). The method is based on the competition between unlabelled cyclic AMP and a fixed quantity of radiolabelled cyclic AMP ($^3$H-cyclic AMP) for binding to a protein which has high specificity and affinity for cyclic AMP (Gilman, 1970). The amount of labelled cyclic AMP-protein complex formed is inversely related to the amount of unlabelled cyclic AMP present in the assay. The concentration of cyclic AMP in the unknown sample was determined by comparison with a linear standard curve. After a two hour incubation period, the protein-bound cyclic AMP was separated from the unbound nucleotide by adsorption of the free nucleotide on charcoal, followed by centrifugation (at 2000 rpm) for 20-25 minutes at 4°C. This method was initially described by Brown et al. (1971). The supernatant was then transferred to liquid scintillation vials containing 5 ml of ACS cocktail. The samples were counted for a 2 minute time period in a Mark III Scintillation Counter.

A straight line was computed from the standard curve data collected, by the method of least squares on a Wang 600 programable calculator. The amount of cyclic AMP in each unknown sample was determined from the standard curve and corrected for dilution and original tissue weight. The cyclic AMP results are expressed as picomoles of cyclic AMP per milligram of wet weight of tissue.

**VIII. Data Presentation and Statistical Analysis:**

The data are presented graphically as cumulative dose-response curves of the agonists alone and in the presence of the antagonists in terms of a change
in tension development (grams) and atrial rate (beats/minute). In some cases the data are also presented in absolute terms. Each graph point is plotted as a mean value representing a sample size of 4-14 animals. The data were statistically analyzed using the Student's t-test and a level of $p < 0.05$ was considered to be statistically significant. The geometric mean $ED_{50}$ values were calculated as described by Fleming et al. (1972).
I. The Action of Phenylephrine on Atrial Rate and Contractile Force

1. Euthyroid State:

The effect of the dual adrenergic agonist phenylephrine on rate and force of euthyroid atria is summarized in Figure 1a. Phenylephrine increased both rate and force of euthyroid atria. The basal developed rate and force were 276 ± 17 beats/minute and 1.04 ± 0.16 grams, respectively. The maximum change in rate was 110 ± 12 beats/minute and the maximum change in tension development was 0.56 ± 0.09 grams. Phenoxybenzamine significantly reduced the contractile effect of 10^-5M phenylephrine, but otherwise had no effect on the inotropic response to all other doses of phenylephrine. Phenoxybenzamine pretreatment also did not reduce the chronotropic response to phenylephrine. On the contrary, phenoxybenzamine actually appeared to cause an increase in the maximum chronotropic response to phenylephrine. Phenoxybenzamine did not significantly decrease the potency of phenylephrine as shown by the negative log ED$_{50}$ of phenylephrine with respect to both rate and force development (Figures 2a and 2b). Propranolol had no effect on the inotropic response to phenylephrine, but significantly reduced the chronotropic response. Propranolol had no effect on the potency of phenylephrine as shown by the negative log ED$_{50}$ of phenylephrine in terms of rate and force development (Figures 2a and 2b). A combination of propranolol and phenoxybenzamine pretreatment completely abolished both the inotropic and chronotropic responses to phenylephrine.

In summary, phenylephrine increased both rate and force in the euthyroid state. Phenoxybenzamine (10^-6M) had little effect on the inotropic and chronotropic responses. Propranolol (10^-6M) depressed the maximum chronotropic response, and had little effect on the inotropic response. However, both antagon-
Figure 1

The effect of phenylephrine on the rate of spontaneously beating right atria and the tension of electrically driven (1 Hz) left atria isolated from euthyroid (ET - A) and hypothyroid (HT - B) rats. Cumulative dose-response curves to phenylephrine were obtained for controls; in the presence of 1 μM phenoxybenzamine (POB) or propranolol (PROP); and in the presence of both adrenoceptor antagonists phenoxybenzamine and propranolol (POB + PROP). The number in brackets indicates the sample size for each curve. Δ Rate (beats/min) - change in atrial rate in beats/minute. Δ T (g) - change in tension development in grams.
Figure 2  The effect of phenoxybenzamine (POB - 1 uM) and propranolol (PROP - 1 uM) on the chronotropic (A) and the inotropic (B) potencies (geometric mean ED$_{50}$ values) of phenylephrine in euthyroid and hypothyroid atria. The number in brackets is the sample size. * - significant difference from control, p<0.05. + - significant difference due to thyroidectomy, p<0.05.
ATRIAL RATE
PHENYLEPHRINE

EUTHYROID

HYPOTHYROID

A

Log MEAN ED_{50}

-6.5

-6.0

-5.5

-5.0

-4.5

-4.0

Control PROP

POB

Control PROP

POB

(9)

(10)

(5)

(13)

(4)

(8)

B

FORCE DEVELOPMENT
PHENYLEPHRINE

EUTHYROID

HYPOTHYROID

Log MEAN ED_{50}

-6.5

-6.0

-5.5

-5.0

-4.5

-4.0

Control PROP

POB

Control PROP

POB

(9)

(14)

(5)

(13)

(4)

(9)
ists together completely abolished the inotropic and chronotropic responses to phenylephrine.

2. Hypothyroid State:

The effect of the dual adrenergic agonist phenylephrine on rate and force of hypothyroid atria is summarized in Figure 1b. Phenylephrine increased both the rate and force of hypothyroid atria. These effects were similar to those on euthyroid atria (Figure 1a). The basal developed rate and force were $235 \pm 7$ beats/minute and $1.01 \pm 0.10$ grams, respectively. The maximum change in rate was $121 \pm 16$ beats/minute and $0.53 \pm 0.11$ grams was the maximum change in tension development. Hypothyroidism significantly increased the potency of phenylephrine with respect to both rate and force development (Figures 2a and 2b) as compared to the euthyroid state. Phenoxybenzamine significantly depressed both the inotropic and chronotropic responses to phenylephrine in hypothyroid atria. These effects were not observed in the euthyroid state (Figure 1a). Phenoxybenzamine pretreatment significantly decreased the potency of phenylephrine as shown by the decreased negative log ED$_{50}$ of phenylephrine with respect to both rate and force development (Figures 2a and 2b). Propranolol pretreatment had no effect on the inotropic response to phenylephrine, but significantly reduced the chronotropic response. Propranolol had no effect on the potency of phenylephrine in terms of force development (Figure 2b), but caused a significant increase in the chronotropic potency of phenylephrine as shown by the negative log ED$_{50}$ in Figure 2a. A combination of propranolol and phenoxybenzamine pretreatment completely abolished both the inotropic and chronotropic responses to phenylephrine in the hypothyroid state. This phenomenon was also observed in the euthyroid state.

In summary, phenylephrine increased both the rate and force in the hypo-
thyroid state to the same extent as was observed in the euthyroid state. Phen­oxybenzamine \(10^{-6}\)M depressed the inotropic and chronotropic responses of phenylephrine on hypothyroid atria. Propranolol \(10^{-6}\)M had little effect on the inotropic response to phenylephrine, and depressed the maximum chronotropic response to the same extent as was observed in the euthyroid state. As in the euthyroid state, both blocking agents together completely abolished both the inotropic and chronotropic responses to phenylephrine. The hypothyroid state increased the potency of phenylephrine.

II. The Effect of Increasing the Concentration of Phenoxybenzamine and Propranolol on the Responses to Phenylephrine in the Euthyroid State

To further investigate the blockade of the phenylephrine responses, increased concentrations of the antagonists were utilized in order to determine if an adequate concentration of antagonist had been used.

The effect of increasing the concentration of phenoxybenzamine and propranolol on the responses to phenylephrine in euthyroid right and left rat atria is summarized in Figure 3. Phenylephrine increased both rate and force of euthyroid right and left atria, respectively, as was similarly shown in Figure 1a. The maximum changes in developed rate and tension were \(158 \pm 23\) beats/minute and \(0.71 \pm 0.11\) grams, respectively. Phenoxybenzamine \(2\times10^{-6}\)M did not affect the rate or force responses to phenylephrine to any greater extent than that observed in Figure 1a. At \(5\times10^{-6}\)M, phenoxybenzamine also did not affect either the rate or force responses to phenylephrine. Propranolol \(2\times10^{-6}\)M almost completely abolished the rate response of the right atria to phenyleph­rine as was observed in Figure 1a with \(10^{-6}\)M propranolol, but did not affect the force response of left atria. At \(5\times10^{-6}\)M, propranolol significantly shift­ed the phenylephrine dose-response curve on force of contraction of the left
Figure 3

The effect of increasing the concentration of phenoxybenzamine and propranolol on the responses to phenylephrine in the euthyroid state. The effect of phenylephrine on the rate of spontaneously beating right atria and the tension of electrically driven (1 Hz) left atria. Cumulative dose-response curves to phenylephrine were obtained for controls; in the presence of $2 \times 10^{-6}$M and $5 \times 10^{-6}$M propranolol (PROP) and phenoxybenzamine (POB). The number in brackets is the sample size for each curve. 

$\Delta$ Rate (beats/min) - change in atrial rate in beats/minute. $\Delta$ Tension (g) - change in tension development in grams.
atria to the right.

In summary, increasing the concentration of phenoxybenzamine (5x10^{-6} M) had little effect on the inotropic and chronotropic responses to phenylephrine in euthyroid atria. Propranolol (2x10^{-6} M) depressed the chronotropic response, as was observed previously, and had little effect on the inotropic response to phenylephrine. Only 5x10^{-6} M propranolol shifted the inotropic dose-response curve of phenylephrine to the right.

III. The Effect of Labetalol on the Responses to Phenylephrine

To further investigate the phenomenon of a complete blockade of the phenylephrine responses, the combined alpha and beta adrenoceptor blocking agent labetalol was utilized in order to determine if the phenylephrine-induced responses were the result of combined alpha and beta adrenoceptor stimulation.

The effect of labetalol on the rate and force responses to phenylephrine in euthyroid right and left atria, respectively, is summarized in Figure 4. Phenylephrine increased both force and rate with a maximum change in force and rate of 0.71 ± 0.11 grams and 142 ± 19 beats/minute, respectively. Labetalol (10^{-6} M) affected both the rate and force responses to phenylephrine. Labetalol (10^{-6} M) significantly shifted the phenylephrine dose-response curves for force and rate to the right, whereas in the presence of 10^{-6} M propranolol, no shift was observed for either the rate or force responses to phenylephrine (Figure 1a). The potency of phenylephrine in the presence of labetalol (10^{-6} M) was significantly decreased in terms of both force and rate. The ED_{50} value, expressed as the negative log, for the phenylephrine control was 5.41 ± 0.08 in left atria and 5.29 ± 0.12 in right atria, while in the presence of 10^{-6} M labetalol the negative log ED_{50} value was 5.10 ± 0.03 in left atria and 4.62 ± 0.13 in right atria. The rate maximum of phenylephrine in the presence of labetalol was
The effect of labetalol on the rate and force responses to phenylephrine in spontaneously beating right and electrically driven (1 Hz) left atria isolated from euthyroid rats. Cumulative dose-response curves to the dual alpha and beta adrenergic agonist phenylephrine were obtained for controls and in the presence of $10^{-6}$M and $5\times10^{-6}$M labetalol (LAB) - an alpha and beta adrenoceptor antagonist. The number in brackets is the sample size for each curve. $\Delta$ Rate (b.p.m.) - change in atrial rate in beats/minute. $\Delta$ Tension (g) - change in tension development in grams.
EUTHYROID STATE

\[
\Delta \text{RATE (b.p.m.)} \quad \Delta \text{TENSION (g)}
\]

- \( \log [\text{PHENYLEPHRINE (M)}] \)

1. CONTROL

\( (5) \)

\( + \text{LAB (10}^{-6} \text{M)} \)

\( (5) \)

\( (5 \times 10^{-6} \text{M}) \)

\( (6) \)

\( (5 \times 10^{-6} \text{M}) \)

\( (5) \)

\( (5) \)

\( (6) \)
significantly depressed over the dose range of $10^{-10}$ to $10^{-4}$ M phenylephrine, as was also observed in Figure 1a in the presence of $10^{-6}$ M propranolol. Increasing the concentration of labetalol to $5 \times 10^{-6}$ M almost completely abolished the rate and force responses to phenylephrine (Figure 4), in a similar manner as was observed in Figure 1a with a combination of phenoxybenzamine and propranolol.

In summary, labetalol ($5 \times 10^{-6}$ M) almost completely abolished both the inotropic and chronotropic responses to phenylephrine in euthyroid atria. At a lower concentration, labetalol ($10^{-6}$ M) shifted the inotropic and chronotropic dose-response curves of phenylephrine to the right.

IV. The Action of Methoxamine on Atrial Rate and Contractile Force

1. Euthyroid State:

The effect of the specific alpha adrenergic agonist methoxamine on rate and force of euthyroid atria is summarized in Figure 5a. The basal developed rate and force were $293 \pm 12$ beats/minute and $1.05 \pm 0.12$ grams, respectively. Methoxamine ($10^{-9}$ to $10^{-5}$ M) had no effect on the rate of euthyroid right atria, but a dose greater than $10^{-5}$ M methoxamine significantly decreased rate of euthyroid right atria. However, methoxamine did effect a positive inotropic response in euthyroid left atria, producing a maximum change in tension of $0.27 \pm 0.03$ grams. Phenoxybenzamine did not prevent the negative chronotropic response, but completely abolished the inotropic response to methoxamine. Thus, no ED$_{50}$ could be obtained for methoxamine alone on right atrial rate, or in the presence of phenoxybenzamine on contractile force of euthyroid left atria. Propranolol pretreatment had no effect on the inotropic response to methoxamine, but blocked the negative chronotropic response to $10^{-4}$ M methoxamine. Propranolol had no effect on the potency of methoxamine in terms of force development.
Figure 5  The effect of methoxamine on the rate of spontaneously beating right atria and electrically driven (1 Hz) left atria isolated from euthyroid (ET-A) and hypothyroid (HT-B) rats. Cumulative dose-response curves for the alpha adrenergic agonist methoxamine were obtained for controls; in the presence of 1 uM propranolol (PROP) or 1 uM phenoxybenzamine (POB). The number in brackets is the sample size for each curve. Δ Rate (beats/min) - change in atrial rate in beats/minute.  
Δ T (g) - change in tension development in grams.
In summary, methoxamine increased force slightly in euthyroid atria, with no effect on rate. At doses greater than $10^{-5}$M, methoxamine produced a pronounced negative chronotropic effect. Propranolol ($10^{-6}$M) had no effect on the tension or rate responses to methoxamine. Phenoxybenzamine ($10^{-6}$M) did not affect the negative chronotropic effect but did completely abolish the inotropic response to methoxamine.

2. Hypothyroid State:

The effect of the specific alpha adrenergic agonist methoxamine on rate and force of hypothyroid atria is summarized in Figure 5b. The basal developed rate and force were $147 \pm 13$ beats/minute and $0.51 \pm 0.07$ grams, respectively. The maximum change in rate was $60 \pm 11$ beats/minute and the maximum change in tension development was $0.87 \pm 0.10$ grams. Hypothyroidism significantly increased the maximum responses to methoxamine as compared to the euthyroid state. In the hypothyroid state methoxamine was significantly more effective, relative to the maximum response of isoproterenol, in terms of force development and atrial rate as shown by a significantly greater percent change in the hypothyroid state as compared to the euthyroid state (Tables II and III). As in the euthyroid atria, phenoxybenzamine abolished the chronotropic response to methoxamine. Hypothyroidism significantly increased the potency of methoxamine with respect to force development (Figure 6) as compared to the euthyroid state. Propranolol pretreatment had no effect on the inotropic and chronotropic responses, and did not affect the potency of methoxamine.

In summary, methoxamine increased both the force and rate to a greater extent in hypothyroid atria as compared to euthyroid atria. The hypothyroid state increased the potency of methoxamine. Propranolol ($10^{-6}$M) had no effect on rate or tension, whereas phenoxybenzamine ($10^{-6}$M) completely abolished both
Table II

EFFECT OF HYPOTHYROIDISM ON THE MAXIMUM INOTROPIC RESPONSES OF RAT LEFT ATRIA TO ISOPROTERENOL AND METHOXAMINE

<table>
<thead>
<tr>
<th>Thyroid State</th>
<th>Agonist</th>
<th>Force Development (g)</th>
<th>Relative Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>Maximal</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>1.36 ± 0.11</td>
<td>2.23 ± 0.15</td>
</tr>
<tr>
<td>(10^{-5}M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euthyroid</td>
<td>Methoxamine</td>
<td>1.05 ± 0.12</td>
<td>1.31 ± 0.14</td>
</tr>
<tr>
<td>(10^{-4}M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>0.76 ± 0.08</td>
<td>1.48 ± 0.13</td>
</tr>
<tr>
<td>(10^{-4}M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>Methoxamine</td>
<td>0.51 ± 0.07</td>
<td>1.36 ± 0.11</td>
</tr>
<tr>
<td>(10^{-5}M)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Doses of each agonist required to maximally increase tension (mean ± SEM) above basal levels are shown in parentheses.

b The relative response of methoxamine versus isoproterenol is the mean ± SEM of the change in tension induced by methoxamine for each atrium, divided by the mean maximum change in tension induced by isoproterenol, times 100.

c p<0.001, compared to euthyroid atria.

d Tension development in grams.
Table III

EFFECT OF HYPOTHYROIDISM ON THE MAXIMUM
CHRONOTROPIC RESPONSES OF RAT RIGHT ATRIA TO
ISOPROTERENOL AND METHOXAMINE

<table>
<thead>
<tr>
<th>Thyroid State</th>
<th>Agonist</th>
<th>Atrial Rate (b.p.m.)</th>
<th>Relative Response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>Maximal</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>Isoproterenol</td>
<td>287 ± 11</td>
<td>423 ± 14</td>
</tr>
<tr>
<td></td>
<td>(10^{-6} M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methoxamine</td>
<td>293 ± 12</td>
<td>294 ± 11</td>
</tr>
<tr>
<td></td>
<td>(10^{-5} M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>Isoproterenol</td>
<td>196 ± 11</td>
<td>372 ± 17</td>
</tr>
<tr>
<td></td>
<td>(10^{-4} M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methoxamine</td>
<td>147 ± 13</td>
<td>213 ± 11</td>
</tr>
<tr>
<td></td>
<td>(10^{-4} M)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Doses of each agonist required to maximally increase rate (mean ± SEM) above basal levels are shown in parentheses.

^b The relative response of methoxamine versus isoproterenol is the mean ± SEM of the change in rate induced by methoxamine for each atrium, divided by the mean maximum change in atrial rate induced by isoproterenol, times 100.

^c p<0.001, compared to euthyroid atria.

^d Rate development in beats/minute.
Figure 6

The effect of hypothyroidism on the inotropic and chronotropic potencies of isoproterenol (ISO) and methoxamine (METH). Histograms of geometric mean ED50 values of the agonists in euthyroid (ET) and hypothyroid (HT) atria. The number in brackets is the sample size. * - and + - significantly different from control, p<0.05.
the inotropic and chronotropic responses to methoxamine.

V. The Action of Isoproterenol on Atrial Rate and Contractile Force

1. Euthyroid State:

The effect of the specific beta adrenergic agonist isoproterenol on rate and contractile force of euthyroid atria is summarized in Figures 7 and 8. Isoproterenol increased both the rate and force of euthyroid atria. The basal developed rate and force were $287 \pm 11$ beats/minute and $1.36 \pm 0.11$ grams, respectively. The maximum change in tension development was $0.67 \pm 0.11$ grams and the maximum change in atrial rate was $136 \pm 15$ beats/minute. Phentolamine pretreatment did not affect the inotropic or chronotropic responses to isoproterenol, and had no effect on the potency of isoproterenol. Propranolol pretreatment affected the response to isoproterenol with respect to both tension and rate, shifting the dose-response curves to the right. Propranolol thus, significantly decreased the negative log ED$_{50}$ of isoproterenol with respect to both rate and force development (Table IV).

In summary, isoproterenol increased both the force and rate of euthyroid atria. Propranolol ($10^{-6}$M) shifted the dose-response curves of isoproterenol to the right in terms of both force and rate, while phentolamine ($10^{-6}$M) had little effect on the responses.

2. Hypothyroid State:

The effect of the specific beta adrenoceptor agonist isoproterenol on rate and contractile force of hypothyroid atria is summarized in Figures 9 and 10. Isoproterenol increased both rate and force of hypothyroid atria. The basal developed rate and force were $196 \pm 11$ beats/minute and $0.76 \pm 0.08$ grams, respectively. The responses of the hypothyroid atria to isoproterenol were similar to the responses of euthyroid atria. The maximum change in tension was
Figure 7 The effect of isoproterenol on the rate of spontaneously beating right atria isolated from euthyroid (ET) rats. Cumulative dose-response curves for the beta adrenoceptor agonist isoproterenol were obtained for controls (n=8); in the presence of 1 µM propranolol (PROP - n=5) or phentolamine (PHENT - n=6). Δ Rate (beats/min) - change in atrial rate in beats/minute.
Figure 8

The effect of isoproterenol on the tension of electrically driven (1 Hz) left atria isolated from euthyroid (ET) rats. Cumulative dose-response curves for the beta adrenoceptor agonist isoproterenol were obtained for controls (n=10); in the presence of 1 uM propranolol (PROP - n=5) or phentolamine (PHENT - n=6). Δ T (g) - change in tension development in grams.
The effect of isoproterenol on the rate of spontaneously beating right atria isolated from hypothyroid (HT) rats. Cumulative dose-response curves for the beta adrenoceptor agonist isoproterenol were obtained for controls (n=10); in the presence of 1 uM propranolol (PROP - n=4) or phentolamine (PHENT - n=10). \( \Delta \) Rate (beats/min) - change in atrial rate in beats/minute.
HT

Δ RATE (beats/min.)

- \text{LOG} \left[ \text{isoproterenol (M)} \right]

- CONTROL
- PHENT
- PROP
The effect of isoproterenol on the tension of electrically driven (1 Hz) left atria isolated from hypothyroid (HT) rats. Cumulative dose-response curves for the beta adrenoceptor agonist isoproterenol were obtained for controls (n=10); in the presence of 1 μM propranolol (PROP - n=4) or phentolamine (PHENT - n=8). Δ.T (g) - change in tension development in grams.
\( \Delta T (\theta) \)

\(-\log [\text{isoproterenol (M)}] \)

- PROP
- Control
- PHENT

HT
0.73 ± 0.11 grams and the maximum change in rate was 176 ± 13 beats/minute. Phentolamine pretreatment did not affect the inotropic or chronotropic responses to isoproterenol, and had no effect on the potency of isoproterenol. Propranolol pretreatment, however, affected the responses to isoproterenol with respect to both the inotropic and chronotropic responses, shifting each dose-response curve for isoproterenol to the right. Propranolol significantly decreased the negative log ED$_{50}$ of isoproterenol and therefore its potency in terms of both rate and force (Table IV). Hypothyroidism did significantly decrease the potency of isoproterenol with respect to both rate and force development as compared to the euthyroid state (Figure 6 and Table IV).

In summary, isoproterenol increased force and rate to the same extent as was observed in the euthyroid state. The hypothyroid state decreased the potency of isoproterenol. Propranolol (10^-6 M) shifted the dose-response curves of isoproterenol to the right in terms of both force and rate. Phentolamine (10^-6 M) had little effect on the responses.

VI. Effectiveness of Isoproterenol and Methoxamine in the Euthyroid and Hypothyroid States

The effect of the hypothyroid state on the responses to methoxamine and isoproterenol is further illustrated by the changes in the effectiveness of the agonists on euthyroid and hypothyroid right and left atria as shown in Figure 11. Methoxamine produced a significantly greater maximum change in rate and contractile force of hypothyroid atria when compared to euthyroid atria. The effectiveness of isoproterenol, however, was not significantly different in the two groups. The basal developed force and rate of the euthyroid state were 1.03 ± 0.08 grams and 300 ± 7 beats/minute, respectively. Those of the hypothyroid state were 0.78 ± 0.08 grams and 195 ± 11 beats/minute, respectively.
Table IV

NEGATIVE LOG MOLAR ED₅₀ VALUES (+ SEM) OF ISOPROTERENOL ON LEFT AND RIGHT ATRIA FROM EUTHYROID AND HYPOTHYROID RATS

<table>
<thead>
<tr>
<th>Thyroid State</th>
<th>Left Atria</th>
<th>Right Atria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n) Control</td>
<td>Propranolol</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>(10) 8.05</td>
<td>+ 0.08</td>
</tr>
<tr>
<td></td>
<td>(5) 6.02ᵃ</td>
<td>+ 0.11</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>(10) 6.61ᵇ</td>
<td>+ 0.15ᵇ</td>
</tr>
<tr>
<td></td>
<td>(4) 4.85ᵃ</td>
<td>+ 0.19ᵃ</td>
</tr>
</tbody>
</table>

ᵃ Significant difference from the corresponding control ED₅₀ value, p<0.05.
ᵇ Significant difference from the euthyroid control, p<0.05.
ᶜ The concentration of propranolol (10⁻⁶ M).
Figure 11

The effect of hypothyroid state on the inotropic and chronotropic effectiveness of isoproterenol (ISO) and methoxamine (METH). Histograms of the maximum change in force development and atrial rate induced by the agonists in the euthyroid (ET) and hypothyroid (HT) states. The number in the brackets is the sample size. The dose at which the maximum response occurred is indicated below each agonist. + - significant difference from control, p < 0.05.
Hypothyroidism significantly decreased the basal rate and force as compared to the euthyroid state.

VII. Presentation of the Data as Percent Change in Tension and Rate of the Isoproterenol Maximum Change in Tension and Rate for the Agonists Phenylephrine and Methoxamine

1. Phenylephrine:

The effect of thyroid state on the rate and force responses to phenylephrine, presented as percent change in rate and force is summarized in Figure 12. Phenylephrine appeared to be less effective, relative to the isoproterenol maximum, in the hypothyroid state (60%) as compared to the euthyroid state (100%) in terms of tension development. In terms of atrial rate, phenylephrine was equi-effective, relative to the isoproterenol maximum, in both the euthyroid and hypothyroid state (70%).

2. Methoxamine:

The effect of thyroid state on the rate and force responses to methoxamine, presented as percent change in rate and tension of the isoproterenol maximum change in rate and tension is summarized in Figure 13. Methoxamine appeared to be more effective, relative to the isoproterenol maximum, in the hypothyroid state (115%) in terms of tension development as compared to the euthyroid state (20%). Methoxamine also appeared to be more effective, relative to the isoproterenol maximum, in the hypothyroid state (30%) in terms of rate development as compared to the euthyroid state (nil%). These data suggest that alpha adrenoceptor stimulation in the hypothyroid state can result in a response as effective as that produced by beta adrenoceptor stimulation.
Figure 12  

The effect of phenylephrine on the rate of spontaneously beating right atria and the tension of electrically driven (1 Hz) left atria isolated from euthyroid (EuT) and hypothyroid (HypoT) rats. The data are presented as percent change in tension (and rate) relative to the isoproterenol (ISO) maximum change in tension (and rate). The number in brackets is the sample size of each curve.
The effect of methoxamine on the rate of spontaneously beating right atria and the tension of electrically driven (1 Hz) left atria isolated from euthyroid (EuT) and hypothyroid (HypoT) rats. The data are presented as percent change in tension (and rate) relative to the isoproterenol (ISO) maximum change in tension (and rate). The number in brackets is the sample size.
% Δ TENSION OF ISO MAX. Δ TENSION

% Δ RATE OF ISO MAX. Δ RATE

- LOG [METHOXAMINE (M)]
VIII. Presentation of the Data for Isoproterenol in Absolute Terms

1. Absolute Tension:

The effect of thyroid state on the force response to isoproterenol is summarized in Figure 14, in terms of absolute force development, to better characterize the effect on the response to the specific beta agonist. The absolute basal force developed was $1.36 \pm 0.11$ grams in the euthyroid state as compared to a significantly lower basal level of $0.76 \pm 0.08$ grams in the hypothyroid state. The maximum change in tension development to isoproterenol was not affected as a result of the significantly different basal levels between the two thyroid states as was observed in Figure 11.

2. Absolute Rate:

The effect of thyroid state on the rate response to isoproterenol is summarized in Figure 15, in terms of absolute rate development, to also better characterize the effect on the response to the specific beta agonist. The absolute basal rate developed was $287 \pm 11$ beats/minute in the euthyroid state as compared to a significantly lower basal level of $195 \pm 11$ beats/minute in the hypothyroid state. The maximum change in rate to isoproterenol was not affected as a result of the significantly different basal levels between the two thyroid states as was observed in Figure 11.

IX. Presentation of the Data for Methoxamine in Absolute Terms

1. Absolute Tension:

The effect of thyroid state on the force response to methoxamine is summarized in Figure 16, in terms of absolute force development to characterize the effect on the response to the specific alpha agonist. The absolute basal force developed in the euthyroid state was $1.05 \pm 0.12$ grams as compared to a significantly lower basal level of $0.51 \pm 0.07$ grams in the hypothyroid state.
Figure 14  The effect of isoproterenol on absolute tension development in grams (g) in euthyroid (EUT) and hypothyroid (HYPOT) electrically driven (1 Hz) rat left atria. Cumulative dose-response curves for the beta adrenoceptor agonist isoproterenol were obtained in both the euthyroid (n=12) and hypothyroid (n=11) states. CONT - the basal level of the left atria in the two thyroid states.
The effect of isoproterenol on absolute rate development (beats/minute) in euthyroid (EUT) and hypothyroid (HYPOT) spontaneously beating rat right atria. Cumulative dose-response curves for the beta adrenoceptor agonist isoproterenol were obtained in both the euthyroid (n=9) and hypothyroid (n=10) states. CONT - the basal level of the right atria in the two thyroid states.
ABSOLUTE ATRIAL RATE (beats/min.)

-LOG [ISOPROTERENOL (M)]

CONT 10 9 8 7 6 5 4 3

EUT

HYPOT
Figure 16  The effect of methoxamine on absolute tension development (grams) in euthyroid (n=10) and hypothyroid (n=12) electrically driven (1 Hz) rat left atria. The dose at which the maximum response occurred is indicated below the agonist methoxamine (METH). * - and + - indicates significant difference from control, p<0.05. CONT - the basal level of the left atria in the two thyroid states.
ABSOLUTE

EUTHYROID

1.5 -

CONT METH (10^-4 M)

TENSION

HYPOTHYROID

+ *

CONT METH (10^-4 M)
Concomitantly, the maximum change in tension developed to methoxamine was affected as a result of the significantly different basal levels between the two thyroid states as was observed in Figure 11.

2. Absolute Rate:

The effect of thyroid state on the rate response to methoxamine is summarized in Figure 17 in terms of absolute rate development to also characterize the effect on the response to the specific alpha agonist. The absolute basal rate developed was 293 ± 12 beats/minute in the euthyroid state as compared to a significantly lower basal level of 147 ± 13 beats/minute in the hypothyroid state. The maximum change in rate to the alpha adrenergic agonist methoxamine was affected as a result of the significantly different basal levels between the two thyroid states as was observed in Figure 11.

X. The Effect of Baseline on the Responses to Methoxamine in Euthyroid and Hypothyroid States

To investigate the effect of altering basal force and rate on the responses produced by the alpha adrenoceptor agonist methoxamine, the muscarinic agonist carbachol was utilized to lower the basal rate and force of euthyroid atria while the beta adrenergic agonist isoproterenol was used to raise the basal rate and force of hypothyroid atria.

1. Effect on Tension Development:

The effect of baseline on absolute tension development of methoxamine in the euthyroid and hypothyroid states is summarized in Figure 18. Methoxamine increased force to a greater extent in the hypothyroid state as compared to the euthyroid state. Lowering the basal developed force of euthyroid left atria with carbachol (10^-6 M) to the hypothyroid control level produced an increase in tension development to methoxamine similar to that observed in hypothyroid con-
The effect of methoxamine on absolute rate development (beats/minute) in euthyroid \((n=10)\) and hypothyroid \((n=11)\) spontaneously beating rat right atria. The dose at which the maximum response occurred is indicated below the agonist methoxamine \((\text{METH})\). 

\* - and \* - indicates significant difference from control, \(p<0.05\). CONT - the basal level of the right atria in the two thyroid states.
Figure 18

The effect of baseline on the force response to methoxamine in the euthyroid and hypothyroid electrically driven left atria. The basal developed force of euthyroid left atria was lowered to that of the hypothyroid basal level by the administration of carbachol (CARB - 1 uM)- at arrow head. The basal developed force of hypothyroid left atria was raised to that of the euthyroid basal level by the administration of isoproterenol (ISO - 1 uM)- at arrow head. Cumulative dose-response curves for the alpha adrenoceptor agonist methoxamine were then obtained. C - the basal level of the left atria in the two thyroid states before drug treatment. Tension in grams (g). The number in brackets is the sample size for each curve. The euthyroid basal levels were reduced to a level of about 50% of their control which closely approximated the basal level of the hypothyroid state by titrating carbachol into the bath - indicated by the dotted line before the C(arrow head).
trols. In addition, the potency of methoxamine was increased. The euthyroid control negative log $ED_{50}$ value was $4.78 \pm 0.17$, while the negative log $ED_{50}$ value for the carbachol-pretreated dose-response curve to methoxamine was $5.14 \pm 0.03$, showing a significantly decreased $ED_{50}$ over the control. Conversely, increasing the basal developed force of hypothyroid left atria with isoproterenol ($10^{-6}$M) to the euthyroid control level produced a small increase in tension development to methoxamine similar to that observed in the euthyroid controls, and significantly decreased the potency of methoxamine as compared to the hypothyroid control. The negative log $ED_{50}$ value of the hypothyroid control was $5.40 \pm 0.06$, while the negative log $ED_{50}$ value of the isoproterenol-pretreated dose-response curve to methoxamine was $4.62 \pm 0.13$, showing a significantly decreased $ED_{50}$ over the control.

2. Effect on Rate Development:

The effect of baseline on absolute rate development of methoxamine in the euthyroid and hypothyroid states is summarized in Figure 19. Methoxamine increased rate in hypothyroid right atria. However, methoxamine did not have any affect on rate of euthyroid right atria up to $10^{-5}$M and then a pronounced negative chronotropic response occurred. Lowering the basal developed rate of euthyroid right atria with carbachol ($10^{-6}$M) to the hypothyroid control level resulted in an increased rate response to methoxamine, similar to that observed in the hypothyroid controls. In addition, the potency of methoxamine was increased over the control. The negative log $ED_{50}$ value was $6.11 \pm 0.05$, while no $ED_{50}$ value could be obtained in the euthyroid control. Conversely, increasing the basal developed rate of hypothyroid right atria with isoproterenol ($10^{-7}$M) to the euthyroid control level produced a response to methoxamine similar to that seen in the euthyroid controls. There was no affect on the rate up to
Figure 19

The effect of baseline on the rate response to methoxamine in the euthyroid and hypothyroid right atria. The basal developed rate of euthyroid right atria was lowered to that of the hypothyroid basal level by the administration of carbachol (CARB - 1 uM) - at arrow head. The basal developed rate of hypothyroid right atria was raised to that of the euthyroid basal level by the administration of isoproterenol (ISO - 10^{-7} M) - at arrow head. Cumulative dose-response curves for the alpha adrenoceptor agonist methoxamine were then obtained. C - the basal level of the right atria in the two thyroid states before drug treatment. The number in brackets is the sample size for each curve. The euthyroid basal levels were reduced to a level of about 50% of their control which closely approximated the basal level of the hypothyroid state by titrating carbachol into the bath - indicated by the dotted line before the C (arrow head).
ABSOLUTE RATE
(beat/min)

EUTHYROID

HYPOTHYROID

CONT (5)

+ CARB (10^{-6} M)

+ ISO (10^{-7} M)

-LOG [METHOXAMINE (M)]
methoxamine, and then a pronounced negative chronotropic response occurred. Thus no $ED_{50}$ value could be obtained.

In summary, adjusting the baseline of one thyroid state to that of the other affected the responses produced by the alpha adrenoceptor agonist methoxamine, such that the rate and force responses observed in hypothyroid atria to methoxamine could be reproduced in carbachol-pretreated euthyroid atria.

XI. Action of Calcium on Atrial Rate and Contractile Force in Euthyroid and Hypothyroid States

To investigate the effect of external calcium concentration on rate and force in euthyroid and hypothyroid states, right and left atria were exposed to an increasing concentration of calcium.

1. Tension Development:

The effect of increasing calcium concentration on the absolute tension development of electrically stimulated left atria of both euthyroid and hypothyroid rats is summarized in Figure 20. Calcium increased force in both euthyroid and hypothyroid left atria. The basal developed force at 2.2 mM calcium was $1.30 \pm 0.08$ grams in the euthyroid state as compared to a significantly lower basal level in the hypothyroid state of $0.56 \pm 0.08$ grams. In the presence of 0.5 mM calcium the absolute tension development of hypothyroid and euthyroid left atria was significantly decreased over that observed in the presence of 2.2 mM calcium. A maximum force development occurred at 6 mM calcium in both the euthyroid and hypothyroid states. The $ED_{50}$ values between the two calcium dose-response curves were found to be significantly different. The negative log $ED_{50}$ values in the euthyroid and hypothyroid state were $2.80 \pm 0.03$ and $2.53 \pm 0.07$, respectively. Calcium effected a maximum change in tension development of $1.41 \pm 0.25$ grams in the euthyroid state and $0.97 \pm 0.12$
Figure 20  The effect of increasing the calcium concentration on absolute tension development in grams (g) in electrically driven (1 Hz) left atria in both euthyroid (n=6) and hypothyroid (n=5) rats. The force developed at the calcium concentration of 2.2 mM is indicated at the C-arrow head.
EUTHYROID

HYPOTHYROID

[CALCIUM (mM)]

ABSORLUTE TENSION (g)

C 2.2 1 2 3 4 5 6 7 8 9 10
grams in the hypothyroid state. The tension changes between the two thyroid states were not significantly different. The maximum tension development in the hypothyroid state (1.09 ± 0.09 grams) was significantly different from the maximum tension development in the euthyroid state (1.71 ± 0.19 grams).

2. Rate Development:

The effect of increasing calcium concentration on the absolute rate development of spontaneously beating right atria of both euthyroid and hypothyroid rats is summarized in Figure 21. Calcium increased rate in both euthyroid and hypothyroid right atria. The basal developed rate at 2.2 mM calcium was 288 ± 12 beats/minute in the euthyroid state as compared to a significantly lower basal level in the hypothyroid state of 183 ± 13 beats/minute. In the presence of 0.5 mM calcium the absolute rate of euthyroid right atria was significantly decreased over that observed in the presence of 2.2 mM calcium. A maximum rate development occurred at 8 mM calcium in both the euthyroid and hypothyroid states. The ED50 values between the two calcium dose-response curves were found to be significantly different. The negative log ED50 values in the euthyroid and hypothyroid states were 2.55 ± 0.024 and 2.46 ± 0.033, respectively. Calcium effected a maximum change in rate development of 156 ± 20 beats/minute in the euthyroid state and 139 ± 20 beats/minute in the hypothyroid state. The difference between the two thyroid states was not significantly different. The maximum rate development in the hypothyroid state (283 ± 13 beats/minute) was significantly different from the maximum rate development in the euthyroid state (384 ± 9 beats/minute).

In summary, calcium effected an increase in both rate and force of euthyroid and hypothyroid atria. There was no difference between the two thyroid states on the maximum change in rate or force developed. However, calcium did
Figure 21 The effect of increasing the calcium concentration on absolute rate development in beats/minute (b.p.m.) in spontaneously beating right atria in both euthyroid (n=5) and hypothyroid (n=5) rats. The atrial rate development at a calcium concentration of 2.2 mM is indicated at the C-arrow head.
produce a greater maximum rate and force in the euthyroid state as compared to the hypothyroid state.

XII. The Effect of Altering Baseline with Calcium Concentration on the Responses to Methoxamine in Euthyroid and Hypothyroid States

To further investigate the effect of altering basal force and rate on the responses produced by the alpha adrenergic agonist methoxamine, the external concentration of calcium was decreased to lower the basal rate and force of euthyroid atria, and increased to raise the basal rate and force of hypothyroid atria. The required calcium concentrations were calculated from the previous calcium dose-response curves.

1. Effect on Tension Development:

The effect of altering baseline on tension development of methoxamine in the euthyroid and hypothyroid states is summarized in Figure 22. Methoxamine increased force to a greater extent in the hypothyroid state as compared to the euthyroid state. Lowering the basal developed force of euthyroid left atria to the hypothyroid control level by reducing the calcium concentration to 0.5 mM produced a trend toward an increase in tension. Although the relative change in tension was not significant, the trend was similar to that seen in the hypothyroid controls. However, the potency of methoxamine was not significantly increased when the baseline was altered in this way (Table V). Conversely, increasing the basal developed force of hypothyroid left atria to that of the euthyroid basal level by increasing the Ca$^{2+}$ concentration to 7.0 mM, produced a trend toward a change in tension similar to that seen in the euthyroid controls. In addition, the potency of methoxamine was significantly decreased as compared to the hypothyroid state, altering the baseline this way (Table V).

2. Effect on Rate Development:

The effect of altering baseline on absolute rate development of methoxamine
The effect of baseline on the force response in grams (g) to methoxamine in the euthyroid and hypothyroid electrically driven left atria. The basal developed force of euthyroid left atria (B) was lowered to that of the hypothyroid basal level (at 2.2 mM calcium concentration) by reducing the calcium to 0.5 mM (A). The basal developed force of hypothyroid left atria (B) was raised to that of the euthyroid basal level (at 2.2 mM calcium concentration) by increasing the calcium concentration to 7.0 mM (A). Cumulative dose-response curves for the alpha adrenoceptor agonist methoxamine were then obtained. The number in brackets is the sample size for each curve.
EUTHYROID

HYPOTHYROID

ABSOLUTE TENSION (g)

-LOG METHOXAMINE (M)

[2.2 mM Ca] (4)

[0.5 mM Ca] (4)

[7.0 mM Ca] (5)

[2.2 mM Ca] (4)
Table V

NEGATIVE LOG MOLAR ED$_{50}$ VALUES (+ SEM) OF METHOXAMINE ON LEFT AND RIGHT ATRIA FROM EUTHYROID AND HYPOTHYROID RATS BEFORE AND AFTER DIFFERENT PRETREATMENTS

<table>
<thead>
<tr>
<th>Thyroid State</th>
<th>Group</th>
<th>(n) Left Atria$^1$</th>
<th>(n) Right Atria$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>Control</td>
<td>(5) 4.78 ± 0.17</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>+ Low Calcium</td>
<td>(4) 5.12 ± 0.14</td>
<td>(4) 6.35 ± 0.28$^b$</td>
</tr>
<tr>
<td></td>
<td>(0.5 mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>Control</td>
<td>(12) 5.40 ± 0.06</td>
<td>(11) 6.33 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>+ High Calcium</td>
<td>(5) 4.75 ± 0.35$^c$</td>
<td>(5) 5.77 ± 0.13$^c$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ ED$_{50}$ could not be obtained as there was no change in atrial rate over the cumulative dose range.

$^b$ Significant difference from euthyroid control, p<0.05.

$^c$ Significant difference from hypothyroid control, p<0.05.

$^1$ left atria measured force effects.

$^2$ right atria measured rate effects.
in the euthyroid and hypothyroid states is summarized in Figure 23. Methoxamine increased rate in hypothyroid atria (at 2.2 mM Ca$^{2+}$) as was observed in Figure 5b. However, methoxamine did not have any effect on rate of euthyroid right atria (at 2.2 mM Ca$^{2+}$) up to $10^{-5}$M, then a pronounced negative chronotropic effect as was observed in Figure 5a. Lowering the basal developed rate of euthyroid right atria to the hypothyroid control level by reducing the calcium concentration to 0.5 mM, resulted in a trend toward an increase in rate development to methoxamine. Although the relative change in rate was not significant, the trend was similar to that seen in the hypothyroid controls. The potency of methoxamine was also increased (Table IV), when the baseline was altered this way. Conversely, increasing the basal developed rate of hypothyroid right atria to that of the euthyroid basal level by increasing the calcium concentration to 8.0 mM, did not produce a noticeable decrease in the rate response to methoxamine. These atria appeared to require more methoxamine in order to produce the same response as was observed in hypothyroid right atria with 2.2 mM calcium; that is, that the dose-response curve for methoxamine in the presence of 8.0 mM calcium had shifted to the right as compared to the dose-response curve for methoxamine, in the presence of 2.2 mM calcium, in the hypothyroid state. The potency of methoxamine was significantly decreased in the presence of 8.0 mM calcium (Table V) as compared to the hypothyroid control, altering the baseline in this fashion.

In summary, adjusting the baseline of one thyroid state to that of the other, utilizing calcium concentrations of different molarities, affected the responses produced by the alpha adrenergic agonist methoxamine. Decreasing the external calcium concentration superfusing euthyroid atria resulted in an apparent increase in the tension and rate responses to methoxamine in a trend similar to that observed in the hypothyroid state. The potency of methoxamine was also increased in terms of both tension and rate, compared to the euthyroid control.
The effect of baseline on the rate response to methoxamine in the euthyroid and hypothyroid spontaneously beating right atria. The basal developed rate of euthyroid right atria (B) was lowered to that of the hypothyroid basal level (at 2.2 mM calcium concentration) by reducing the calcium concentration to 0.5 mM (A). The basal developed rate of hypothyroid right atria (B) was raised to that of the euthyroid basal level (at 2.2 mM calcium concentration) by increasing the calcium concentration to 8.0 mM (A). Cumulative dose-response curves for the alpha adrenoceptor agonist methoxamine were then obtained. Atrial rate in beats/minute (b.p.m.). The number in brackets is the sample size for each curve.
EUTHYROID

[2.2 mM Ca] (4)

[0.5 mM Ca] (4)

HYPOTHYROID

[8.0 mM Ca] (5)

[2.2 mM Ca] (4)

ABSOLUTE RATE (b.p.m.)

-LOG METHOXAMINE (M)
Increasing the external calcium concentration superfusing hypothyroid atria resulted in an apparent decrease in tension similar to that observed in the euthyroid state, with no change on rate. The potency of methoxamine was, however, decreased in terms of both tension and rate, compared to the hypothyroid control.

XIII. The Effect of Altering the Temperature of the Bathing Medium on the Responses to Methoxamine

To further investigate the altering of basal rate and force levels on the responses to the alpha adrenergic agonist methoxamine, the temperature of the bathing medium was decreased on euthyroid right atria to lower the basal rate to the hypothyroid basal level, and on hypothyroid left atria to raise the basal tension to the euthyroid basal level.

1. Euthyroid State - Rate Development:

The effect of altering the temperature of the bathing medium on the rate response to methoxamine is summarized in Figure 24. The basal rate of euthyroid right atria at 37°C was lowered to the level of the hypothyroid controls by decreasing the temperature of the bath from 37°C to 25°C. The response to methoxamine was found to be similar at both temperatures. There was no response up to 10^-5 M methoxamine, and then a pronounced negative chronotropic response was observed at both the 37°C and at 25°C bathing-medium temperatures.

2. Hypothyroid State - Tension Development:

The effect of altering temperature of the bathing medium on the tension response to methoxamine is summarized in Figure 25. The basal level of hypothyroid left atria at 37°C was raised to that of the euthyroid basal level by decreasing the temperature of the bath from 37°C to 25°C. The maximum change in force to methoxamine was found to be considerably decreased in hypothyroid atria at 25°C as compared to hypothyroid atria at 37°C. The maximum change in
Figure 24

The effect of changing temperature on the rate response to methoxamine in euthyroid right atria (beats/minute). The basal rate of euthyroid atria (C37°C; n=5) was lowered to the hypothyroid basal level by reducing the temperature of the bath from 37°C to 25°C (C25°C; n=4). Cumulative dose-response curves for the alpha adrenoceptor agonist methoxamine were obtained at 37°C and at 25°C.
EUTHYROID STATE

ABSOLUTE RATE

(beat/min.)

-LOG [METHOXAMINE (M)]

37°C

25°C
Figure 25

The effect of changing temperature on the tension response to methoxamine in hypothyroid left atria (grams). The basal tension of hypothyroid left atria (C37; n=5) was raised to a basal level above that observed in hypothyroid left atria at 37°C, by reducing the temperature of the bath from 37°C to 25°C (C25; n=7). Cumulative dose-response curves for the alpha adrenoceptor agonist methoxamine were obtained at both 37°C and at 25°C.
HYPOTHYROID STATE

ABSOLUTE TENSION (g)

-LOG [METHOXAMINE (M)]

25°C

37°C

C 9 8 7 6 5 4
tension development at 37°C was 0.87 ± 0.10 grams, whereas at 25°C it was 0.34 ± 0.03 grams. There was a significant shift in the dose-response curve at 25°C to the right indicating a decrease in the potency of methoxamine compared to the hypothyroid control at 37°C. The negative log $ED_{50}$ value for methoxamine at 37°C was 5.40 ± 0.06, and at 25°C it was 5.15 ± 0.11. The $ED_{50}$ values for the dose-response curves at the two temperatures were significantly different.

In summary, lowering the temperature of the bathing medium had no affect on the chronotropic response to methoxamine on euthyroid atria. However, the maximum inotropic response to methoxamine on hypothyroid atria was decreased at a lower temperature. The potency of methoxamine was also decreased to the magnitude of the euthyroid $ED_{50}$ value.

XIV. Effect of Altering the Temperature of the Bathing Medium on the Responses to Isoproterenol in the Euthyroid State

The basal rate of euthyroid atria was lowered to the hypothyroid basal level, by decreasing the temperature of the bathing medium to determine the effect on the response to the beta adrenoceptor agonist isoproterenol.

The effect of changing the temperature of the bathing medium on the rate response to isoproterenol in the euthyroid state is summarized in Figure 26. The absolute basal rate was lowered, compared to the control at 37°C (287 ± 11 beats/minute), when the temperature was decreased to 25°C (120 ± 5 beats/minute). There was no difference between the maximum change in rate to isoproterenol at 37°C (136 ± 15 beats/minute) and at 25°C (117 ± 16 beats/minute). The dose-response curve to isoproterenol at 25°C was shifted significantly to the left compared to the dose-response curve to isoproterenol at 37°C. The negative log $ED_{50}$ at 37°C was 8.16 ± 0.11, and at 25°C it was 8.92 ± 0.09, showing a significantly increased potency of isoproterenol at 25°C.
The effect of changing temperature on the rate response to isoproterenol in euthyroid right atria. The basal rate of euthyroid right atria (C37°C; n=8) was lowered to the hypothyroid basal level, by reducing the temperature of the bath from 37°C to 25°C (C25°C; n=4). Cumulative dose-response curves for the beta adrenoceptor agonist isoproterenol were then obtained at both 37°C and at 25°C.
EUTHYROID STATE
ABSOLUTE RATE
(beat/min.)

37°C

25°C

-LOG [ISOPROTERENOL (M)]
In summary, decreasing the euthyroid basal rate with temperature (25°C) shifted the dose-response curve to the left and therefore increased the potency of isoproterenol.

XV. Effect of Carbachol Pretreatment on the Responsiveness of Euthyroid Atria to Methoxamine and Isoproterenol

It is apparent from the studies previously described that alterations in baseline values of rate and force affect the responsiveness of euthyroid and hypothyroid atria to adrenergic amines.

To further study and illustrate the relationship between the hypothyroid atria and the carbachol-pretreated euthyroid atria, euthyroid right and left atria were pretreated with the muscarinic agonist carbachol and then exposed to the alpha adrenoceptor agonist methoxamine and the beta adrenoceptor agonist isoproterenol.

1. Methoxamine:

(i) Rate Response

The effect of carbachol pretreatment on the responsiveness of euthyroid right atria to methoxamine is summarized in Figure 27. Methoxamine effected a negative chronotropic response in the euthyroid state and a positive chronotropic response in the hypothyroid state as was observed in Figures 5a and 5b. After pretreatment with \(10^{-6} M\) carbachol, euthyroid right atria responded to methoxamine as was observed in the hypothyroid controls. The maximum change in rate was \(60 \pm 11\) beats/minute in hypothyroid atria and \(76 \pm 10\) beats/minute in euthyroid atria pretreated with carbachol \(10^{-6} M\). The negative log ED\(_{50}\) value for the carbachol-pretreated dose-response curve to methoxamine was \(6.11 \pm 0.05\) which was significantly increased over the control state.
Figure 27  The effect of carbachol pretreatment on the responsiveness of euthyroid atria to methoxamine; comparing the effect to euthyroid (EuT) and hypothyroid (HypoT) controls. Cumulative dose-response curves for the alpha adrenoceptor agonist methoxamine were obtained from euthyroid right atria (n=5) alone and in the presence of 1 uM carbachol (CARB - n=5); and from hypothyroid controls (n=11).
ABSOLUTE RATE (beats/min.)

-LOG [METHOXAMINE (M)]

○ EuT
■ EuT + CARB
□ HypoT (10^-6 M)
(ii) **Tension Response**

The effect of carbachol pretreatment on the responsiveness of euthyroid left atria to methoxamine is summarized in Figure 28. Methoxamine effected a slight positive inotropic response in the euthyroid state and a greater positive inotropic response in the hypothyroid state, as was observed in Figures 5a and 5b. After pretreatment with carbachol \((10^{-6} M)\) the euthyroid left atria responded to methoxamine as was observed in the hypothyroid controls. The maximum change in tension development was \(0.87 \pm 0.11\) grams in the hypothyroid atria and \(0.70 \pm 0.19\) grams in the euthyroid atria pretreated with \(10^{-6} M\) carbachol. The potency of methoxamine was significantly increased after pretreatment with \(10^{-6} M\) carbachol. The negative log ED\(_{50}\) value of the euthyroid control was \(4.78 \pm 0.17\), while the negative log ED\(_{50}\) value of the carbachol-pretreated left atria to methoxamine was \(5.14 \pm 0.03\), showing a significantly decreased ED\(_{50}\) over the control.

2. **Isoproterenol:**

(i) **Rate Response**

The effect of carbachol pretreatment on the responsiveness of euthyroid right atria to isoproterenol is summarized in Figure 29. Isoproterenol effected a positive chronotropic response in both the euthyroid and hypothyroid states, as was observed in Figures 7 and 9. After pretreatment with carbachol \((5 \times 10^{-7} M)\) the euthyroid right atria responded to isoproterenol as was observed in the hypothyroid controls. The maximum change in rate was \(176 \pm 13\) beats/minute in the hypothyroid atria and \(180 \pm 23\) beats/minute in the euthyroid atria pretreated with \(5 \times 10^{-7} M\) carbachol.

(ii) **Tension Development**

The effect of carbachol pretreatment on the responsiveness of euthyroid
Figure 28

The effect of carbachol pretreatment on the responsiveness of euthyroid atria to methoxamine; comparing the effects to euthyroid (EuT) and hypothyroid (HypoT) controls. Cumulative dose-response curves for the alpha adrenoceptor agonist methoxamine were obtained from euthyroid left atria (n=5) alone and in the presence of 1 uM carbachol (CARB - n=6); and from hypothyroid controls (n=12).
ABSOLUTE TENSION (g)

-LOG [METHOXAMINE (M)]

- EuT
- EuT + CARB (10^{-6}M)
- HypoT
The effect of carbachol pretreatment on the responsiveness of euthyroid atria to isoproterenol; comparing the effects to euthyroid (EuT) and hypothyroid (HypoT) controls. Cumulative dose-response curves for the beta adrenoceptor agonist isoproterenol were obtained from euthyroid right atria (n=8) alone and in the presence of $5 \times 10^{-7}$M carbachol (CARB - n=4); and from hypothyroid controls (n=10).
ABSOLUTE RATE
(letts/min.)

- LOG [ISOPROTERENOL (M)]

- □ EuT
- ★ EuT + CARB $\left(5 \times 10^{-7}M\right)$
- ☆ HypoT
left atria to isoproterenol is summarized in Figure 30. Isoproterenol effected a positive inotropic response in both euthyroid and hypothyroid states as was observed in Figures 8 and 10. After pretreatment with carbachol (10^-6 M) the euthyroid left atria responded to isoproterenol as was observed in the hypothyroid controls. The maximum change in tension development was 0.73 ± 0.11 grams in the hypothyroid atria and 0.6 ± 0.19 grams in the euthyroid atria pretreated with 10^-6 M carbachol.

In summary, the responses produced in carbachol-pretreated euthyroid atria by methoxamine and isoproterenol were almost identical to the responses produced in hypothyroid atria. The ED50 values of the dose-response curves in the presence of carbachol were almost identical in magnitude to the ED50 values obtained in the hypothyroid control atria.

XVI. Effect of Carbachol Pretreatment, Low Calcium and Low Temperature on the Responses to Phenylephrine

It is apparent from the studies previously described that pretreatment of euthyroid atria with carbachol almost completely duplicated the responses observed in the hypothyroid state to both methoxamine and isoproterenol.

To further investigate the effect of baseline alteration on the responses to phenylephrine, the basal rate and force of euthyroid atria were decreased to the hypothyroid basal level using the muscarinic agonist carbachol, external calcium concentration or temperature. The euthyroid developed tensions and rates were reduced and stabilized as closely as possible to the mean value of the hypothyroid control atria in one of three ways: 1) carbachol - a stock solution of carbachol was added in 20 ul increments to the tissue bath until the desired tensions or rates were achieved; 2) low calcium - the buffer superfusing the left atria was switched from 2.2 mM to 0.5 mM calcium, and fine adjustments
Figure 30 The effect of carbachol pretreatment on the responsiveness of euthyroid atria to isoproterenol; comparing the effects to euthyroid (EuT) and hypothyroid (HypoT) controls. Cumulative dose-response curves for the beta adrenoceptor agonist isoproterenol were obtained from euthyroid left atria (n=10) alone and in the presence of 1 μM carbachol (CARB - n=5); and from hypothyroid controls (n=10).
ABSOLUTE TENSION (g)

-LOG [ISOPROTERENOL (M)]

- LOG [ISOPROTERENOL (M)]
in tension to the desired level were then achieved by 20 μl additions of calcium chloride solution; or 3) low temperature - the buffer superfusing the right atria was cooled from 37°C to that temperature which achieved the desired basal rate.

1. **Tension Development:**

The effect of carbachol pretreatment and low calcium concentration on tension development to phenylephrine in left atria in grams (g) from euthyroid rats as well as the action of phenylephrine on hypothyroid left atria, is summarized in Figure 31a. Phenylephrine increased the force of both euthyroid and hypothyroid left atria. The basal developed force was $1.24 \pm 0.12$ grams in the euthyroid atria and $0.85 \pm 0.07$ grams in the hypothyroid atria. The basal tensions developed between the two thyroid states were significantly different from each other. The maximum change in force was $0.91 \pm 0.09$ grams in the euthyroid state and $0.55 \pm 0.11$ grams in the hypothyroid state. The changes in force developed between the two thyroid states were significantly different from each other. Carbachol pretreatment (0.4 μM) shifted the dose-response curve of phenylephrine to the left and therefore increased the potency of phenylephrine as compared to the euthyroid state (Table VI) in a similar fashion as that observed in the hypothyroid state. However, the same maximum response was achieved as was observed in the euthyroid state. In the presence of low calcium buffer (1.1 mM) phenylephrine produced the same maximum response as in the euthyroid state even though the initial basal developed tension was significantly different from the euthyroid state. The ED$_{50}$ was not different from the euthyroid control (Table VI).

2. **Rate Development:**

The effect of carbachol pretreatment and low temperature on atrial rate
The effect of phenylephrine on tension development of left atria (A) in grams (g) and on atrial rate of right atria (B) in beats/minute (b/min) from euthyroid and hypothyroid rats. The experimental subgroups consist of the following: euthyroid controls (o—o; n=9, left; n=10, right); hypothyroid controls (●—●; n=13, left and right); euthyroid pretreated with carbachol (○—○; n=4, left, 0.4 uM; n=4, right, 0.3 uM); euthyroid in low calcium buffer (△—△; n=4, left, 1.1 mM); euthyroid at low temperature (▽—▽; n=4, right, 33°C). Cumulative dose-response curves were obtained for the alpha and beta adrenergic agonist phenylephrine. C-indicates the control basal level before the addition of the agonist.
A - Left Atrial Tension (g)  B - Right Atrial Rate (b/min)

[PHENYLEPHRINE] (-log M)
### Table VI

NEGATIVE LOG MOLAR ED$_{50}$ VALUES (+ SEM) OF PHENYLEPHRINE ON LEFT AND RIGHT ATRIA FROM EUTHYROID AND HYPOTHYROID RATS

<table>
<thead>
<tr>
<th>Thyroid State</th>
<th>Group</th>
<th>(n)</th>
<th>Left Atria</th>
<th>(n)</th>
<th>Right Atria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>Control</td>
<td>(9)</td>
<td>5.16 ± 0.03</td>
<td>(9)</td>
<td>5.14 ± 0.08</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>Control</td>
<td>(13)</td>
<td>5.59 ± 0.14$^{a}$</td>
<td>(13)</td>
<td>5.70 ± 0.17$^{a}$</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>+ Carbachol$^{b}$</td>
<td>(4)</td>
<td>5.42 ± 0.09$^{a}$</td>
<td>(4)</td>
<td>5.57 ± 0.07$^{a}$</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>+ Low Calcium (1.1 mM)</td>
<td>(4)</td>
<td>5.18 ± 0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>+ Low Temperature (33°C)</td>
<td>-</td>
<td>-</td>
<td>(4)</td>
<td>5.21 ± 0.10</td>
</tr>
</tbody>
</table>

$^{a}$ Significant difference from euthyroid controls, $p<0.05$.

$^{b}$ Concentration of carbachol: 0.4 uM, left atria; 0.3 uM, right atria.
development to phenylephrine in right atria in beats/minute (b/min) from euthyroid rats as well as the action of phenylephrine on hypothyroid right atria is summarized in Figure 31b. Phenylephrine increased the rate of both euthyroid and hypothyroid atria. The basal developed rate was $307 \pm 6$ beats/minute in euthyroid atria and $221 \pm 8$ beats/minute in hypothyroid atria. The basal developed rates between the two thyroid states were significantly different from each other. The maximum change in rate was $101 \pm 15$ beats/minute in the euthyroid state and $146 \pm 21$ beats/minute in the hypothyroid state. The changes in rate development between the two thyroid states were not significantly different from each other. Carbachol pretreatment (0.3 uM) shifted the dose-response curve of phenylephrine to the left and therefore increased the potency of phenylephrine (Table VI), as compared to the euthyroid state in as similar fashion as that observed in the hypothyroid state. The same maximum response was achieved as compared to the hypothyroid state. At low temperature ($33^\circ C$) phenylephrine produced the same change in rate development as was observed in the euthyroid state. The $ED_{50}$ value was unaffected compared to the euthyroid control (Table VI).

In summary, carbachol pretreatment on euthyroid left atria increased the maximum tension development and increased the potency of phenylephrine. Altering the external calcium concentration had no effect on the phenylephrine tension response. However, carbachol pretreatment on euthyroid right atria duplicated the phenylephrine responses of the hypothyroid state and increased the potency of phenylephrine. At a lower temperature ($33^\circ C$) the maximum change in rate was similar to the euthyroid control with no change in the $ED_{50}$.
The Effect of Carbachol Pretreatment, Low Calcium and Low Temperature on the Responses to Isoproterenol

To further investigate the effect of baseline alteration on the responses to isoproterenol, the basal developed rates and forces of euthyroid atria were reduced and stabilized as closely as possible to the mean value of the hypothyroid control atria in one of three ways: 1) carbachol - a stock solution of carbachol was added in 20 ul increments to the tissue bath until the desired tensions and rates were achieved; 2) low calcium - the buffer superfusing the left atria was switched from 2.2 mM to 0.5 mM calcium and fine adjustments in tension to the desired level were then achieved by 20 ul additions of calcium chloride solution; or 3) low temperature - the buffer superfusing the right atria was cooled from 37°C to that temperature which achieved the desired basal rate.

1. Tension Development:

The effect of carbachol pretreatment and low calcium concentration on tension development to isoproterenol in left atria in grams (g) from euthyroid rats as well as the action of isoproterenol on hypothyroid left atria is summarized in Figure 32a. Isoproterenol increased the force of both euthyroid and hypothyroid left atria. The basal developed force was $1.27 \pm 0.08$ grams in euthyroid atria and $0.76 \pm 0.08$ grams in hypothyroid atria. The basal developed tensions between the two thyroid states were significantly different from each other. The maximum change in force was $0.87 \pm 0.11$ grams in the euthyroid state and $0.73 \pm 0.11$ grams in the hypothyroid state. The changes in tension development between the two thyroid states were not significantly different from each other. Carbachol pretreatment (1.0 um) shifted the dose-response curve to isoproterenol to the right and therefore decreased the potency of isoproterenol.
Figure 32

The effect of isoproterenol on tension development of left atria (A) in grams (g) and on atrial rate of right atria (B) in beats/minute (b/min) from euthyroid and hypothyroid rats. The experimental subgroups consist of the following: euthyroid controls (○—○; n=10, left; n=8, right); hypothyroid controls (●—●; n=10, left and right); euthyroid pretreated with carbachol (□—□; n=5, left, 10^-6M; n=4, right, 5x10^-7M); euthyroid in low calcium buffer (▲—▲; n=4, left, 0.8 mM); euthyroid and low temperature (▼—▼; n=4, right, 32°C). Cumulative dose-response curves were obtained for the beta adrenoceptor agonist isoproterenol. C—indicates the control basal level before the addition of the agonist.
A - Left Atrial Tension (g)

B - Right Atrial Rate (b/min)

[ISOPROTERENOL] (-log M)
as compared to the euthyroid state in a similar fashion as that observed in the hypothyroid state. The same maximum response was achieved as was observed in the hypothyroid state. In the presence of calcium buffer (0.8 mM) isoproterenol produced the same maximum force response as in the euthyroid state even though the initial basal developed tension was significantly different from the euthyroid state. The ED$_{50}$ value was not different from the euthyroid control (Table VII).

2. Rate Development:

The effect of carbachol pretreatment and low temperature on atrial rate development to isoproterenol in right atria in beats/minute (b/min) from euthyroid rats as well as the action of isoproterenol on hypothyroid atria is summarized in Figure 32b. Isoproterenol increased the rate of both euthyroid and hypothyroid right atria. The basal developed rate was $287 \pm 11$ beats/minute in euthyroid atria and $196 \pm 11$ beats/minute in hypothyroid atria. The basal developed rates between the two thyroid states were significantly different from each other. The maximum change in rate was $136 \pm 15$ beats/minute in the euthyroid state and $176 \pm 13$ beats/minute in the hypothyroid state. The changes in rate development between the two thyroid groups were not significantly different from each other. Carbachol pretreatment (1.0 uM) shifted the dose-response curve to isoproterenol to the right and therefore decreased the potency of isoproterenol (Table VII) as compared to the euthyroid state in a similar fashion as that observed in the hypothyroid state. At low temperature (32°C) isoproterenol produced the same maximum rate development as was observed in the hypothyroid state. The ED$_{50}$ value was unaffected (Table VII) compared to the euthyroid control. However, at an even lower temperature (25°C - Figure 26) the ED$_{50}$ was significantly decreased indicating a supersensitivity at temperatures
### Table VII

NEGATIVE LOG MOLAR ED$_{50}$ VALUES (+ SEM) OF ISOPROTERENOL ON LEFT AND RIGHT ATRIA FROM EUTHYROID AND HYPOTHYROID RATS

<table>
<thead>
<tr>
<th>Thyroid State</th>
<th>Group</th>
<th>(n)</th>
<th>Left Atria</th>
<th>(n)</th>
<th>Right Atria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>Control</td>
<td>(10)</td>
<td>8.05 ± 0.08</td>
<td>(8)</td>
<td>8.16 ± 0.11</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>Control</td>
<td>(10)</td>
<td>6.61 ± 0.15$^a$</td>
<td>(10)</td>
<td>6.75 ± 0.12$^a$</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>+ Carbachol$^b$</td>
<td>(5)</td>
<td>6.66 ± 0.06$^a$</td>
<td>(4)</td>
<td>7.06 ± 0.20$^a$</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>+ Low Calcium</td>
<td>(4)</td>
<td>7.97 ± 0.11</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.8 mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euthyroid</td>
<td>+ Low Temperature</td>
<td>(4)</td>
<td>8.21 ± 0.17</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(32°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Significant difference from euthyroid controls, p<0.05.

$^b$ Concentration of carbachol: 1.0 uM, left atria; 0.5 uM, right atria.
lower than 30°C.

In summary, carbachol pretreatment of euthyroid atria duplicated the isoproterenol force and rate responses to the same extent as that observed in the hypothyroid state, and also decreased the potency of isoproterenol. Altering the external calcium concentration had no effect on the isoproterenol tension response. At a lower temperature (32°C) the same maximum change in rate was similar to the hypothyroid maximum change in rate, with no change in the ED\textsubscript{50}.

XVIII. The Effect of Carbachol Pretreatment, Low Calcium and Low Temperature on the Responses to Methoxamine

To further investigate the effect of baseline alteration on the responses to methoxamine, the basal developed tensions and rates of euthyroid atria were reduced and stabilized as closely as possible to the mean value of the hypothyroid control atria in one of three ways: 1) carbachol - a stock solution of carbachol was added in 20 ul increments to the tissue bath until the desired tensions or rates were achieved; 2) low calcium - the buffer superfusing the left atria was switched from 2.2 mM to 0.5 mM calcium, and fine adjustments in tension to the desired level were then achieved by 20 ul additions of calcium chloride solution; or 3) low temperature - the buffer superfusing the right atria was cooled from 37°C to that temperature which achieved the desired basal rate.

1. Tension Development:

The effect of carbachol pretreatment and low calcium on tension development to methoxamine in left atria in grams (g) from euthyroid rats as well as the action of methoxamine on hypothyroid left atria is summarized in Figure 33a. Methoxamine increased the force of both euthyroid and hypothyroid left atria to the same maximum. The basal developed force was 1.05 ± 0.12 grams in
Figure 33: The effect of methoxamine on tension of left atria (A) in grams (g) and on atrial rate (B) in beats/minute (b/min) from euthyroid and hypothyroid rats. The experimental subgroups consist of the following: euthyroid controls (○—○; n=5, left; n=10, right); hypothyroid controls (●—●; n=12, left; n=11, right); euthyroid pretreated with carbachol (□—□; n=4, left, 0.5 uM; n=5, right, 0.4 uM); euthyroid in low calcium buffer (▲—▲; n=4, left, 0.5 mM); euthyroid and low temperature (▼—▼; n=4, right, 25°C). Cumulative dose-response curves were obtained for the alpha adrenoceptor agonist methoxamine. 

C indicates the control basal level before the addition of the agonist.
A - Left Atrial Tension (g)

B - Right Atrial Rate (b/min)

[METHOXAMINE] (-log M)
euthyroid atria and 0.51 ± 0.07 grams in hypothyroid atria. The basal developed tensions between the two thyroid states were significantly different from each other. The maximum change in force was 0.27 ± 0.03 grams in the euthyroid state and 0.87 ± 0.10 grams in the hypothyroid state. The changes in tension development were significantly different between the two thyroid states. Carbachol pretreatment (0.5 μM) shifted the dose-response curve to methoxamine to the left and therefore increased the potency of methoxamine (Table VIII) as compared to the euthyroid state in a similar fashion as that observed in the hypothyroid state. The same maximum response was achieved as was observed in the hypothyroid state. In the presence of low calcium buffer (0.5 mM) methoxamine produced the same maximum change in force as was observed in the euthyroid state. The ED₅₀ value was not different from the euthyroid control (Table VIII).

2. Rate Development:

The effect of carbachol pretreatment and low temperature on atrial rate development to methoxamine in right atria in beats/minute (b/min) from euthyroid rats as well as the action of methoxamine on hypothyroid right atria is summarized in Figure 33b. Methoxamine had no effect on rate of euthyroid right atria. At doses greater than 10⁻⁵M a negative chronotropic response occurred. However, methoxamine increased rate of hypothyroid right atria. The basal developed rate was 293 ± 12 beats/minute in the euthyroid state and 147 ± 13 beats/minute in the hypothyroid state. The basal rates developed in the two thyroid states were significantly different from each other. The maximum change in rate was 60 ± 11 beats/minute in the hypothyroid atria which was significantly greater compared to the euthyroid atria (1 ± 8 beats/minute). Carbachol pretreatment (0.4 μM) of euthyroid atria shifted the dose-response curve to meth-
Table VIII

NEGATIVE LOG MOLAR ED<sub>50</sub> VALUES (+ SEM) OF METHOXAMINE ON LEFT AND RIGHT ATRIA FROM EUTHYROID AND HYPOTHYROID RATS

<table>
<thead>
<tr>
<th>Thyroid State</th>
<th>Group</th>
<th>(n)</th>
<th>Left Atria</th>
<th>(n)</th>
<th>Right Atria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>Control</td>
<td>(5)</td>
<td>4.78 + 0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>Control</td>
<td>(12)</td>
<td>5.49 + 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(11)</td>
<td>6.33 + 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>+ Carbachol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(4)</td>
<td>5.46 + 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(5)</td>
<td>6.11 + 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>+ Low Calcium</td>
<td>(4)</td>
<td>5.12 + 0.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant difference from euthyroid controls, p<0.05.

<sup>b</sup> Concentration of carbachol: 0.5 μM, left atria; 0.4 μM, right atria.

<sup>c</sup> No ED<sub>50</sub> could be obtained as there was no change in atrial rate over the cumulative dose range.
oxamine to the left and therefore increased the potency of methoxamine (Table VIII) as compared to the control euthyroid state. A similar shift was observed between the euthyroid and the hypothyroid state. At low temperature (25°C) methoxamine did not have an effect on rate. At doses greater than $10^{-5} M$, a negative chronotropic response occurred. No $ED_{50}$ could be obtained as was the case in the euthyroid state (Table VIII).

In summary, carbachol pretreatment of euthyroid atria duplicated the methoxamine force and rate responses observed in the hypothyroid state, and increased the potency of methoxamine. Altering the external calcium concentration did not significantly increase the potency of methoxamine. At a lower temperature (25°C) the rate response to methoxamine was similar to the euthyroid control.

XIX. Effect of Phenoxybenzamine on the Force and Rate Responses to Phenylephrine in Euthyroid, Hypothyroid and Carbachol-Pretreated Euthyroid Rat Atria

It is apparent from the studies previously described that carbachol pretreatment of euthyroid atria duplicated the responses to adrenergic agonists in hypothyroid atria.

To further investigate the effect of pretreating euthyroid atria with carbachol, the responses to phenylephrine in the presence of the alpha adrenergic antagonist phenoxybenzamine were studied in euthyroid, hypothyroid and carbachol-pretreated left and right atria.

The effect of phenoxybenzamine on the force and rate responses to phenylephrine in euthyroid, hypothyroid and carbachol-pretreated euthyroid rat atria is summarized in Table IX. The data are presented as negative log $ED_{50}$ values to illustrate the potency changes to phenylephrine under the three conditions.
Table IX
NEGATIVE LOG MOLAR ED\textsubscript{50} VALUES (+ SEM) OF PHENYLEPHRINE ON EUTHYROID, HYPOTHYROID AND CARBACHOL-PRETREATED EUTHYROID RAT LEFT AND RIGHT ATRIA

<table>
<thead>
<tr>
<th>Group</th>
<th>Left Atria</th>
<th></th>
<th></th>
<th>Right Atria</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Control</td>
<td>POB\textsuperscript{c}</td>
<td>Log Ratio</td>
<td>Control</td>
<td>POB\textsuperscript{c}</td>
</tr>
<tr>
<td>Euthyroid Control</td>
<td>(9)</td>
<td>5.16</td>
<td>+ 0.03</td>
<td>0.28</td>
<td>(10)</td>
<td>5.14</td>
</tr>
<tr>
<td>Hypothyroid Control</td>
<td>(13)</td>
<td>5.59\textsuperscript{b}</td>
<td>+ 0.14</td>
<td>0.78</td>
<td>(13)</td>
<td>5.70\textsuperscript{b}</td>
</tr>
<tr>
<td>Euthyroid + Carbachol\textsuperscript{e}</td>
<td>(4)</td>
<td>5.43\textsuperscript{b}</td>
<td>+ 0.09</td>
<td>0.47</td>
<td>(4)</td>
<td>5.57\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Significant difference from the corresponding control value, p<0.05.
\textsuperscript{b} Significant difference from the euthyroid control, p<0.05.
\textsuperscript{c} The concentration of phenoxybenzamine (POB - 10\textsuperscript{-6}M).
\textsuperscript{d} The log concentration ratio equals the mean ED\textsubscript{50} of phenylephrine alone minus the mean ED\textsubscript{50} of phenylephrine after pretreatment with the antagonist phenoxybenzamine, ED\textsubscript{50} as the negative log.
\textsuperscript{e} Concentration of carbachol: 0.3 uM, left atria; 0.8 uM, right atria.
and to illustrate the effectiveness of the \textit{alpha} adrenoceptor blocking agent in the hypothyroid and carbachol-pretreated euthyroid atria as compared to the euthyroid control. The euthyroid tension and rate responses to phenylephrine were unaffected by phenoxybenzamine (10^{-6} M) as indicated by the unchanged negative log ED_{50} values. However, in the hypothyroid state the potency of phenylephrine was increased compared to the euthyroid state, as indicated by the significantly different negative log ED_{50} values. There was an increased blocking effectiveness of phenoxybenzamine in the hypothyroid state as indicated by the apparent increase in the log dose ratio over the euthyroid control.

Similarly, in the carbachol-pretreated euthyroid atria, the potency of phenylephrine was increased compared to the euthyroid atria as indicated by the significantly different negative log ED_{50} values. An increased blocking effectiveness of phenoxybenzamine was also observed in the carbachol-pretreated atria, as indicated by the apparent increase in the log dose ratio over the euthyroid control.

In summary, phenoxybenzamine appeared more effective in blocking the rate and force responses to phenylephrine in the hypothyroid state and carbachol-pretreated euthyroid state as compared to the euthyroid control.

XX. \textbf{Effect of Propranolol on the Force and Rate Responses to Isoproterenol in Euthyroid, Hypothyroid and Carbachol-Pretreated Euthyroid Rat Atria}

To further investigate the effect of pretreating euthyroid atria with carbachol, the responses to isoproterenol in the presence of the \textit{beta} adrenergic antagonist propranolol were studied in euthyroid, hypothyroid and carbachol-pretreated euthyroid left and right atria.

The effect of propranolol on force and rate responses to isoproterenol in euthyroid, hypothyroid and carbachol-pretreated euthyroid rat atria is summar-
ized in Table X. The data are presented as negative log $ED_{50}$ values to illustrate the potency changes to isoproterenol under the three conditions and to illustrate the effectiveness of the beta adrenoceptor blocking agent in the hypothyroid and carbachol-pretreated euthyroid atria as compared to the euthyroid control. The euthyroid tension and rate responses to isoproterenol were affected by propranolol ($10^{-6}$ M) as indicated by the significantly different negative log $ED_{50}$ values. However, in the hypothyroid state the potency of isoproterenol was decreased compared to the euthyroid state, as indicated by the significantly different negative log $ED_{50}$ values. There was a decreased blocking effectiveness of propranolol in the hypothyroid state, as indicated by the apparent decrease in the log dose ratio over the euthyroid control. Similarly, in the carbachol-pretreated euthyroid atria, the potency of isoproterenol was decreased compared to the euthyroid state, as indicated by the significantly different negative log $ED_{50}$ values. A decreased blocking effectiveness of propranolol was also observed in the carbachol-pretreated atria, as indicated by the apparent decrease in the log dose ratio over the euthyroid control.

In summary, propranolol appeared less effective in blocking the rate and force responses to isoproterenol in the hypothyroid state and carbachol-pretreated euthyroid state as compared to the euthyroid control.

XXI. The Influence of Methoxamine and Isoproterenol on Cyclic AMP Production

A time response study was carried out to study the effect of methoxamine and isoproterenol on cyclic AMP production, rate and force in right and left atria, respectively, in both the euthyroid and hypothyroid states, and is summarized in Figure 34 and Tables XI and XII. The basal rate of euthyroid right atria was $290 \pm 7$ beats/minute, while the basal rate of hypothyroid atria was $165 \pm 10$ beats/minute. The basal tension development of euthyroid left
Table X

NEGATIVE LOG MOLAR ED$_{50}$ VALUES (+ SEM) OF ISOPROTERENOL ON EUTHYROID, HYPOTHYROID AND CARBACHOL-PRETREATED EUTHYROID RAT LEFT AND RIGHT ATRIA

<table>
<thead>
<tr>
<th>Group</th>
<th>Left Atria</th>
<th></th>
<th>Right Atria</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Control     PROP$^c$</td>
<td>Log Ratio</td>
<td>(n)</td>
</tr>
<tr>
<td>Euthyroid Control</td>
<td>(10)</td>
<td>8.05, + 0.08</td>
<td>6.02, + 0.11</td>
<td>(8)</td>
</tr>
<tr>
<td>Hypothyroid Control</td>
<td>(10)</td>
<td>6.61, + 0.15</td>
<td>4.85*; a</td>
<td>(10)</td>
</tr>
<tr>
<td>Euthyroid + Carbachol$^e$</td>
<td>(5)</td>
<td>6.66, + 0.06</td>
<td>5.05*; a</td>
<td>(4)</td>
</tr>
</tbody>
</table>

$^a$ Significant difference from the corresponding control ED$_{50}$ value, p<0.05.

$^b$ Significant difference from the euthyroid control; p<0.05.

$^c$ The concentration of propranolol (PROP - 10$^{-6}$M).

$^d$ The log concentration ratio equals the mean ED$_{50}$ of isoproterenol alone minus the mean ED$_{50}$ of isoproterenol after pretreatment with the antagonist propranolol. ED$_{50}$ - as the negative log.

$^e$ Concentration of carbachol: 1.1 uM, left atria; 0.3 uM, right atria.
The effect of the alpha adrenoceptor agonist methoxamine, with time, on cyclic AMP production, rate of spontaneously beating right atria and tension development of electrically driven (1 Hz) left atria, in both the euthyroid (EuT) and hypothyroid (HypoT) states. The data are presented in absolute terms (picomoles/mg tissue) for cyclic AMP (cA) production with time (0 - 180 seconds) to the single dose of $10^{-5}$M methoxamine in the right (R.) atrium and of $10^{-4}$M methoxamine in the left (L.) atrium. $\Delta$ R (b/m) - change in atrial rate in beats/minute. $\Delta$ T (g) - change in tension development in grams. The number in brackets at each graph point of each thyroid state is the sample size.

Euthyroid state: cyclic AMP (cA) - (■—■); tension (T) or rate (R) - ($\Delta$—$\Delta$).

Hypothyroid state: cyclic AMP (cA) - (□—□); tension (T) or rate (R) - ($\blacktriangledown$—$\blacktriangledown$).
METHOXAMINE

R. ATRIUM \((10^{-5} M)\)

L. ATRIUM \((10^{-4} M)\)
Table XI

EFFECT OF ISOPTHRETENOL ON INOTROPIC RESPONSES AND CYCLIC AMP LEVELS OF LEFT ATRIA FROM EUTHYROID AND HYPOTHYROID RATS

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>(n)</th>
<th>Cyclic AMP&lt;sup&gt;b&lt;/sup&gt; (pmol/mg tissue)</th>
<th>Tension&lt;sup&gt;c&lt;/sup&gt; (g)</th>
<th>Time (sec)</th>
<th>(n)</th>
<th>Cyclic AMP&lt;sup&gt;b&lt;/sup&gt; (pmol/mg tissue)</th>
<th>Tension&lt;sup&gt;c&lt;/sup&gt; (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>(16)</td>
<td>0.38 ± 0.04</td>
<td>0</td>
<td>(9)</td>
<td>0.36 ± 0.07</td>
<td>0.28 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>(5)</td>
<td>0.65 ± 0.06&lt;sup&gt;a&lt;/sup&gt; 0.60 ± 0.12</td>
<td>0.54 ± 0.12</td>
<td>(11)</td>
<td>0.49 ± 0.07</td>
<td>0.28 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>(4)</td>
<td>0.65 ± 0.15&lt;sup&gt;a&lt;/sup&gt; 0.58 ± 0.11</td>
<td>0.60 ± 0.05</td>
<td>(4)</td>
<td>0.63 ± 0.09&lt;sup&gt;a&lt;/sup&gt; 0.44 ± 0.11</td>
<td>0.44 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>(5)</td>
<td>0.67 ± 0.06&lt;sup&gt;a&lt;/sup&gt; 0.58 ± 0.11</td>
<td>0.60 ± 0.12</td>
<td>(4)</td>
<td>0.66 ± 0.14&lt;sup&gt;a&lt;/sup&gt; 0.49 ± 0.13</td>
<td>0.49 ± 0.13</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant difference from the zero time point in each thyroid state, p < 0.05.

<sup>b</sup> Concentration of cyclic AMP in picomoles/mg tissue.

<sup>c</sup> Change in tension development in grams.
Table XII

EFFECT OF ISOPROTERENOL ON CHRONOTROPIC RESPONSES AND CYCLIC AMP LEVELS OF RIGHT ATRIA FROM EUTHYROID AND HYPOTHYROID RATS

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>(n)</th>
<th>Cyclic AMP&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Rate&lt;sup&gt;c&lt;/sup&gt;</th>
<th>(n)</th>
<th>Cyclic AMP&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Rate&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18</td>
<td>0.35 ± 0.05</td>
<td>0</td>
<td>15</td>
<td>0.33 ± 0.05</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>0.64 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123 ± 17</td>
<td>6</td>
<td>0.53 ± 0.14</td>
<td>162 ± 20</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>0.72 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>119 ± 15</td>
<td>4</td>
<td>0.59 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>156 ± 14</td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>0.64 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>166 ± 15</td>
<td>7</td>
<td>0.44 ± 0.09</td>
<td>194 ± 6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant difference from the zero time point in each thyroid state, p<0.05.

<sup>b</sup> Concentration of cyclic AMP in picomoles/mg tissue.

<sup>c</sup> Atrial rate development in beats/minute.
atria was $1.13 \pm 0.06$ grams, while that of the hypothyroid atria was $0.51 \pm 0.07$ grams. The basal rate and force levels between the two thyroid states were found to be significantly different. The basal cyclic AMP levels in the euthyroid and hypothyroid left atria were $0.38 \pm 0.04$ picomoles/mg tissue, and $0.36 \pm 0.07$ picomoles/mg tissue, respectively (Figure 34 and Table XI). The basal cyclic AMP levels in euthyroid and hypothyroid right atria were $0.35 \pm 0.05$ picomoles/mg tissue, and $0.33 \pm 0.05$ picomoles/mg tissue, respectively (Figure 34 and Table XII). No significant difference in the basal levels of cyclic AMP could be detected between euthyroid and hypothyroid right and left atria.

No change in cyclic AMP production over the basal level to a single dose of methoxamine ($10^{-5}$ M in left atria; $10^{-4}$ M in right atria) was observed during the entire 180 second time period, in either left or right atria of both euthyroid and hypothyroid states. There was however, a significantly greater increase in the change in tension development in the hypothyroid left atria ($0.62 \pm 0.06$ grams) as compared to the euthyroid state ($0.35 \pm 0.06$ grams). Similarly, there was a significant increase in the change in atrial rate of right atria in the hypothyroid state ($75 \pm 12$ beats/minute) as compared to the euthyroid state ($20 \pm 8$ beats/minute).

Isoproterenol significantly increased cyclic AMP levels over the basal levels in right and left atria of both euthyroid and hypothyroid states (Tables XI and XII). No significant difference in cyclic AMP levels between the two thyroid states could be detected over the entire time period. The maximum cyclic AMP level in the left atrium was $0.67 \pm 0.06$ picomoles/mg tissue in the euthyroid state and $0.66 \pm 0.14$ picomoles/mg tissue in the hypothyroid state (Table XI). The maximum cyclic AMP level in the right atrium was $0.72 \pm 0.07$.
picomoles/mg tissue in the euthyroid state and 0.59 ± 0.13 picomoles/mg tissue in the hypothyroid state (Table XII). The cyclic AMP levels returned to control levels in hypothyroid right atria while the cyclic AMP level in the euthyroid state remained significantly elevated at the 60 second time point (Table XII). Isoproterenol increased force and rate (Tables XI and XII) with no significant difference in the change in force or rate between the two thyroid states over the entire time period. The maximum change in rate was 166 ± 15 beats/minute in the euthyroid state and 194 ± 6 beats/minute in the hypothyroid state (Table XII). The maximum change in force was 0.58 ± 0.11 grams in the euthyroid state and 0.49 ± 0.13 grams in the hypothyroid state (Table XI).

In summary, methoxamine had no affect on cyclic AMP production in either the euthyroid or hypothyroid states, but increased tension and rate to a greater extent in the hypothyroid state as compared to the euthyroid state. However, isoproterenol significantly increased the cyclic AMP levels over the basal levels in right and left atria of both euthyroid and hypothyroid states, with no significant difference detected in the maximum change in force and rate produced between the two thyroid states.

XXII. The Effect of Paired-Pacing on Euthyroid and Hypothyroid Left Atria

To further investigate the responsiveness of both euthyroid and hypothyroid left atrial tissue the technique of paired-pacing was used.

The effect of paired-pacing euthyroid and hypothyroid left atria tissue is summarized in Figure 35. Euthyroid and hypothyroid left atria developed significantly different basal forces of 1.49 ± 0.11 grams and 0.43 ± 0.04 grams, respectively. The left atria were paced by paired-pulses at a frequency of 1 Hz and with a known time interval (in milliseconds) between the stimulations. A Grass Stimulator (Model SD9D) was used to obtain the paired-pulses. The time
The effect of paired-pacing on euthyroid (EUT) and hypothyroid (HYPOT) left atrial tissue measured as tension development in grams (g). The time-interval delay is measured in milliseconds (msec). The number in brackets is the sample size. CONT (at arrow head) indicates the zero millisecond delay between the paired-pulses. Left atria were stimulated at a frequency of 1 Hz with pulses of 5 msec duration at a voltage slightly above threshold. The atria were paired paced with a time interval of 20 to 100 milliseconds between the stimulations.
interval was then varied from 20 to 100 milliseconds. Paired-pacing with a 75 millisecond delay demonstrated that the euthyroid tissue was able to achieve a maximal force of $2.10 \pm 0.02$ grams, while the hypothyroid tissue could only achieve a maximal tension development of $0.91 \pm 0.07$ grams. There was no significant difference in the maximal change in tension development between the two thyroid states. The maximal change in force in the euthyroid state was $0.64 \pm 0.10$ grams and $0.48 \pm 0.04$ grams in the hypothyroid state.
DISCUSSION

The work of the present study: 1) investigated the hypothesis that hypothyroidism produces an alteration in cardiac responsiveness to adrenergic amines, by examining the effects of selective $\alpha$ and $\beta$ adrenoceptor agonists on isolated right and left rat atria; 2) re-examined the proposal of Kunos (1977) that this alteration in cardiac responsiveness is due to an interconversion of $\alpha$ and $\beta$ adrenoceptors, by investigating the effects of $\alpha$ and $\beta$ adrenoceptor antagonists on the responses induced by the selective $\alpha$ and $\beta$ agonists and the dual agonist, phenylephrine; 3) explored the possibility that the alteration in responsiveness of cardiac tissue is due to characteristic differences between the euthyroid and hypothyroid basal atrial rates and forces, by adjusting the basal levels of one thyroid state to that of the other, using ambient temperature, external calcium concentration, the muscarinic agonist carbachol or the $\beta$ adrenoceptor agonist isoproterenol; and 4) investigated the effect of specific $\alpha$ and $\beta$ adrenoceptor agonists on the production of cyclic AMP in both euthyroid and hypothyroid left and right atria.

Due to the complexity of the data presented in this study, a brief summary of the results may give perspective. The influence of hypothyroidism upon cardiac responsiveness to adrenergic amines was profiled from isolated right and left atria of thyroidectomized and euthyroid rats. The negative log $\text{ED}_{50}$ values, and therefore the potencies, of methoxamine and phenylephrine were increased, while the negative log $\text{ED}_{50}$ value of isoproterenol was decreased in the hypothyroid state as compared to the euthyroid control state. Methoxamine was more effective in producing a maximum change in tension and rate development in the hypothyroid state than in the euthyroid state. Isoproterenol and phenylephrine, on the other hand, were similar in producing a maximum change
in tension and rate in the hypothyroid state and the euthyroid state. Phenoxybenzamine appeared to block the rate and force responses to phenylephrine more effectively in the hypothyroid state as compared to the euthyroid control state. Moreover, propranolol appeared to block the rate and force responses to isoproterenol less effectively in the hypothyroid state than in the euthyroid state. Altering the basal rates and forces of one thyroid state to that of the other affected the responses to, and the potencies of, the adrenergic agonists methoxamine, isoproterenol and phenylephrine. Raising the hypothyroid basal force to that of the euthyroid basal level by increasing the external calcium concentration, by decreasing the temperature of the bathing medium, or by adding a single dose of isoproterenol produced responses to methoxamine similar to those observed in the euthyroid controls. The potency of methoxamine was concurrently reduced to the magnitude of the euthyroid control. Lowering the euthyroid basal force to that of the hypothyroid basal level by decreasing the external concentration of calcium or by using a single dose of carbachol produced responses to methoxamine, isoproterenol and phenylephrine similar to those observed in hypothyroid controls. The potencies of methoxamine and phenylephrine were increased, while the potency of isoproterenol was decreased, to the magnitude of the hypothyroid controls. Raising and lowering the basal rates of hypothyroid and euthyroid right atria, respectively, produced results similar to the data obtained when the basal forces were altered by thyroid state alone. However, lowering the rate by means of decreasing the temperature had no effect on the rate responses to methoxamine, isoproterenol or phenylephrine. The potencies and effectiveness of methoxamine, isoproterenol and phenylephrine in terms of both rate and force in euthyroid atria were affected by carbachol pretreatment in a similar way to that observed in
hypothyroidism. Further, methoxamine was found to have no effect on the produc-
tion of cyclic AMP in either euthyroid or hypothyroid right or left atria.
However, isoproterenol increased the cyclic AMP levels to the same extent in
both euthyroid and hypothyroid right and left atria.

Kunos et al. (1974) and Kunos (1977) reported an increased inotropic and
chronotropic potency of phenylephrine in hypothyroid rat atria. The results
of the present study utilizing the selective alpha-adrenoceptor agonist meth-
oxamine and the dual agonist phenylephrine, as representative adrenergic ag-
onists, generally support those data. The inotropic and chronotropic potenc-
ies of methoxamine and phenylephrine were found to be significantly increased
in hypothyroidism (Figures 2a and 2b; 6 and Tables VI and VIII) as compared to
the euthyroid state. The results of Nakashima et al. (1971; 1973) and Nakash-
ima and Hagino (1972) are also in agreement with these findings. They found
that propylthiouracil pretreatment potentiated the positive effect of both
methoxamine and phenylephrine on rat atria and shifted the inotropic dose-
response curves to the left. Propylthiouracil pretreatment also was found to
increase the chronotropic potency of methoxamine and phenylephrine. It is
therefore evident from the results of the present study and those of other
workers that thyroidectomy or chronic propylthiouracil pretreatment alters the
responsiveness of cardiac tissue to adrenergic amines.

The blocking effectiveness of the alpha-adrenoceptor antagonist phen-
oxymethamphetamine was also affected by hypothyroidism. Phenoxymethamphetamine signifi-
cantly reduced the maximum inotropic and chronotropic effect of phenylephrine
on hypothyroid, but not euthyroid, atria and decreased the potency of phenyle-
phrine more effectively in hypothyroid atria (Figures 2a and 2b; Table IX).
This supports the hypothesis that there is a greater alpha-receptor involve-
ment in the hypothyroid state. These findings are similar to those of Kunos et al. (1974) and Kunos (1977). Further, Nakashima et al. (1971) and Nakashima and Hagino (1972) reported a reduced maximum inotropic effect of phenylephrine on hypothyroid atria in the presence of phentolamine. However, other investigators (Lee and Yoo, 1964; Benfey and Varma, 1967; Yoo and Lee, 1970) have not observed an inhibition of the inotropic response to phenylephrine, in euthyroid rabbit atria, with phentolamine.

The increase in alpha adrenergic responsiveness, associated with the hypothyroid state, is accompanied by a decrease in beta adrenergic responsiveness. The inotropic and chronotropic potency of isoproterenol was decreased in hypothyroid atria (Figure 6 and Table IV). In addition, propranolol decreased the inotropic and chronotropic potencies of isoproterenol to a lesser extent in the hypothyroid state (Table X). However, the inotropic and chronotropic effectiveness of isoproterenol was unaffected by hypothyroidism (Figure 11). Kunos (1977) also reported a decreased blocking effectiveness of propranolol in hypothyroid atria, but he employed noradrenaline as the agonist rather than isoproterenol. In addition, Kunos (1977) reported that hypothyroidism markedly decreased the potency of isoproterenol, but did not decrease the potency of noradrenaline to the same extent.

The use of phenylephrine as a representative alpha adrenergic agonist does present problems because the drug possesses intrinsic alpha and beta adrenergic activity. Phenylephrine is considered to be primarily an alpha adrenergic agonist at lower concentrations and a beta adrenergic agonist at higher concentrations (Ahlquist and Levy, 1959; Govier, 1968; McNeill and Verma, 1973). In addition, phenylephrine is reported to release endogenous catecholamines (Daly et al., 1966; Martinez and McNeill, 1977). Due to the
fact that phenylephrine is a dual agonist, there are difficulties associated with the interpretation of data which employ this agonist.

There is no satisfactory explanation for the observed increase in the maximum chronotropic response to phenylephrine in the euthyroid state in the presence of phenoxybenzamine (Figure 1a). A similar finding was reported by Bennett and Kemp (1978) who used phentolamine as the α-adrenoceptor antagonist. They also did not offer an explanation for the observed effect. The stimulation of α-adrenoceptors in euthyroid atria may indeed inhibit the β-adrenoceptor-mediated chronotropic response of the dual agonist phenylephrine. Therefore, one can suggest that in the presence of phenoxybenzamine the phenylephrine-induced increase in rate, which is due to β-adrenoceptor stimulation, is no longer affected by α-adrenoceptor stimulation. The net result of such a mechanism would be to increase the atrial rate with phenylephrine, in the presence of phenoxybenzamine, to a greater extent than would be observed with phenylephrine alone.

Moreover, the phenylephrine-induced inotropic response was only blocked at very high doses (5x10^{-6}M) of the β-antagonist propranolol (Figure 3). Propranolol (10^{-6}M, Figure 1a; or, 2x10^{-6}M, Figure 3) did not affect the inotropic response to phenylephrine. The ability of a β-antagonist to affect the inotropic response of phenylephrine is controversial. Benfey and Varma (1967) observed that propranolol (3x10^{-7}M) did not inhibit the effect of phenylephrine on the contractile force of rabbit atria. Govier (1968) reported that pronethanol (2x10^{-6}M) did not block the phenylephrine-induced positive inotropic response on guinea-pig atria. However, Yoo and Lee (1970) and Bennett and Kemp (1978) reported that propranolol (5x10^{-6}M) caused a depression of the inotropic response to phenylephrine on left atria from normal rabbits.
and rats, respectively.

In this study it also was observed that a combination of phenoxybenzamine and propranolol pretreatment completely abolished the inotropic and chronotropic responses to phenylephrine in both euthyroid and hypothyroid atria, whereas neither blocking agent alone was effective in abolishing the responses (Figures 1a and 1b). Interactions between \textit{alpha} and \textit{beta} adrenoceptor blocking agents have been reported in other tissues (Hull et al., 1960; Olivaries et al., 1967; Yamamura and Horita, 1968). Similarly, Nakashima et al. (1971) and Nakashima and Hagino (1972) also showed that a combination of phentolamine and propranolol almost completely abolished the inotropic and chronotropic responses to phenylephrine in both euthyroid and hypothyroid atria, whereas each antagonist alone did not abolish the responses. In addition, Wenzel and Su (1966) reported that the inotropic response to phenylephrine on rat right ventricle strips was completely abolished with a combination of phentolamine and pronethalol. Moreover, Kunos (1977) showed that a combination of a \textit{beta} adrenoceptor blocking agent, H 93/26, and phenoxybenzamine, almost completely blocked the phenylephrine-induced chronotropic response in hypothyroid atria, but that the combination had little effect in euthyroid atria. It would appear that in order to completely abolish the inotropic and chronotropic effects of the dual adrenergic agonist phenylephrine, a combination of \textit{alpha} and \textit{beta} adrenoceptor blockade is required. A second interpretation of the combined \textit{alpha} and \textit{beta} adrenoceptor blockade of the phenylephrine-induced responses would be that binding of one antagonist to one receptor affects the responsiveness of the other receptor, such that the addition of a second blocking agent, in a dose which was previously ineffective, now produces significant blockade. Furthermore, Schumann et al. (1977), utilizing the isolated rabbit papillary
muscle preparation, reported that a combination of **alpha** and **beta** adrenoceptor blockade was more effective against dopamine than either antagonist alone. Only a combination of phentolamine \(10^{-6}\) M and pindolol \(3\times10^{-8}\) M was able to shift the whole dose-response curve for dopamine to the right. Schumann and co-workers (1977) concluded that dopamine produced its positive inotropic effect by directly stimulating **alpha** and **beta** adrenoceptors to about the same extent, in such a way that if one type of adrenoceptor is blocked, the other may compensate. In the presence of an **alpha** antagonist, most of the dopamine molecules could act on the **beta** adrenergic receptors and vice versa, since dopamine has nearly the same affinity for both types of cardiac adrenoceptors (Schumann et al., 1977). This line of reasoning may help to explain the similar situation observed for the dual agonist phenylephrine in this study.

Since the presence of both an **alpha** and **beta** adrenoceptor blocking agent completely abolished both the rate and force responses to phenylephrine it was felt that the reported dual antagonist labetalol, possessing both **alpha** and **beta** adrenoceptor blocking properties (Farmer et al., 1972; Hansson, 1976; Maconochie et al., 1977; Blakeley and Summer, 1978; Raftery, 1978) would be a useful pharmacological tool to investigate this phenomenon. It was observed that at a concentration of \(10^{-6}\) M, labetalol did not completely abolish either the rate or tension responses to phenylephrine, but did shift the dose-response curves to the right (Figure 4). However, at a concentration of \(5\times10^{-6}\) M, labetalol did effectively abolish the rate and force responses to phenylephrine, as was observed with a combination of both the **alpha** antagonist phenoxybenzamine and the **beta** adrenoceptor antagonist propranolol (Figure 1a). Thus, the effect of labetalol on the inotropic and chronotropic responses of phenylephrine would further suggest that these responses are produced through the
stimulation of both alpha and beta adrenoceptors.

The use of a selective alpha adrenergic agonist, such as methoxamine in the present study, has helped to resolve some of the problems observed when utilizing the dual adrenergic agonist phenylephrine. Since methoxamine possesses neither the ability to release catecholamines nor an intrinsic beta adrenergic activity (Rabinowitz et al., 1975), it had no effect on rate of euthyroid atria. However, methoxamine significantly increased the rate of hypothyroid atria and this effect was completely abolished by phenoxybenzamine (Figures 5a and 5b). Methoxamine also significantly increased the force of contraction in hypothyroid atria to a much greater extent than in euthyroid atria (Figures 5a and 5b). In addition, the inotropic potency of methoxamine was significantly increased by hypothyroidism (Figure 6). Rabinowitz et al. (1975) also reported a positive inotropic effect of methoxamine on euthyroid cat papillary muscles. In addition, Nakashima et al. (1973) observed a positive inotropic response to methoxamine in propylthiouracil treated rats which was greater than that produced in euthyroid controls. The responses to methoxamine were unaffected by propranolol ($3 \times 10^{-7} \text{M}$), while in the presence of phentolamine ($3 \times 10^{-7} \text{M}$), both the chronotropic and inotropic responses to methoxamine were almost completely abolished. However, using the normal whole dog preparation, Brewster et al. (1960) stated that methoxamine lacked a significant direct positive inotropic effect upon ventricular muscle. The difference between the results of Brewster et al. (1960), Rabinowitz et al. (1975) and the present study may be attributed to differences in experimental preparation and species. It should also be noted, from the figure shown by Brewster et al. (1960), that a slight positive inotropic effect to methoxamine does appear to occur. Overall, the data in the present study suggest that alpha adrenoceptors...
are involved in the chronotropic and inotropic responses to a greater extent in hypothyroid than in euthyroid atria and that this phenomenon is demonstrated more clearly by the use of methoxamine, rather than phenylephrine, as a representative alpha adrenergic agonist.

In the present study, it was observed that at doses greater than $10^{-5}$ M, methoxamine elicited a pronounced negative chronotropic response (Figure 5a) in euthyroid atria. James et al. (1968) reported that after methoxamine was administered directly into the isolated canine sinus node artery, a marked decrease in sinus rate occurred on a dose dependent basis. This effect was altered by phenoxybenzamine or phentolamine. James et al. (1968) interpreted the negative chronotropic action of methoxamine as being due to alpha adrenoceptor stimulation. The data of the present study do not agree with those of James et al. (1968) in that the negative chronotropic effect of methoxamine was not blocked by phenoxybenzamine. Nakashima et al. (1973), on the other hand, reported a positive chronotropic response to methoxamine in euthyroid right atria, while in the present study, a consistently pronounced negative chronotropic effect was always observed at doses of methoxamine greater than $10^{-5}$ M. Nakashima et al. (1973), however, did not report their data in absolute terms. It is therefore possible that the basal rate of the euthyroid right atria might have been as low as the hypothyroid atrial basal level. As shown in the present study (Figures 19 and 33), when the basal rate of euthyroid atria was low (comparable to the hypothyroid state), methoxamine produced a marked chronotropic response as opposed to the negligible response in the euthyroid state with an initially higher basal level. Thus, the discrepancy between the results of the present study and those of Nakashima et al. (1973) might be explained if their data had been presented in absolute terms.
Thus, it is possible that the conclusions of the study might depend on whether the data are presented in absolute or relative terms. Kunos (1977) presented his data as percent of the maximum control response to noradrenaline. Accordingly, in the present study, the maximum inotropic and chronotropic responses to methoxamine (Figure 13; Tables II and III) and phenylephrine (Figure 12) in euthyroid and hypothyroid atria were normalized in terms of the maximum response to isoproterenol in the same group. Presentation of the data in this fashion did not alter the overall conclusions of this study. When the phenylephrine data were expressed in this manner (Figure 12) phenylephrine was found to be equi-effective in both the euthyroid and hypothyroid state in terms of both force and rate development. However, when the methoxamine data were expressed in this manner, methoxamine was found to have a significantly greater effectiveness in the hypothyroid state in terms of both force development and atrial rate (Figure 13; Tables II and III). This would support the hypothesis that there is an increased involvement of alpha adrenoceptors in the hypothyroid state. It would appear therefore, that the hypothyroid state preferentially affects alpha adrenoceptors, such that selective alpha adrenergic agonists have a greater inotropic and chronotropic effectiveness in the hypothyroid state. It also is evident that the data are best presented in absolute terms in order to fully illustrate the effect of hypothyroidism on the responses to adrenergic agonists.

Kunos et al. (1974) and Kunos (1977) reported that thyroidectomy did not significantly alter the basal contractile force of left atria, but significantly decreased the rate of right atria. In the present study it was observed that cardiac preparations from hypothyroid animals developed significantly lower basal rates and tensions than those from euthyroid animals, and that
these differences selectively influenced the \textit{alpha} and \textit{beta} adrenoceptor agonist effects. Altering the basal developed force of euthyroid and hypothyroid right and left atria affected the responsiveness of the atria to the actions of methoxamine and isoproterenol. When the basal developed tension and rate of euthyroid left and right atria, respectively, were lowered to the basal level of hypothyroid atria with carbachol, the responses to methoxamine and isoproterenol on euthyroid atria were observed to be similar to those seen in the hypothyroid control atria (Figures 18, 19, 27, 28, 32, and 33). In addition, the potency of methoxamine was increased and that of isoproterenol was decreased (Tables VII and VIII). On the other hand, when the basal developed tension and rate of hypothyroid left and right atria, respectively, were raised to the basal level of euthyroid atria with isoproterenol, the responses of the hypothyroid atria to methoxamine were similar to those seen in the euthyroid control atria (Figures 18 and 19). There also was an apparent decrease in the potency of methoxamine as indicated by the decreased negative log $ED_{50}$ value. However, interpretation of these changes in $ED_{50}$ values should be considered with caution since the ability of the hypothyroid tissue to develop force was already limited by an elevated baseline. The reason for this apparent limitation being that left atria from hypothyroid rats are only able to produce a maximal developed force of 1.5 grams as shown in Figures 31, 32 and 33.

Acute administration of carbachol to atria from euthyroid rats enhanced the \textit{alpha}, and diminished the \textit{beta} adrenoceptor responsiveness, as did thyroidectomy. In the presence of carbachol, the inotropic response to methoxamine was increased over that observed in the euthyroid controls (Figure 33). Endoh and Motomura (1979) reported that carbachol pretreatment further increased the
tension development by phenylephrine in the presence of pindolol. On the other hand, in the presence of carbachol, the maximum inotropic response to isoproterenol was decreased compared to the euthyroid controls (Figure 32). However, the maximum change in tension development to isoproterenol was not significantly different between the euthyroid controls and carbachol-pretreated euthyroid atria (Figures 29, 30 and 32). Endoh and Motomura (1979) also reported that carbachol pretreatment resulted in a pronounced inhibition of the beta adrenoceptor-mediated action of isoproterenol or phenylephrine in the presence of phentolamine. Adjusting the basal developed rates and forces of euthyroid right and left atria, respectively, to the hypothyroid basal levels with carbachol also affected the responsiveness to the dual agonist phenylephrine (Figure 31). Carbachol pretreatment produced an increase in the ED_{50} for phenylephrine from that of the euthyroid controls in terms of both force and rate (Table VI). Thyroidectomy and carbachol pretreatment produced quantitatively indistinguishable effects on the rate response to phenylephrine. However, in terms of the force response, carbachol pretreatment potentiated the effect of the dual agonist phenylephrine.

The following discussion may help to explain the effect of carbachol pretreatment on the responses to the agonists used. In all cases, the maximum responsiveness attainable by the hypothyroid left atrial tissue to the agonists methoxamine, isoproterenol or phenylephrine was about 1.5 grams, whereas the maximum responsiveness attainable by the euthyroid left atrial tissue to the three agonists was about 2.0 grams. Carbachol pretreatment potentiated the tension response to the alpha adrenoceptor agonist methoxamine and reduced the tension response to the beta adrenoceptor agonist isoproterenol. The potentiated tension response observed in response to the dual adrenergic agonist
phenylephrine may be explained in part by the fact that phenylephrine possesses both alpha and beta adrenoceptor properties. Carbachol pretreatment therefore, potentiated the alpha adrenoceptor component, while reducing the maximum effect attained through the beta adrenoceptor component. Further, the potentiated tension response to phenylephrine may be explained by the fact that euthyroid tissue itself is more responsive to adrenergic agonists. Moreover, while mediating tension effects through both alpha and beta adrenoceptors, phenylephrine was found to mediate the rate effects predominantly through beta adrenoceptors since the rate response was effectively blocked with propranolol. Therefore, the effect of carbachol pretreatment on the phenylephrine-induced rate response was similar to the effect seen on the isoproterenol-induced rate response in that carbachol pretreatment reduced the beta adrenoceptor-mediated responses.

Equivalent reductions of basal rates and tensions of euthyroid atria by means other than using the muscarinic agonist carbachol did not affect the responses to adrenergic amines to the same extent as was observed using carbachol. The use of a decreased external calcium concentration to lower the basal rate and tension level of euthyroid right and left atria, respectively, to the basal level of the hypothyroid atria, barely affected the responsiveness of the atria to methoxamine (Figures 22, 23 and 33). Methoxamine in the presence of 0.5 mM calcium produced a positive response which was greater than that observed in the euthyroid controls at 2.2 mM calcium concentration, but the atria did not achieve the same maximum tension or rate as was observed in the hypothyroid state. In contrast, low calcium merely decreased the initial tension and did not otherwise affect the responses to phenylephrine or isoproterenol (Figures 31 and 32). The effect of alpha adrenoceptor agonists has been
reported to be calcium dependent (Endoh et al., 1975), and the data of the present study would support this concept. At a concentration of calcium of 0.5 mM, the agonist methoxamine was not as effective in producing inotropic and chronotropic responses, as it was in the presence of 2.2 mM calcium even though the euthyroid baseline was in the hypothyroid basal range. It has been reported that the resting state of contraction of rat myocardium is dependent on the external calcium concentration (Forester and Mainwood, 1974). The releasable pool of calcium within the myocardium can be filled adequately with calcium in the resting state if the external calcium level is high enough (i.e., greater than 2.5 mM); below this level, filling of the releasable pool of calcium is incomplete in the resting state, but the pool fills as a result of stimulation involving the inward movement of calcium (Niedergerke, 1957; Forester and Mainwood, 1974). At a low calcium concentration therefore, methoxamine would not be able to draw on the releasable pool of calcium, as the pool would be incompletely filled, so that the response produced by the agonist would be reduced compared to the response in the hypothyroid state at 2.2 mM calcium concentration. Landmark (1972) reported that the external calcium concentration determined the force of contraction of isolated rat atria, in that the responses to adrenaline and noradrenaline were augmented at a low external calcium concentration. The same relationship between the external calcium concentration and amplitude of contraction has been demonstrated in guinea-pig atrial muscle (Jork et al., 1967). However, Tuttle and Moran (1969) reported that the noradrenaline dose-response curve maximum on rabbit aortic strips was reduced in low external calcium concentration as compared to the control.

When the hypothyroid basal level was increased to that of the euthyroid
basal range by the addition of 7-8 mM calcium, the response to methoxamine was reduced as compared to that seen in the hypothyroid controls (Figures 22 and 23). West (1968) suggested that a drug response, whose mechanism of action depends on the intracellular mobilization of calcium, may be increased at a low external calcium concentration and decreased in a high external calcium concentration. In addition, Graham and Lamb (1967) reported that the relative response of frog ventricular muscle to epinephrine in the presence of a reduced calcium concentration was greater than in an augmented calcium concentration, perhaps because the increased external calcium concentration alone produced the maximum tension which the muscle could develop. This line of reasoning may help to explain the similar situation observed for methoxamine in the presence of a high external calcium concentration.

In the present study it was observed that calcium produced an increase in rate and tension development in both the euthyroid and hypothyroid states (Figures 20 and 21). Calcium plays an important role in the muscle contraction (Entman, 1970; Mori, 1978). Furthermore, the transition from the resting state to the active state in heart muscle cells depends upon the increase in intracellular calcium availability. The calcium is thought to come from internal stores in the sarcoplasmic reticulum or from the external medium (Nayler and Seabra-Gomes, 1975; Mori, 1978). Drugs such as catecholamines, cardiac glycosides and verapamil alter cardiac contractile force by increasing or decreasing the amount of calcium which becomes available for participation in the events associated with excitation-contraction coupling (Grossman and Furchgott, 1964; Nayler and Szeto, 1972; Nayler, 1973). The positive inotropic action of calcium also was reported by Brodde et al. (1980). However, they did not observe a significant shift in the calcium dose-response curves on atria from euthyroid
and propylthiouracil treated rats. On the other hand, they did not observe a difference between the two thyroid states in the maximum change in force development by the atria, as was observed in the present study. The data reported by Brodde et al. (1980) were not presented in absolute terms. In the present study calcium effected a greater maximum developed force in the euthyroid atria than in the hypothyroid atria. Also, an apparent shift in the calcium dose-response curve to the right was observed in the hypothyroid state as compared to the euthyroid state. Similar results were obtained in terms of the rate response of calcium between the two thyroid states. Although there was a statistically significant difference between the ED$_{50}$ values of the two thyroid states in terms of both rate and force, the difference was not marked and such a difference between the values may not warrant a conclusion suggesting that there is a difference in the potency of calcium between the two thyroid states. Even though the difference is small between the negative log ED$_{50}$ values it does not rule out the possibility that there may be a difference in the utilization of calcium between the two thyroid states. It is also possible that the ability of hypothyroid atria to respond is decreased as indicated by the decreased maximal rate and force responses attainable to calcium compared to the euthyroid state. Calcium ion also has been implicated in cardiac chronotropism (Katz, 1970). Somjen and Baskerville (1968) demonstrated that the administration of calcium caused an increase in heart rate in situ. On the other hand, Baetjer (1978) reported that rats on a low calcium diet showed a significantly decreased heart rate. The apparent differences in the responsiveness of the atria to calcium can be interpreted as being due to the fact that the increased external calcium concentration alone produced the maximum rate and force which the muscle could develop.
It has been reported that paired-pacing provides an index of the tissue maximal developed force (Haacke et al., 1970; Lee et al., 1970). Therefore, it was decided to use this technique to further investigate the responsiveness of both euthyroid and hypothyroid left atria tissue. The maximal developed force achieved by paired-pacing (Figure 35), in both euthyroid and hypothyroid left atria tissue, closely approximated the maximal developed force achieved using calcium (Figures 20 and 21). This technique therefore, demonstrated the maximal force attainable by the tissue in the two thyroid states, and further, helped to explain the difference in responsiveness of those tissues to calcium.

Lowering the temperature of the bathing medium in order to decrease the basal rate of spontaneously beating euthyroid right atria to the basal level of hypothyroid right atria did not result in a positive chronotropic response to methoxamine as was observed in the hypothyroid controls (Figures 24 and 33). These results, and those of other workers (Martinez and McNeill, 1977; Benfey, 1979; Mori et al., 1979) are not in agreement with the hypothesis that adrenoceptor interconversion occurs with temperature decrease (Kunos and Nickerson, 1976). According to the hypothesis proposed by Kunos and Nickerson (1976; 1977) and Kunos (1977) the effect of a low temperature environment is exhibited as a greater alpha adrenoceptor involvement in the inotropic response to adrenergic amines. Lowering the temperature of the bathing medium to increase the hypothyroid basal levels of left atria to that observed for the euthyroid control left atria (Figure 25) affected the responsiveness of the hypothyroid left atria to methoxamine. At 25°C, the maximum tension developed was significantly less than the hypothyroid controls at 37°C. In addition, the potency of methoxamine returned to the magnitude of the euthyroid control as indicated
by the decreased negative log ED$_{50}$ value. The reservation on the interpretation of data with respect to alteration of baselines has already been discussed in the case of the effect of isoproterenol and external calcium concentration on the basal developed force of hypothyroid left atria. Likewise, it is quite conceivable that the ability of hypothyroid left atria to develop force would similarly be diminished by an elevated baseline due to the decreased temperature of the bathing medium. In addition, since the hypothyroid state increases the involvement of alpha adrenoceptors and it is alleged that low temperature does the same (Kunos and Nickerson, 1976), one would have expected an enhanced alpha adrenoceptor response to methoxamine at $25^\circ$C in the hypothyroid atria compared to the hypothyroid controls at $37^\circ$C. In terms of the rate response to methoxamine one would have also expected an increased involvement of alpha adrenoceptors showing a positive chronotropic effect in right atria of euthyroid rats compared to the euthyroid control state, if the interconversion hypothesis was correct. It would therefore appear that the rat heart atria contain the same alpha adrenoceptors at both normal ($37^\circ$C) and low ($25^\circ$C) temperatures (Figures 24 and 25) in euthyroid and hypothyroid states, and that the alpha adrenoceptors have not 'interconverted' from beta adrenoceptors.

Lowering the temperature of the bathing medium to $25^\circ$C did affect the responsiveness of euthyroid right atria to isoproterenol such that an apparent supersensitivity to isoproterenol developed (Figure 26). The sensitivity of isolated guinea-pig right atria to sympathomimetic amines, as shown by the displacement of the dose-response curve to the left, was also reported to be increased at low temperature by Broadley and Duncan, 1977). Lowering the temperature of the bathing medium to $25^\circ$C did not, however, affect the maximum change in rate development as compared to the euthyroid controls at $37^\circ$C. Thus,
decreasing the temperature in order to alter the basal level of euthyroid right atria to that of hypothyroid atria resulted in a shift of the dose-response curve in the opposite direction as would have been expected. Since a decreased beta adrenoceptor responsiveness was observed in the hypothyroid state as indicated by the decrease in chronotropic potency of isoproterenol (Figure 6) one would have also expected a similar decrease in potency to isoproterenol after altering the euthyroid basal rate with temperature. This would have been exhibited as a shift in the dose-response curve to the right, and not to the left as was observed. Therefore, this would provide further evidence against the interconversion hypothesis.

The results of the present study, utilizing the specific alpha adrenoceptor agonist methoxamine, are in agreement with those of other investigators (Osnes and Øye, 1975; Verma and McNeill, 1976) who did not observe an increase in cyclic AMP to phenylephrine in the presence of propranolol on isolated perfused guinea-pig hearts. Brückner et al. (1978) also were unable to detect any phenylephrine-produced increase in cyclic AMP under conditions in which the drug increased the force of contraction of the cat papillary muscles. They concluded that the presumably alpha adrenoceptor-related positive inotropic effect of phenylephrine was not mediated by cyclic AMP.

The concept that the alpha adrenoceptor-mediated positive inotropic effect of adrenergic amines is not mediated by cyclic AMP has been proposed by other investigators (Schumann et al., 1975; Endoh et al., 1976; Watanabe et al., 1977). The biochemical mechanism of the positive inotropism seems to be independent of cyclic AMP. However, most of the studies cited were done in the presence of a beta adrenoceptor blocking agent to exclude the beta adrenoceptor mediated effects of high concentrations of phenylephrine. Methoxamine has been
shown to produce its positive inotropic effect on isolated rabbit papillary muscle without any significant increase in cyclic AMP levels (Schümann et al., 1975). In addition, Rabinowitz et al. (1975) reported that there was no increase in cyclic AMP to the agonist methoxamine in cat atria even though an increase in contractile force was observed.

The marked inotropic response to alpha adrenergic stimulation of atria from hypothyroid rats, as compared to euthyroid rats, occurred without any cyclic AMP elevation (Figure 34). Osnes (1976) reported a similar finding using phenylephrine in the presence of propranolol on propylthiouracil treated rats. He observed an increase in contractile force in perfused hypothyroid rat hearts without a corresponding increase in cyclic AMP. Osnes (1976) also reported that isoproterenol increased both the contractile force and cyclic AMP levels in isolated perfused hypothyroid rat hearts. The results of the present study are in agreement with the results of Osnes (1976). However, Kunos et al. (1976) reported an alpha adrenoceptor-mediated rise in cyclic AMP in hypothyroid atria because of the reciprocal blocking effectiveness of alpha and beta antagonists to the dual agonist phenylephrine. They also based their conclusions from the results obtained over a very narrow dose range of the agonist phenylephrine.

The cyclic nucleotide data presented in this thesis also do not support the hypothesis that hypothyroidism induces an interconversion of the adrenoceptors which mediate increases in cyclic AMP levels (Kunos et al., 1976). If there was an interconversion of adrenoceptor type in the hypothyroid state, from beta to alpha, one would expect an increase in cyclic AMP levels following methoxamine. Such an increase did not occur although the inotropic and chronotropic effects of methoxamine were increased in atria from hypothyroid
animals (Figure 34). Isoproterenol, at least at one dose, produced increases in mechanical responses and cyclic AMP levels of a similar magnitude in euthyroid and hypothyroid atria (Tables XI and XII). The observed increase in cyclic AMP with isoproterenol, a beta adrenoceptor agonist known to increase cyclic AMP (Robison et al., 1965; George et al., 1970; Kukovetz et al., 1973; Donges et al., 1977), established that it was possible, with the method employed, to detect a change in cyclic AMP production. Therefore, it was concluded that the absence of an increase with methoxamine was a valid observation. The basal levels of cyclic AMP were not found to be significantly different between the two thyroid states (Figure 34; Tables XI and XII), which is in agreement with the results of Hagino and Shigéi (1976).

The data of the present study are not in general agreement with the data of Brodde et al. (1980). They reported that isoproterenol (3×10^{-8} M) increased the cyclic AMP level in left atria by about 60% after 60 seconds on euthyroid atria, while on hypothyroid left atria the effect of the same isoproterenol concentration on the adenylate cyclase system was diminished showing a cyclic AMP increase of only 25%. They concluded that the reduced sensitivity of cardiac beta adrenoceptors is associated with a decreased responsiveness of the adenylate cyclase system. They reported extremely low basal tensions of both euthyroid and hypothyroid atria compared to the basal tensions of the two thyroid states observed in the present study. Brodde et al. (1980) also did not observe a significant difference between the basal tension levels of the two thyroid states, as was observed in the present study. The basal levels of cyclic AMP reported by them were about twice that observed in the present study. The differences between the present study and that of Brodde et al. (1980) in terms of the cyclic AMP levels obtained after the administration of isoproter-
enol may also be due to the difference in drug concentrations used. In the present study there was no difference in the maximum cyclic AMP levels obtained between the thyroid states.

The positive inotropic and chronotropic effects of alpha and beta adrenoceptor agonists probably involve different mechanisms. One mechanism may be dependent on and the other independent of cyclic AMP. Osnes (1976) postulated that adrenoceptor agonists influence myocardial calcium translocation through different mechanisms, in such a way that a single physiological response to one agonist may be mediated by more than one mechanism. Such an adaptation may serve to maintain responsiveness of the heart under various conditions such as altered thyroid state.

Several binding studies have indicated a change in the number of alpha and beta adrenoceptors in the hypothyroid, euthyroid and hyperthyroid states. However, the conclusions from these studies differ. Recently, McConnaughey et al. (1979) reported that the ratio of beta to alpha adrenoceptors approximately doubled in hyperthyroid rats, but remained unchanged in hypothyroid rats when compared to the euthyroid controls. They used $^3$H-dihydroergocryptine and $^3$H-dihydroalprenolol and reported that in propylthiouracil-treated hypothyroid rats there was a decrease in the total number of both alpha and beta adrenoceptors relative to the euthyroid controls. Conversely, they found a decrease in the number of alpha adrenoceptors and an increase in the number of beta adrenoceptors in the hyperthyroid state as compared to the number in the euthyroid controls. Smith et al. (1978) also found a decrease in the number of beta adrenergic receptors in the hypothyroid myocardium. Williams et al. (1977) and Ciaraldi and Marinetti (1977) reported similar results to the above studies. However, Kunos et al. (1974) reported that the number of alpha...
adrenoceptors had increased in the hypothyroid state since the binding by $^3$H-phenoxybenzamine was significantly increased over that in the euthyroid controls. Sharma and Banerjee (1978) also found an increased content of myocardial alpha adrenoceptors in hypothyroidism. On the other hand, Williams and Lefkowitz (1979) found that the hypothyroid alpha adrenoceptor density did not change. There is, therefore, a lack of consistency in the data of the binding studies. From the results of the present study and those of other investigators (Nakashima et al., 1971; Nakashima and Hagino, 1972; Kunos, 1977) it was observed that there was an increased alpha and a decreased beta adrenoceptor responsiveness in hypothyroidism. Receptor number alone may not be an indication of, nor an explanation for, the responses associated with hypothyroidism. Moreover, one must also consider the results of the present study in which euthyroid atria were pretreated with carbachol. The responses observed in these atria were very similar to the responses observed in hypothyroid atria. It seems likely that carbachol pretreatment alone does not alter adrenoceptor number, but it is clear that it influences the atrial responsiveness in such a way as to mimic the hypothyroid state.

From the results of the present study it would appear that the alpha and beta-adrenoceptors have not interconverted in the rat myocardium, but remain as two separate pools of receptors, in agreement with the results of Buckley and Jordan (1970). In the euthyroid state the alpha receptors are silent, possibly because in this thyroid state a post-receptor component is absent or an inhibitor is present which acts in such a way as to make the alpha adrenoceptor-mediated responses less predominant. However, in the hypothyroid state and in carbachol-pretreated euthyroid atria alpha receptor-induced responses increase. The component that allows the emergence of these responses in the
hypothyroid state to a greater degree than in the euthyroid state is at present unknown. However, evidence in the literature suggests that this component might be cyclic GMP (see below). It is clear that it could not be cyclic AMP related because methoxamine was unable to increase cyclic AMP production in either the euthyroid or the hypothyroid state (Figure 34). In addition, the basal levels of cyclic AMP were not significantly different between the two thyroid states.

The ability of acute carbachol pretreatment to mimic the effects of chronic hypothyroidism on cardiac responsiveness to adrenergic amines might have been predicted on the basis of previous evidence. For example, Amer and Byrne (1975) recently proposed that myocardial alpha adrenoceptor responses are mediated through cyclic GMP. In addition, Kunos et al. (1976) showed a two fold increase in the basal cyclic GMP concentration in hypothyroid right atria as compared to the euthyroid control basal levels, but did not comment on the significance of the different levels. Furthermore, the parasympathomimetic carbachol has been shown to increase cyclic GMP levels (Keely et al., 1978; Endoh, 1979). Therefore, cholinergic antagonism of the positive inotropic and chronotropic actions in the rat myocardium may increase the cyclic GMP levels. Moreover, it has been shown that the positive inotropic action of beta adrenoceptor stimulation was accompanied by an increase in cyclic AMP levels and by a concomitant decrease in cyclic GMP levels in the isolated perfused rat heart (George et al., 1970). The acetylcholine-induced negative inotropic and chronotropic effects were associated with an elevation of the cyclic GMP levels and a lowering of the cyclic AMP levels in the same preparation (George et al., 1970; 1973). Furthermore, based on findings in the guinea-pig heart (Watanabe and Besch, 1975) it has been proposed that the antiad-
Renergic effects of cholinergic stimulation in the mammalian ventricular muscle may be mediated by the specific antagonism of the intracellular actions of cyclic AMP by cyclic GMP. However, Brooker (1977) showed that low concentrations of carbachol decreased guinea-pig atrial contractile force without increasing myocardial cyclic GMP levels, suggesting that cyclic GMP may not be involved in mediating the negative inotropic action of acetylcholine on the heart. From these studies, cyclic GMP may indeed be involved with respect to the increased α, and decreased β adrenoceptor responsiveness in the hypothyroid and carbachol-pretreated euthyroid state. Further work is required.
The study of the responsiveness of hypothyroid atria to adrenergic amines led to the following conclusions:

1. That the induction of the hypothyroid state produces changes in adrenoceptor responsiveness to adrenergic amines;

2. That the data do not agree with the hypothesis proposed by Kunos (1977) that adrenoceptors interconvert, but can possibly be explained on the basis of two separate pools of adrenoceptors;

3. That the data on rate and tension indicate that myocardial adaptation to the hypothyroid state is manifested as an increased responsiveness to alpha adrenergic amines, and a decreased responsiveness to beta adrenergic amines;

4. That the basal levels of rate and tension development can also influence the degree of responsiveness to the adrenergic amines;

5. That hypothyroidism appears to influence cardiac responsiveness to adrenergic amines at a level beyond the adrenoceptor, which may be selectively duplicated by carbachol pretreatment; and

6. That the influence of carbachol does not appear to be secondary to reductions in basal rates or forces per se but to specific consequences of muscarinic activation, possibly through increased cyclic GMP levels.
REFERENCES


