The Effect of Beta-Adrenergic Receptor Blockade on the Atrial Refractory Period of Hyperthyroid Rabbits

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ABSTRACT

Treatment of rabbits with thyroxine 1 mg/kg daily for three to six days produced a hyperthyroid state as evidenced by increase in the heart rate and PBI. The hyperthyroid animals exhibited a significant shortening of the atrial refractory period. Beta adrenergic receptor blocking agents, propranolol (10^{-6}M) pronethalol (3\times10^{-6}M) or MJ-1999 (3\times10^{-4}M), completely blocked the shortening of the atrial refractory period produced by the treatment with thyroxin, and with norepinephrine. Consequently, the shortening of the atrial refractory period of rabbits treated with thyroxin appears to be mediated through stimulation of the beta adrenergic receptor in the heart. From these results, it may be concluded that the stimulation of the beta adrenergic receptor plays an important role in the alteration of cardiac function found in hyperthyroidism, and that the beta adrenergic blocking agents may be useful in treatment of the cardiac complications of hyperthyroidism.

INTRODUCTION

Accumulated evidence has suggested that the hemodynamic changes in hyperthyroidism can be attributed to an increased activity of the sympathetic nervous system. This hypothesis is further supported by the observations that the symptoms of hyperthyroidism were alleviated by reserpine (Wayne, 1960; de Groot et al. 1961), guanethidine (Gaffney et al., 1961; Leak, 1963; Goldstein and Killip, 1965) and propranolol (Howitt and Rowlands, 1966). The exaggerated response of hyperthyroid animals to epinephrine and norepinephrine suggests that the appearance of increased sympathetic activity may be due to an increased sensitivity to catecholamines (Hoffman et al., 1947; Schneckloth et al., 1953). Recently this laboratory has demonstrated that the cardiac effects of treatment with thyroxine were largely dependent upon the capacity of thyroxine to increase the catecholamine content of the heart (Lee et al. 1965).

In the course of studying the effect of thyroxine on the cardiac activity it was found that the refractory period of atria isolated from rabbits treated with thyroxine was markedly shortened when compared with that of normal rabbits. The similarity between the shortening of the atrial refractory period observed in hyperthyroid animal and that seen in increased sympathetic activity of normal animals led us to consider the possible role of the sympathetic nervous system in the hyperthyroid state. The present study was undertaken to evaluate whether or not shortening of the cardiac refractory period in hyperthyroid rabbits is mediated through the beta adrenergic receptors.

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in hearts.

METHODS

Preparation of atria

Under ether anesthesia the heart was rapidly removed from the rabbits of both sexes weighing approximately 2 kg of body weight. Ventricular muscle, connective tissue, fat and blood vessels were excised from the atria which were then suspended in muscle chambers containing 100 ml of modified Tyrode’s solution, pH 7.4 (Lee and Shideman, 1958) maintained at a constant temperature of 38°C. A mixture of 95% oxygen and 5% carbon dioxide was bubbled through a bathing fluid via a sintered glass plate at the bottom of the chamber. The solution also contained the disodium salt of ethylenediamine tetraacetic acid (EDTA) in a concentration of 10^{-5}M to exclude the effects of any heavy metal contaminants in the bathing fluid (Lee et al., 1965).

Determination of atrial refractory period

For electrical stimulation a dual square-wave stimulation was used. Both paired stimuli were varied in intensity and duration and interval. Two concentric electrodes made from 19 gauge hypodermic needles were used for recording the electrical phenomena. One electrode was placed on the right auricle in the vicinity of the sinus node, and the other was placed approximately two centimeters apart on the left auricle. All records were obtained by a Grass Model 7 Polygraph. Isometric tension signals from the transducer were transmitted to the recorder by way of a strain-gauge amplifier connected to the DC amplifier in the recorder.

The refractory period was determined by injecting paired supramaximal stimuli at successively shorter intervals. The time at which there was no response to the second stimulus was taken as the refractory period.

Thyroxine treatment and measurement of serum protein bound iodine (PBI)

To produce a hyperthyroid state, rabbits were injected intraperitoneally with thyroxine daily 1 mg/kg for three to six days. The animals were given food ad libitum. The experiment was conducted twenty-four hrs after the last thyroxine injection, rabbits were killed and atria were isolated for the experiment.

Serum protein bound iodine was measured by the method of Boutwell (1961).

RESULTS

Effect of thyroxine treatment on atrial refractory period

Table 1 gives the figures for the atrial refractory period of normal and thyroxine-treated rabbits. The heart rate and PBI at the time of the experiment were also included in the table. It can be seen that the atrial refractory periods were markedly shortened and the heart rate and PBI were elevated in the thyroxine-treated animals as compared to those in normal rabbits. From the data presented in Table 1 it may be concluded that treatment of animals with thyroxine 1 mg/kg daily for three to six days satisfactorily produced hyperthyroid state (Rosen and Moran, 1963), which in turn produced shortening of the atrial refractory period.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rabbits</th>
<th>Mean atrial refractory period (per min.)</th>
<th>Mean atrial rate (beats per min.)</th>
<th>Mean serum PBI (μg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>11</td>
<td>146.0±2.91</td>
<td>146.0±6.16</td>
<td>3.40±0.35</td>
</tr>
<tr>
<td>Thyroxine for 3 days</td>
<td>13</td>
<td>128.8±3.67**</td>
<td>194.5±6.70</td>
<td>49.88±4.49</td>
</tr>
<tr>
<td>Thyroxine for 6 days</td>
<td>9</td>
<td>123.4±2.34**</td>
<td>235.2±3.34</td>
<td>75.20±6.55</td>
</tr>
</tbody>
</table>

*: Thyroxine (1 mg/kg) was injected into rabbits intraperitoneally.

**: Significantly different from untreated rabbits (P<0.1).
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>150±3.80</td>
<td>161±4.55</td>
<td>155±7.31</td>
<td>159±5.60</td>
<td>141.8±5.42</td>
<td>150±6.30</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>14</td>
<td>127.2±4.08*</td>
<td>160±5.88</td>
<td>123±6.10*</td>
<td>150.4±10.10</td>
<td>126.7±6.84*</td>
<td>159.3±4.18</td>
</tr>
<tr>
<td>Animal</td>
<td>N</td>
<td>Mean atrial rate±SE (per min.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>141±7.27</td>
<td>124±5.24</td>
<td>147±8.02</td>
<td>140±5.15</td>
<td>152.4±14.60</td>
<td>141±9.10</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>14</td>
<td>210±8.88</td>
<td>158±10.72</td>
<td>200±13.24</td>
<td>161±11.30</td>
<td>198±11.00</td>
<td>159.3±4.18</td>
</tr>
</tbody>
</table>

* : Significantly different from control animals (P<.01).

**Effects of propanol, pronethalol and MJ-1999 on atrial refractory period of hyperthyroid rabbits**

Before assessing the nature of the shortening of the atrial refractory period observed in hyperthyroid animals, the relative potency of beta adrenergic receptor blocking agents in blocking the shortening of atrial refractory period induced by norepinephrine was examined in normal rabbits. After careful evaluation of responses of the atrial refractory period to norepinephrine at concentrations ranging from 10^{-8}M to 10^{-5}M, 10^{-5}M norepinephrine was chosen as an agonist since the pattern of decrease in the atrial refractory period by this concentration of the drug was quite similar to that observed in hyperthyroid animals.

As shown in Figure 1, all adrenergic beta blocking agents used in this study significantly depressed or blocked the response to norepinephrine (10^{-5}M). The concentration of these blocking agents necessary to completely block the shortening of atrial refractory period produced by norepinephrine were 10^{-6}M for propanol, 5×10^{-6}M for pronethalol and 5×10^{-6}M for MJ-1999. It is evident from this result that the order of potency of the three blocking agents for blocking the response to norepinephrine was propanol, pronethalol, and MJ-1999.

Table 2 summarizes the effects of beta-receptor blocking agents on the atrial refractory period of hyperthyroid rabbits. It may be seen that the presence of propanol (10^{-6}M), pronethalol (5×10^{-6}M), or MJ-1999 (5×10^{-6}M) nearly completely abolished the shortening of the atrial refractory period, as well as the increase in heart rate observed in hyperthyroid animals.

**DISCUSSION**

It is generally accepted that increased activity of the sympathetic nervous system may play an important role in producing some of the signs of hyperthyroidism. In favor of this view is the observations that the symptoms of hyperthyroidism were alleviated by blockade of the central sympathetic pathways by procaine injected into the subarachnoid space (Knight, 1945) or applied to the whole of the epidural area (Brewster et al. 1956). This was further supported by the findings.

![Fig. 1. Blockade of the refractory-period response to 10^{-5}M norepinephrine by propanol, pronethalol or MJ-1999.](image_url)
that the tachycardia of hyperthyroidism could be reduced by reserpine (Canary et al. 1957), guanethidine (de Groot et al. 1961) and α-methyl dopa (Theilen et al. 1963). More direct evidence favoring of this concept was presented by Lee et al. (1965) who observed that the faster rate of atria isolated form thyroxine-treated animals was largely dependent upon the capacity of thyroxine to increase the catecholamine content of the heart.

The present study demonstrates that intraperitoneal injection of thyroxine into rabbits 1 mg/kg daily for 3-6 days produced hyperthyroidism as evidenced by the increase in heart rate and PBL. The refractory period of the atria isolated from rabbits treated with thyroxine, when as well as with norepinephrine, was shown to be markedly shortened compared to that of atria isolated from normal rabbits. The demonstration that the beta adrenergic blocking agents such as propranolol, pronethalol or MJ-1999 completely blocked the shortening in atrial refractory period produced by both thyroxine and norepinephrine appears to indicate that changes in the cardiac refractory period in hyperthyroidism are mediated through stimulation of the beta adrenergic receptor in the heart. This is in agreement with the experimental results provided by Howitt and Rowlands (1966) and by Turner et al. (1965) that propranolol produced a consistent reduction in the heart rates of hyperthyroidism. From the results obtained from the present study, it is conceivable to assume that stimulation of the beta adrenergic receptor plays an important role in the cardiac symptoms of hyperthyroidism and that the blocking agents such as propranolol, pronethalol or MJ-1999 may be useful in their treatment.

REFERENCES


