Muscarnic Cholinergic Receptors in Rat Heart

EFFECTS OF THYROIDECTOMY*

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SUMMARY

The effects of thyroid status on muscarinic cholinergic receptors in the rat myocardium were investigated. The potent muscarinic antagonist \(^{3}H\)quinuclidinyl benzilate was used to identify muscarinic cholinergic receptors in rat heart particulate fractions from control, hypothyroid, and hyperthyroid rats. Thyroidectomy increased specific \(^{3}H\)quinuclidinyl benzilate binding to heart particulate fractions by about 60% as compared to euthyroid rat cardiac preparations. Administration of triiodothyronine to euthyroid rats decreased specific binding to muscarinic cholinergic receptors by about 20%. Scatchard analysis revealed that the cardiac particulate fraction from thyroidectomized rats contained 134 fmol of \(^{3}H\)quinuclidinyl benzilate binding sites per mg of protein, as compared with 85 fmol/mg of protein found in the heart preparation of thyroidectomized rats chronically treated with triiodothyronine. The equilibrium dissociation constant for the interaction of receptors with quinuclidinyl benzilate was the same (1 nM) in the heart particulate fractions derived from these two groups of rats. The results of this study demonstrate that thyroid hormone can regulate the number of cardiac muscarinic cholinergic receptors. Thus, the parasympathetic nervous system may participate in the cardiovascular abnormalities of different thyroid states.

There is considerable controversy concerning the biochemical mechanisms responsible for the cardiovascular abnormalities in hyperthyroidism (1, 2). Several well controlled and carefully performed studies have provided evidence for increased cardiac sensitivity to catecholamines in hyperthyroidism (3–6). There are other reports, however, suggesting that cardiac abnormalities may not be entirely due to alterations of myocardial sensitivity to catecholamines. For example, thyroxine and triiodothyronine have been shown to increase conversion of ATP to cyclic AMP in rat heart homogenates which is not blocked by propranolol (7). Similarly, propranolol does not significantly change oxygen consumption, heart rate, systemic mean arterial pressure, cardiac output, or total systemic resistance in subjects treated with triiodothyronine (8). Finally, there is evidence that the intrinsic contractile properties of the myocardium are altered in thyrotoxicosis (9, 10).

Many organs receive both sympathetic and parasympathetic fibers and are influenced in opposite ways by the two divisions of the autonomic nervous system. In some instances, for example, heart and intestine, the organs are endowed with inherent activity and require dual innervation with opposing actions in order to elevate or suppress inherent activity when appropriate. The cells of these organ systems are directly influenced by both sympathetic and parasympathetic nerves (11). In other instances, control of function is regulated by opposing actions on different effector cells. Thus, thyroid hormones may influence the intrinsic contractility of the myocardium by regulating adrenergic as well as muscarinic cholinergic activity of the cardiac cells. One possible mechanism for such regulation would be an alteration in the number or affinity (or both) of \(\beta\)-adrenergic and muscarinic cholinergic receptors in different thyroid states. Recently, three laboratories independently reported that thyroid hormones can regulate the number of cardiac \(\beta\)-adrenergic receptors and that the decreased number of receptors may be responsible, at least in part, for the reduced catecholamine sensitivity of \(\beta\)-adrenergic-coupled cardiac responses in the hypothyroid state (12–14). In the present study, \(^{3}H\)quinuclidinyl benzilate is used to demonstrate a highly significant decrease in the specific binding to muscarinic receptors in cardiac membranes from hyperthyroid rats and an increase in the specific binding to these receptors in thyrotoxicimized rat hearts.

Male Sprague-Dawley rats (initial weight 120 to 160 g) were divided into four groups containing six rats per group. Thyroidectomized (7 to 8 weeks after surgery) or euthyroid rats were injected with 30 \(\mu\)g of triiodothyronine per 100 g of body weight on alternate days for a total of 11 or 12 doses. Animals were killed 24 h after the last dose of triiodothyronine. The other two groups received only the solvant of triiodothyronine. The average weights for euthyroid, thyroidectomized, euthyroid plus triiodothyronine, and thyroidectomized plus triiodothyronine were 325, 290, 302, and 315 g, respectively. Similarly the average whole heart weights were 1.12, 0.65, 1.26, and 1.21 g, respectively. Finally, triiodothyronine levels in the serum in euthyroid rats were 128 ng/dl as compared with 44 ng/dl in thyroidectomized rats and 365 ng/dl and 773 ng/dl in thyroidectomized and euthyroid rats, respectively, treated chronically with triiodothyronine.

The crude membrane preparation was assayed for \(^{3}H\)quinuclidinyl benzilate binding as previously described for \(\beta\)-adrenergic receptor binding (14–16) and for muscarinic receptor binding (17, 18). \(^{3}H\)Quinuclidinyl benzilate, a potent muscarinic receptor antagonist, has been used to identify muscarinic receptors in membrane from several tissues (17–19). In particulate fractions from rat heart, \(^{3}H\)quinuclidinyl benzilate binds to sites which have the affinity and specificity expected of muscarinic cholinergic receptors,1 as previously

demonstrated in other mammalian tissues, including rat brain (17) and guinea pig ileum (18). Binding is displaced by muscarinic cholinergic agonists and antagonists in proportion to their pharmacologic potency. Nicotinic cholinergic drugs and noncholinergic agents have negligible affinity for the quinuclidinyl benzilate binding sites in heart particulate fraction. In each set of experimental tubes, nonspecific accumulation of radioactivity was determined by measuring the amount of radioactive counts retained on filters when incubations were performed in the presence of 100 μM oxotremorine. Specific binding was determined by subtracting nonspecific counts from total accumulation of radioactivity. Specific binding constituted 70 to 85% of the total counts retained on filters. The effect of thyroid status on postjunctional muscarinic cholinergic receptors was determined by measuring specific [3H]quinuclidinyl benzilate binding to rat heart particulate fractions (Table I). The specific binding to cholinergic muscarinic receptors increased by about 60% (from 25.29 to 40.02 fmol/mg of protein) following surgical thyroidectomy of euthyroid rats. Administration of triiodothyronine decreased specific [3H]quinuclidinyl benzilate binding by about 50% (to 20.96 fmol/mg of protein). These differences are statistically highly significant and exactly opposite to the effects of thyroid status on β-adrenergic receptors. Surgical as well as chemical thyroidectomy decreased specific [3H]dihydroalprenolol binding to rat heart membranes (12, 14). Administration of triiodothyronine to surgically thyroidectomized rats increased specific [3H]quinuclidinyl benzilate binding to cardiac particulate fractions from thyroidectomized (hypothyroid) rats and thyroidectomized rats chronically treated with triiodothyronine (hyperthyroid). Cardiac particulate fractions were incubated with a series of concentrations of [3H]quinuclidinyl benzilate and specific binding was determined as described in Table I. The lines for hypothyroid rat (r = 0.86) and hyperthyroid rat (r = 0.91) heart particulate fractions were determined by linear regression analysis. Each point represents the mean of triplicate determinations. The negative reciprocal of the slope provides an estimate of the equilibrium dissociation constant (Kᵦ) for the interaction of [3H]quinuclidinyl benzilate with the binding sites, and for both, the value of Kᵦ was found to be 1 nM. The maximal number of binding sites (B_max) for hypothyroid and hyperthyroid rat heart preparations were found to be 134 and 85 fmol/mg of protein, respectively.

**Table I**

<table>
<thead>
<tr>
<th>Thyroid status</th>
<th>Specific Binding (fmol/mg protein)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Euthyroid</td>
<td>25.29 ± 0.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thyroidectomed</td>
<td>40.02 ± 1.24</td>
<td></td>
</tr>
<tr>
<td>B Thyroidectomed</td>
<td>40.02 ± 1.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thyroidectomed + triiodothyronine</td>
<td>20.96 ± 0.97</td>
<td></td>
</tr>
<tr>
<td>C Euthyroid + triiodothyronine</td>
<td>25.29 ± 0.82</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Fig. 1.** Scatchard plot of [3H]quinuclidinyl benzilate (QNB) binding to cardiac particulate fractions from thyroidectomized (hypothyroid) rats and thyroidectomized rats chronically treated with triiodothyronine (hyperthyroid). Cardiac particulate fractions were incubated with a series of concentrations of [3H]quinuclidinyl benzilate and specific binding was determined as described in Table I. The lines for hypothyroid rat (r = 0.86) and hyperthyroid rat (r = 0.91) heart particulate fractions were determined by linear regression analysis. Each point represents the mean of triplicate determinations. The negative reciprocal of the slope provides an estimate of the equilibrium dissociation constant (Kᵦ) for the interaction of [3H]quinuclidinyl benzilate with the binding sites, and for both, the value of Kᵦ was found to be 1 nM. The maximal number of binding sites (B_max) for hypothyroid and hyperthyroid rat heart preparations were found to be 134 and 85 fmol/mg of protein, respectively.

**Fig. 2.** Reciprocal plot of [3H]quinuclidinyl benzilate binding to cardiac particulate fractions from thyroidectomized (hypothyroid) rats and thyroidectomized rats chronically treated with triiodothyronine (hyperthyroid). Cardiac particulate fractions were incubated with a series of concentrations of [3H]quinuclidinyl benzilate and specific binding was determined as described in Table I. The lines for hypothyroid rat (r = 0.99) and hyperthyroid rat (r = 0.99) heart particulate fractions were determined by linear regression analysis. Each point represents the mean of triplicate determinations and some are averages of two experiments run in triplicate. The equilibrium dissociation constant for both the preparations was found to be 1 nM and the maximal number of binding sites for hypothyroid and hyperthyroid rat heart preparations were found to be 133.3 and 77 fmol/mg of protein, respectively.
Regulation of Muscarinic Receptors by Thyroid Hormone

Alterations in specific \(^{3}H\)quinuclidinyl benzilate binding in different thyroid status may be due to changes in the number or affinity of muscarinic cholinergic receptors, or both. The number and affinity of \(^{3}H\)quinuclidinyl benzilate binding sites in cardiac particulate fractions from thyroidectomized and triiodothyronine-treated thyroidectomized rats were assessed by Scatchard analysis (Fig. 1) and reciprocal plots (Fig. 2). Treatment of rats with triiodothyronine resulted in a decrease in number of binding sites from 134 to 85 fmol/mg of protein (Fig. 1). No significant alterations in affinity of receptors for the muscarinic antagonist, quinuclidinyl benzilate, could be detected (Figs. 1 and 2).

In conclusion, hyperthyroidism decreases and hypothyroidism increases muscarinic receptors for acetylcholine on cardiac plasma membranes. This may help to provide a biochemical explanation for the inability of previous workers to reverse cardiovascular effects of triiodothyronine by \(\beta\)-adrenergic receptor antagonists (8). Although secretion of the autonomic nervous system is known to influence intrinsic activity of the cardiac muscle (11), the results of this study and our previous report (14) provide the first direct demonstration that intrinsic activity of cardiac cells may be regulated in part by the alterations in the number of postjunctional neurotransmitter receptors on the surface of these cells. Finally, the regulation of number of membrane receptors by thyroid hormones has been suggested to involve protein synthesis (13, 20). A similar mechanism may not be involved in the regulation of muscarinic cholinergic receptors by thyroid hormones, unless protein synthesis is required in the degradation of these receptors.

REFERENCES
