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Effect of propylthiouracil treatment on the regulation of adenylate cyclase activity in rat myocardium *

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Thyroid hormones have been shown to exert permissive action on biological responses to catecholamines [1, 2]. In heart, contractile and metabolic responses are decreased in hypothyroidism [3, 4], whereas hearts from hyperthyroid animals are more sensitive to adrenergic stimulation [5–8]. Furthermore, hypothyroidism leads to reduced sensitivity of β-adrenergic stimulation in atria and liver [9] and in ventricles [10, 11]; thyroxine treatment of these animals returns the sensitivity of β-receptors to normal levels [10, 11]. Recently, Williams and Lefkowitz [12], using (-)-[^{3}H]dihydroalprenolol, have shown that the number of β-adrenergic receptors in hyperthyroid rat heart membranes is increased significantly, and have concluded that the increased number of receptors may be responsible, at least in part, for the enhanced catecholamine sensitivity of β-adrenergic coupled cardiac responses in the hyperthyroid state. Alternatively, the altered physiological response can be attributed to a change in the responsiveness of adenylate cyclase and, in turn, of the cellular cyclic AMP levels. Although several attempts have been made to elucidate the relationship between thyroid hormone and β-adrenergic receptor sensitivity [9–11], very little information is available on the mechanism of regulation of adenylate cyclase by thyroid hormone and the role of guanine nucleotide in this process. In order to gain an insight into the possible role of thyroid hormones in the regulation of adenylate cyclase, we have studied the stimulation of adenylate cyclase in myocardium of normal and 6-propyl-2-thiouracil (PTU)-treated rats. Our results show that stimulation by GTP and Gpp(NH)p of adenylate cyclase activity, measured in the absence and presence of isoproterenol, is significantly low in PTU-treated rat heart, whereas basal, solubilized and fluoride-stimulated activities remains unchanged. These data suggest that thyroid hormones modulate the guanine nucleotide stimulation of adenylate cyclase enzyme.

Materials. 6-Propyl-2-thiouracil (PTU) was purchased from ICN Pharmaceuticals. The source of other chemicals and radiochemicals was the same as described earlier [13]. Male albino rats, weighing 90–120 g, were purchased from the Holtzman Co., Madison, WI, and were divided into control and treated groups. The treated rats were given subcutaneous injections of 20 mg PTU daily for 21 days before they were killed.

Enzyme preparations. Rats were killed by heart puncture while they were under light ether anesthesia. The hearts were quickly removed and transferred to homogenizing buffer (0.25 M sucrose in 0.05 M Tris–HCl, pH 7.4). The ventricles were dissected free of atria and washed with cold homogenizing buffer to get rid of blood. The tissue was homogenized, in 7–10 vol. of cold homogenizing buffer with a polytron (Brinkman) at a rheostat setting of 3, for 10 sec. The homogenate was filtered through two layers of cheesecloth and centrifuged at 8500 *g* for 10 min to yield the pelleted crude plasma membranes. Pelleted membrane fraction was suspended in 0.025 M Tris–HCl, pH 7.4, for assaying of adenylate cyclase activity and GTP binding. Soluble adenylate cyclase was prepared as described earlier [14].

Adenylate cyclase assay. Adenylate cyclase activity was measured according to the procedure of Salomon *et al.* [13] as described previously [14]. The activity present in the tubes without tissue protein was subtracted from the experimental values. All assays were performed under conditions of linearity with respect to protein concentration and time of incubation.

GTP binding assay. GTP binding was carried out according to Salomon and Rodbell [15], with slight modifications, in a final volume of 100 μl containing 25 mM Tris–HCl, pH 7.4, 1 mM dithiothreitol, 1 mM EDTA, 10 μM cyclic AMP, and 50 nM to 50 μM [^{3}H]GTP; 40–50 μg of cardiac membrane was added to start the reaction. Incubations were carried out at 30° for 5 min, and terminated by adding 3.0 ml of ice-cold 25 mM Tris–HCl, pH 7.4, and filtering the mixture immediately through Millipore filters (HA, 0.45 μm). The filters were washed three times with the same buffer, dried in the oven at 80° and counted in Econo fluor (NEN). Two types of controls were used to correct the nonspecific binding to membranes and Millipore filters. In one control, 1 mM unlabeled GTP was added along with labeled nucleotide and cardiac membranes, whereas in the second set the plasma membranes were omitted from the incubation medium. Both controls showed 0.25 to 0.5 per cent binding of the total radioactivity added to the assay medium.

Protein was measured by the method of Lowry *et al.* [16] with bovine serum albumin as standard. Appropriate blanks were used to correct for Tris and Lubrol-PX interference in Lowry's method.

Effects of GTP and isoproterenol on myocardial adenylate cyclase activity. The effects of GTP (100 μM), Gpp(NH)p (10 μM) and isoproterenol (10 μM) on myocardial membrane adenylate cyclase were studied for PTU-treated and control rats (Table 1). Because of the day to day experimental variation in basal and agonist-stimulated values, data from four different experiments were normalized by calculating percent stimulation, keeping basal values at 100 per cent. The percentage stimulation by various agonists was then subjected to Student's *t*-test. The results, given in Table 1, show that there was a significant decrease in stimulation of adenylate cyclase ($P < .05$) by GTP, Gpp(NH)p or isoproterenol in PTU-treated rats. Although isoproterenol plus Gpp(NH)p stimulation was not significantly different in PTU and control rats, the percentage stimulation was consistently low in PTU groups.

[^{3}H]GTP binding to myocardial membranes. Previous studies have shown that GTP and Gpp(NH)p bind to myocardial plasma membranes and stimulate adenylate cyclase activity [17]. In this study, we have measured the binding of [^{3}H]GTP under adenylate cyclase assay conditions in PTU-treated and control rat cardiac membranes. Our results suggest that the apparent affinities of GTP binding did not differ in PTU-treated and control rats (0.55 to 0.6 μM). As determined from the ordinate intercept, the number of binding sites observed for GTP was 75–80 pmoles/mg of membrane protein in both control and PTU-treated rats.

Effect of Lubrol-PX solubilization on basal and fluoride-stimulated myocardial adenylate cyclase activity. To test the influence of membrane environment on adenylate cyclase activity, the membranes were solubilized in the nonionic detergent Lubrol-PX. The protein solubilized from control and PTU-treated myocardial membranes was 12–15 per cent, showing no differences in the extent of solubilization. The

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Table 1. Stimulation of myocardial adenylate cyclase from normal and PTU-treated rats*

Agonist	Normal (Pmoles cAMP formed/mg protein/10 min)	PTU-treated (Pmoles cAMP formed/mg protein/10 min)
None	41.2 ± 4.4 (100)	41.6 ± 6.2 (100)
Isoproterenol (10 μM)	63.5 ± 7.4 (157 ± 18)	51.1 ± 7.4 (124 ± 7.7)†
Gpp(NH)p (10 μM)	115.4 ± 19.0 (279 ± 25)	77.2 ± 18.0 (180 ± 19.4)†
GTP (100 μM)	61.6 ± 7.0 (151 ± 8.9)	46.5 ± 5.0 (116 ± 11.0)†
Isoproterenol (10 μM) + Gpp(NH)p (10 μM)	179.2 ± 34.0 (439 ± 75.5)	147.2 ± 39.5 (357 ± 69.9)

* Adenylate cyclase was assayed under standard assay conditions described in Experimental Procedure. Values represent means ± S.E. of four different experiments. Statistical analysis was performed by Student's *t*-test, using the percentage stimulation over basal values presented in parentheses.

† Significantly different ($P < 0.05$) from the normal control values.

basal and fluoride-stimulated activities in PTU-treated and control rat myocardial membranes as well as Lubrol-PX soluble enzyme were comparable, showing that both basal and fluoride activities are unaltered in PTU-treated rats (Table 2).

In this communication, the activities of adenylate cyclase in the particulate and the solubilized preparations from the hearts of PTU-treated and control rats were compared. In the particulate preparations, when basal and fluoride-stimulated activities were compared, no differences were observed in the control and PTU-treated groups. However, the stimulation of adenylate cyclase by GTP, Gpp(NH) or isoproterenol was consistently decreased in the PTU-treated group, and the magnitude of the difference was markedly increased when Gpp(NH)p and isoproterenol were studied in combination. This apparent decrease in guanine nucleotide- and catecholamine-stimulated enzyme activity, with no change in basal or fluoride-stimulated activity, would suggest that possibly the receptor or the coupler moiety of adenylate cyclase is altered in the PTU-treated groups without any change in the catalytic unit. To test this hypothesis further, the membranes were solubilized by Lubrol-PX. The solubilization leads to dissociation between the receptor molecules and the catalytic moiety [18]. When basal and fluoride-stimulated activities were compared in the soluble enzyme, once again, no differences were observed in the two treatment groups. These observations further support the contention that the mechanism for loss in enzyme stimulability could be due to a change in either of these three sites, i.e. (1) receptor affinity or number (2) nucleotide site, and/or (3) coupler unit.

Studies carried out in the past on receptor binding suggest that, although the affinity of binding remains the same, the number of β-adrenergic receptors decreases in hypothyroidism [10, 19] and increased in hyperthyroidism [12], suggesting a possible link between the circulating thyroid hormone level and the number of β-adrenergic receptors. These observations may, in part, explain our findings of reduced stimulability by isoproterenol of myocardial adenylate cyclase in PTU-treated animals.

It is difficult to explain the reduced GTP stimulation of adenylate cyclase activity in PTU-treated rat myocardium in view of no change in the affinity and number of binding sites. A possible explanation could be that the GTP-binding site is an integral part of the β-adrenergic receptor–adenylate cyclase complex [20]. Therefore, a reduction in the number of β-adrenergic receptor sites in PTU-treated animals could result in reduced stimulation by GTP without affecting the GTP-binding sites. It would be interesting to examine if the replacement therapy with T_3 or T_4 could restore the GTP and isoproterenol stimulation in the PTU-treated group.

In summary, the effects of hypothyroidism induced by propylthiouracil (PTU) injections (20 mg/day for 21 days) on the adenylate cyclase activity in myocardial membranes were investigated. GTP and Gpp(NH)p stimulation in the absence and presence of isoproterenol was reduced significantly ($P < .05$) in the myocardial membranes of PTU-treated rats. However, the binding constants (0.55 to 0.60 μM) and number of binding sites (75–80 pmoles/mg of protein) for [^{3}H]GTP were similar in PTU-treated and control rat myocardial membranes. Basal and fluoride-stimulated

Table 2. Adenylate cyclase activities in the myocardial membranes of normal and PTU-treated rats*

Preparation	Animals	Adenylate cyclase activities (pmoles cAMP formed/10 min/mg protein) Basal	Fluoride (10 mM)
Membranes (8500 *g* pellet of homogenate)	Control	62 ± 5	578 ± 20
	PTU-treated	65 ± 8	572 ± 36
Lubrol-PX solubilized enzyme	Control	464 ± 12	3667 ± 68
	PTU-treated	437 ± 19	3786 ± 54

* Membranes were solubilized as described under Experimental Procedure. Adenylate cyclase was assayed under standard conditions described in the text. Values are the means ± S.E. The means are from three different experiments in triplicate.

activities were similar in the two types of animals. Similarly, Lubrol-PX soluble adenylate cyclase activity was comparable in treated and control groups, suggesting that the catalytic moiety is not altered in the treated group. Specific reduction in isoproterenol and guanine nucleotide stimulation of adenylate cyclase in hypothyroid rat heart may be due to the changes at the receptor or coupler site of the adenylate–cyclase complex.

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