THYROXINE AND PROPYLTHIOURACIL EFFECTS IN VIVO ON ALPHA AND BETA ADRENERGIC RECEPTORS IN RAT HEART

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SUMMARY: Dihydroalprenolol and dihydroergocryptine were used to measure β and α adrenergic receptors respectively in heart ventricles from control, thyroxine (T_4)-treated and propylthiouracil (PTU)-treated rats. Ventricles from T_4-treated rats show an increase in the number of β receptors and a decrease in the number of α receptors. The β to α receptor ratio increases six fold. No change in binding affinity of the β receptor is observed but a decrease occurs in the affinity of the α receptor in ventricles from T_4-treated hearts. Ventricles from PTU-treated hearts show a small decrease in the number of β receptors but a large decrease in the number of α receptors. The binding affinity for both the α and β receptor is increased in the PTU-treated rats. The total number of α plus β receptors is increased in the T_4-treated rats and decreased in the PTU-treated rats.

INTRODUCTION: Thyroid hormones modulate or exert a permissive action on the biological responses to catecholamine hormones (1-3). Hyperthyroidism leads to a state resembling adrenergic overactivity. In heart, contractile and metabolic responses are decreased in hypothyroidism (4-5), whereas hyperthyroid hearts are more sensitive to adrenergic stimulation (6-10). In the hypothyroid heart, the receptor mediating the positive inotropic response appears to change from the normal β in the euthroid to α as determined by potency ratios of agonists and by a new susceptibility to inhibition by phentolamine (4-5). This α inotropic response acts independently of cAMP, unlike the β-response (11).

Adipocytes from hyperthyroid rats show an increased responsiveness to the lipolytic actions of catecholamines (12). Human and rat adipocytes from hypothyroid subjects are less sensitive to catecholamines (13-15).

Responses such as lipolysis, glycogenolysis and cardiac contractility which are enhanced in hyperthyroidism are those mediated mainly by β-adrenergic receptors (16). The decreased catecholamine responsiveness in hypothyroidism appears to be the combined effect of decreased β-receptor sensi-

tivity and increased α-receptor activity (17,18). The lipolytic effects of norepinephrine, a mixed α and β agonist, are diminished in hypothyroid fat cells but can be restored to near normal by addition of the α-blocking agent phentolamine (17,18), indicating an anti-lipolytic effect of α receptor stimulation.

The relationship between α and β adrenergic receptors and the biochemical mechanism whereby thyroxine influences the biological actions of catecholamines are not well understood. We wished to see if the alterations in adrenergic sensitivity elicited by thyroxine (T_4) were reflected in the affinity or number of α and β adrenergic receptors in ventricular membranes from the rat. For this study we used the specific binding of (-)-^{3}H-dihydroalprenolol (19,20) and ^{3}H-dihydroergocryptine (21) to measure β and α-adrenergic receptors respectively in ventricles from control, T_4-treated and propylthiouracil (PTU)*-treated rats. We find that the number and affinity of these receptors are altered by T_4 and PTU-treatment.

METHODS: Age and weight (140-160 gms) matched male Sprague-Dawley rats were divided into three groups containing six rats per group. Six control animals were maintained on a normal diet of Purina lab chow and water ad libitum. Six rats were injected subcutaneously with L-thyroxine (Sigma Chemical Co.) (75 μg/100 gms body wt) once daily for seven days. This method was used by Will-Shahab and Wollenberger (22) to produce hyperthyroid rats. The rats were sacrificed on the eighth day. Six rats were given 0.1% propylthiouracil in their drinking water for ten days. This method was used by Debons and Schwartz (23) to produce hypothyroid rats. The rats were sacrificed on the eleventh day. Hearts from T_4-treated rats increased in weight by 39% whereas those from PTU-treated rats showed a 6% decrease in weight. Fat pads from T_4-treated rats decreased in weight by 33% whereas fat pads from PTU-treated rats increased in weight by 12%. These findings provide some evidence that the thyroxine and propylthiouracil treatment did produce the desired effects on the rats. We used the same method of others (22,23) to produce hyperthyroid and hypothyroid rats, and although we did not measure T_4 or T_3 levels in these rats we believe it is fair to assume on the basis of the changes in heart and fat pad weights that the rats were indeed made hyperthyroid by T_4-treatment and made hypothyroid by propylthiouracil treatment.

A ventricular membrane fraction rich in adenylate cyclase activity was prepared by the method of Lefkowitz et.al. (19). Protein was determined by the method of Lowry et.al. (24).

* PTU represents propylthiouracil

Table 1

Specificity of Binding of (-)-^{3}H-Dihydroalprenolol and ^{3}H-Dihydroergocryptine to Rat Ventricle Membranes

	f moles bound/mg protein	
	(-)-^{3}H-Dihydroalprenolol	^{3}H-Dihydroergocryptine
Total binding	55.1 ± 10.3	142 ± 22.4
plus phentolamine, 10 μM	60.1 ± 11.6	75.6 ± 3.8
plus (-) - propranolol, 1 μM	39 ± 2.3	144 ± 9.1
plus (-)-alprenolol, 2 μM	26.8 ± 6.1	
plus (+) - alprenolol, 2 μM	51.1 ± 2.1	

Binding of (-)^{3}H-dihydroalprenolol (12.7 nM) and ^{3}H-dihydroergocryptine (11 nM) was carried out as described in the text.

RESULTS: The binding of (-)-^{3}H-dihydroalprenolol is stereospecific and has the characteristics of the β-adrenergic receptor in ventricular membranes of the dog (19,20). We find that the binding of (-)-^{3}H-dihydroalprenolol to rat ventricular membranes is sterospecifically inhibited by unlabeled (-) alprenolol and (-) propranolol (Table 1) and reaches equilibrium within 5 min. The binding of (-)-^{3}H dihydroalprenolol is not inhibited by the α-antagonist phentolamine (Table 1) and shows this binding to be a valid measure of β-receptors in these tissues. Our results agree with the work of Lefkowitz et.al. on dog heart (19,20).

A binding site with characteristics of the α-adrenergic receptor has been identified by Williams and Lefkowitz (21) in rat uterus using the α-antagonist ^{3}H-dihydroergocryptine. We have used this ligand to study α-receptors in rat heart ventricle membranes and have found a high-affinity specific binding site. In our studies binding to this site in ventricle membranes is inhibited by the α-antagonist phentolamine and not by the β-antagonist pro-

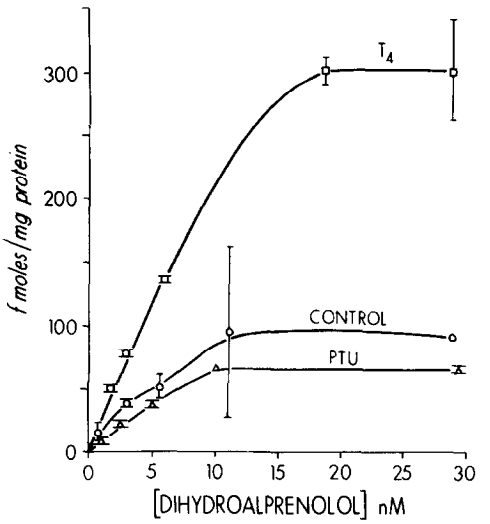

Fig. 1. Concentration dependence of the binding of (-)-^{3}H-dihydroalprenolol to ventricular membranes from rat heart. Ventricular membranes were incubated for 10 minutes at 37°C in a reaction volume of 150 µl. Each reaction contained: 75 mM Tris-HCl, pH 7.4, 25 mM $MgCl_2$, membranes (200-250 µg protein), and varying concentrations (10-100 nCi per reaction) of (-)-^{3}H-dihydroalprenolol (New England Nuclear, 30 Ci/mmole,). The reaction was stopped by the addition of 2 ml ice cold reaction buffer. The diluted mixtures were mixed and rapidly filtered through Whatman GFC glass fiber filters (19). Tubes were rinsed twice with 5 ml cold buffer and the rinses poured over the filters. The filters were washed two additional times with 5 ml of cold buffer. Nonspecific binding was corrected for by including 1 µM (-)-alprenolol (Hassle). Specific binding represented 85-90% of the total binding and is the difference between total binding and binding seen with added 1 µM (-) alprenolol. Binding in the text and figures refers to specific binding. Ventricles were prepared from 6 pooled rat hearts for each group. The points on the curves represent the mean ± SD of triplicate analyses.
O = control rats; ◻ = T_4 treated rats; Δ = PTU treated rats.

pranolol (Table 1). The binding of ^{3}H-dihydroergocryptine to ventricle membrane is rapid and saturable.

The stereospecific binding of (-)-^{3}H-dihydroalprenolol displayed a hyperbolic curve, with saturation occurring at a ligand concentration of 20 nM for control rats and between 10-15 nM for treated rats (Figure 1). In hearts from T_4-treated rats there is a marked increase in β receptors and a decrease in α receptors such that the β/α receptor ratio increases 6-fold (Table 2). There is also a decrease in affinity for the α-receptor

Table 2

Relationship of Alpha and Beta-Adrenergic Receptors from Ventricle Membranes of Control, T_4-Treated and PTU-Treated Rats.

Receptor Type	Binding Capacity (Vm) fmoles/mg protein			Binding Affinity (Km) nM		
	Control	T_4	PTU	Control	T_4	PTU
β	92	302	66	6.2	6.7	4.3
α	307	165*	52	4.5	18.5*	2.5
β/α ratio	0.3	1.83	1.27			
β + α	399	467*	118			

* Represent minimal values since saturation of binding was not attained over the concentration range studied. These values are calculated from the data in Figures 1 and 2.

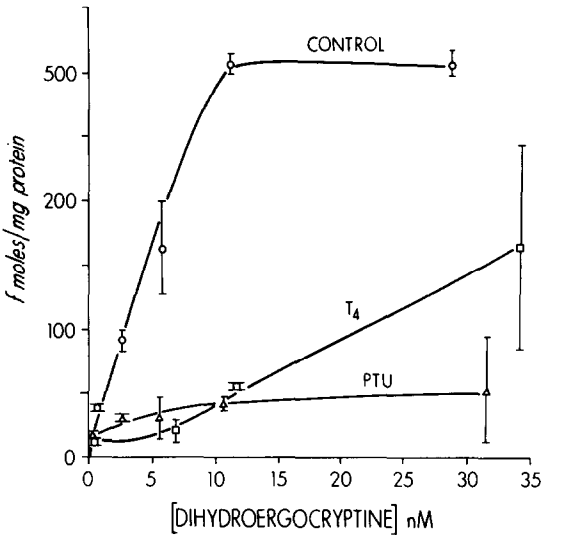

Fig. 2. Concentration dependence of ^{3}H-dihydroergocryptine binding to ventricular membranes from rat heart. The binding method employed was the same as described in Figure 1 with modification of the reaction conditions. Incubations were for 15 minutes at 25°C. The reaction system consisted of 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, membranes (200 µg protein) and varying concentrations (6.7 - 67 nCi per reaction) of ^{3}H-dihydroergocryptine (New England Nuclear, 21 Ci/mmole). Non-specific binding blanks were obtained by using 1 µM phentolamine. Specific binding represented 40-50% of the total binding. Ventricles were prepared from 6 pooled hearts for each group. The points on the curves represent the mean ± SD of triplicate analyses. O = control rats; ◘ = T_4 treated rats; Δ = PTU treated rats.

and no change in affinity of the β-receptor. These changes can be expected to lead to a much enhanced β-adrenergic response.

The profiles for the binding of dihydroergocryptine are shown in Figure 2. In the hearts from PTU-treated rats there is a small decrease in the number of β-receptors and a surprisingly large decrease in the number of α-receptors (Table 2). The β/α receptor ratio is increased 4-fold relative to the control rats but decreased relative to the T_4-treated animals. There appears to be a small compensatory increase in affinity of both the α and β receptor.

The change in receptor number and affinity may explain the enhanced physiologic β-adrenergic response in the T_4-treated rats. However our bind-

ing data does not explain the enhanced α-adrenergic response in hypothyroid (17,18) rats except for our finding that the ventricular membranes from PTU-treated animals have the smallest number of total α and β receptors.

Considering the observation that hearts from all three thyroid states are reported to be equally sensitive to exogenous cAMP or its analogues (12), the alteration in adrenergic sensitivity may be regulated by β-receptor concentration and affinity. Kunos and Nickerson propose that α and β receptors in rat heart may be interconverted by thyroid hormone (25). It is noteworthy that in the T_4-treated rats the increase in β-receptors is accompanied by a decrease in α-receptors over the concentration range studied, but there is also an increase in total number of α and β receptors as compared to the control animals.

Recent work has shown that the level of membrane receptors for a given hormone is regulated by the concentration of the hormone and varies inversely with hormone concentration (26,27). Our present work adds new insight into hormonal regulation since it suggests that one hormone can influence the number and affinity of the receptors for another hormone. It is of interest that more than one hormone can regulate the level of β-adrenergic receptors since Wolf et.al. (28) have found a 3 to 5 fold increase in β-receptors in rat liver after adrenalectomy and partial restoration to normal levels after administration of cortisone. Our studies show the advantage of measuring both α and β receptors with specific ligands to give a more complete understanding of the biochemical basis for adrenergic regulation of a given tissue. The finding that thyroxine binds to nuclear receptors (29-31) makes it likely that thyroxine can regulate the synthesis of adrenergic receptors. The thyroxine effect is not limited to adrenergic receptors since Madsen and Sonne (32) have reported recently an increase in glucagon receptors in fat cells from hyperthyroid rats.

Our observation on the increase in β-receptors in heart ventricle mem-

branes from T_4-treated rats has been confirmed in ventricle slices incubated in vitro with T_4 (33).

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(1) S. S. Waldstein, Ann. Rev. Medicine 17, 123 (1966).
(2) T. S. Harrison, Physiol. Rev. 44, 161 (1964).
(3) S. W. Spaulding and R. H. North, Med. Clin. N. Amer. 59, 1123 (1975).
(4) V. Felt, J. Nedvidkova, S. Hynie, B. Mosinger, M. Vavrinkova, Endokrinologie 66, 301 (1975).
(5) G. Kunos, I. Vermes-Kunos, M. Nickerson, Nature 250, 779 (1974).
(6) B. A. Young and J. H. McNeill, Can. J. Physiol. Pharmacol. 52, 375 (1974).
(7) H. Kaciuba-Uscilko, Z. Brzezinska, J. E. Greenleaf, Experimentia 32, 68 (1976).
(8) K. R. Hornbrook, P. V. Quinn, J. H. Siegel, T. M. Brody, Biochem. Pharmacol. 14, 925 (1965).
(9) D. A. K. Roncain and V. K. Murthy, J. Biol. Chem. 250, 4134 (1975).
(10) M. J. Fregly, E. L. Nelson, G. E. Resch, F. P. Field, L. O. Lutherer, Amer. J. Physiol. 229, 916 (1975).
(11) J. B. Osnes, Acta Pharmacol. et Toxicol. 39, 232 (1976).
(12) R. G. Van Inwegen, G. A. Robison, W. J. Thompson, K. J. Armstrong, J. E. Stouffer, J. Biol. Chem. 250, 2452 (1975).
(13) U. Rosenqvist, S. Efendic, B. Jereb, J. Ostman, Acta Med. Scand. 189, 381 (1971).
(14) G. A. Bray, Endocrinology 79, 554 (1966).
(15) G. Krishna, S. Hynie, B. B. Brodie, Proc. Natl. Acad. Sci. USA 59, 884 (1968).
(16) R. P. Ahlquist, Amer. J. Physiol. 135, 586 (1948).
(17) U. Rosenqvist, Acta Med. Scand. 192, 353 (1972).
(18) J. P. D. Reckless, C. H. Gilbert, D. J. Galton, J. Endocr. 68, 419 (1976).
(19) R. J. Lefkowitz, Biochem. Pharmacol. 24, 1651 (1975).
(20) R. W. Alexander, L. T. Williams, R. J. Lefkowitz, Proc. Natl. Acad. Sci. USA, 72, 1564 (1975).
(21) L. T. Williams and R. J. Lefkowitz, Science 192, 791 (1976).
(22) D. Will-Shahab and A. Wollenberger, Acta Biol. Med. Germ. 32, K 1 (1974).
(23) A. F. Debons and I. A. Schwartz, J. Lipid Res. 2, 86 (1961).
(24) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem. 193, 265 (1951).
(25) G. Kunos and M. Nickerson, J. Physiol. 256, 23 (1976).
(26) J. V. Mickey, R. Tate, D. Mullikin, R. J. Lefkowitz, Mol. Pharmacol. 12, 409 (1976).
(27) M. A. Lesniak and J. Roth, J. Biol. Chem. 251, 3730 (1976).
(28) B. B. Wolfe, T. K. Harden, P. B. Molinoff, Proc. Natl. Acad. Sci. USA 73, 1343 (1976).
(29) M. I. Surks, D. Koerner, W. Dillman and J. H. Oppenheimer, J. Biol. Chem. 248, 7066 (1973).
(30) H. H. Samuels, J. S. Tsai, J. Casonova, and J. Stanley, J. Clin. Invest. 54, 853 (1974).
(31) M. A. Charles, G. U. Ryffel, O. Obinata, B. J. McCarthy and J. B. Baxter, Proc. Natl. Acad. Sci. USA 72, 1787 (1975).
(32) S. N. Madsen and O. Sonne, Nature 262, 793 (1976).
(33) S. Kempson and G. V. Marinetti (work in progress).