EFFECT OF HYPOTHYROID STATUS ON MYOCARDIAL RESPONSES TO SYMPATHOMIMETIC DRUGS

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The physiological relationship between thyroid hormone and the catecholamines has been investigated for many years (1, 2). In spite of numerous studies it is still not clear whether thyroid status alters the sensitivity of the heart to catecholamines. Coville (3) has reported that thyroid hormone increased the magnitude of the response to catecholamines, while some authors have failed to show that altering the thyroid status affects the response to catecholamines (4, 5). Recently, it was tried to explore some effects of thyroidectomy on the response of alpha and beta adrenergic receptor mechanisms to catecholamines (6). A few workers have reported the existence of both alpha and beta receptors in the hearts of mammals (7, 8).

Present studies reported here were made in an attempt to clarify some of the discrepancies in many reports by seeking a quantitative difference between different types of cardiac adrenergic receptor mechanisms after 6-propyl-2-thiouracil (PTU) treatment.

METHODS

Male Wistar strain rats (170 to 200 g) were used for experiments. The rats were divided into two groups. Group A was served as control. Group B was in a hypothyroid state produced by feeding on 0.15% PTU diet for 6 to 8 weeks. This group developed dryness of fur, slow heart rate and a great retardation in growth.

Each rat was killed by a blow on the head and the heart was rapidly removed and the left atrium dissected free. Isolated left atria were suspended in a 25-ml tissue bath containing Ringer solution of the following composition (mM): NaCl, 158; KCl, 5.6; CaCl₂, 3.6; NaHCO₃, 3.6; and glucose, 15.6. The solution was maintained at a temperature of 30±0.5°C and saturated with pure oxygen. The preparations were driven at a rate of 180 beats/min. through an electrode by a square wave pulse of 6 msec duration and voltage of approximately twice threshold delivered by a stimulator (Nihon Kohden MSE-3). A force displacement transducer (Nihon Kohden SB-1T) was used for measurement of isometric contractile force which was recorded by an ink writing oscillograph. A resting tension of 0.5 g was placed on each atrium. The preparations were allowed to equilibrate in Ringer solution for about 60 min. before exposing them to drugs. Drugs were dissolved before use in 0.9% saline. The drugs used in these experiments were 1-phenylephrine hydrochloride, 1-isoproterenol
hydrochloride, phentolamine hydrochloride and propranolol hydrochloride. Drug concentrations refer to the final concentration in the tissue bath. Analysis of data for significance was performed by means of the Student's t test.

RESULTS

Fig. 1 shows effects of phenylephrine (PHE) and isoproterenol (ISO) on contractile force of atria from groups A and B. Eleven control and 11 PTU fed rats were used for PHE experiment and 10 and 7 for ISO. The magnitude of changes was expressed as percent change to the amplitude existing just prior to the addition of the drugs.

The increases in contractile force produced by PHE of graded doses in PTU fed rats were significantly greater than those obtained in control rats (p<0.005 for each) (Fig. 1A). The positive inotropic effect of ISO decreased at lower concentrations than $3 \times 10^{-7}$ M in PTU fed rats (p<0.025 for each) (Fig. 1B).

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**Fig. 1.** Dose response curves for the inotropic action of PHE (A) and ISO (B) on atria from control and PTU fed rats.

- control rats,
- PTU fed rats

The curves of this figure and Fig. 2 were obtained by the intermittent dose method. Each point represents a mean value. The standard error of the mean is indicated by vertical bars. Ordinate: percentage of changes of contractile amplitude relative to those existing prior to addition of PHE or ISO, abscissa: molar concentrations of PHE or ISO on a logarithmic scale.
FIG. 2. Dose response curves for the inotropic action of PHE and ISO on atria from control and PTU fed rats.
- PHE on control rats, ○ PHE on PTU fed rats
- ISO on control rats, △ ISO on PTU fed rats
Ordinate: effects as percentage of maximal response to PHE or ISO.

L-Phenylephrine and L-isoproterenol (M)

FIG. 3. The effects of phentolamine and propranolol on the dose response curve of PHE in control (A) and PTU fed rats (B).

- PHE alone,
  × PHE with phentolamine $3 \times 10^{-7}$ M
  □ PHE with propranolol $3 \times 10^{-7}$ M
  ■ PHE with phentolamine $3 \times 10^{-7}$ M and propranolol $3 \times 10^{-7}$ M

The curves of this figure and Fig. 4 were obtained by the cumulative dose method. Ordinate: percent increase in contractile force.
As shown in Fig. 2, increase in contractile force produced by each molar concentration calculated as a percentage of the maximum effect of PHE and ISO, respectively, was plotted against the corresponding concentration. The dose response curve of PHE was significantly shifted to the left at ED_{50} level after PTU treatment (p<0.005). On the other hand, the dose response curve of ISO was shifted to the right (p<0.005).

Effects of alpha and beta blockers on the positive inotropic response to PHE were examined. Phentolamine (3 x 10^{-7} M), an alpha blocker, or propranolol (3 x 10^{-7} M), a beta blocker was allowed to interact with the tissue for 30 minutes before the addition of PHE. When phentolamine was used in combination with propranolol, both drugs were added simultaneously. Fig. 3 shows the effects of the alpha and beta blockers on the dose response curves of PHE obtained in the isolated atria from control (A) and PTU fed rats (B). The numbers of control rats used for these experiments were 7 for each phentolamine and propranolol experiment and 5 for combination of phentolamine and propranolol. The numbers of PTU fed rats were 6, 4 and 5, respectively.

In control rats, phentolamine depressed significantly the responses to 10^{-6} and 3 x 10^{-6} M PHE (p<0.005), but unaffected the responses to higher concentrations of PHE (p>0.05). Propranolol did not affect the effects of lower concentrations of PHE, but depressed that of higher concentrations. The combined effects of two blockers were additive in inhibiting of PHE effects in all cases.

In PTU fed rats, the dose response curve of PHE was unaffected by propranolol, but it was significantly affected by phentolamine. Effects of the combination of phentolamine and propranolol were a little more remarkable than those of phentolamine alone.

In Fig. 4, height of contraction produced by each molar concentration, calculated as a percentage of the maximum effect of PHE alone in each isolated atria was plotted against the

![Fig. 4. The effects of phentolamine and propranolol on the dose response curve of PHE in control (A) and PTU fed rats (B). Ordinate: effects as percentage of maximal response to PHE alone.](image-url)
logarithm of the corresponding concentration. The standard error was calculated in each molar concentration. The effects of both adrenergic blocking agents were shown more clearly in Fig. 4 than in Fig. 3.

DISCUSSION

It has been generally considered that the adrenergic receptors of the myocardium are entirely of beta type. However, PHE, which is believed to affect directly only alpha adrenergic receptors (9), was found to produce a positive inotropic action in the guinea pig (8) and rabbit (7, 10, 11) atria. Daly et al. (12) found that PHE released tritiated norepinephrine from the mouse heart. Govier (8) reported that the positive inotropic response to PHE in electrically driven guinea pig atria was specifically blocked by phentolamine or phenoxybenzamine, and he concluded that alpha adrenergic receptors are present in the myocardium. Benfey et al. (7) also observed that propranolol did not inhibit the action of PHE on contractility of rabbit atria; whereas phentolamine inhibited the effects of low concentrations of PHE. In contrast, Lee and Yoo (10, 11) reported that the positive inotropic action of PHE was effectively blocked by dichloroisoproterenol and propranolol and not altered by phenoxybenzamine and phentolamine on the isolated rabbit atria. They considered that PHE exerts its cardiostimulant action through the beta adrenergic receptor in the heart.

In present experiments, PTU treatment potentiated the positive inotropic response to PHE in the rat atria, but decreased the sensitivity to ISO. These phenomena suggest that positive inotropic action of PHE in hypothyroid rats is not ascribable to its action on beta adrenergic receptor. In control rats, the positive inotropic effects of lower concentrations of PHE are selectively blocked by an alpha blocking agent, phentolamine, but not by a beta blocking agent, propranolol. On the contrary, the effects of higher concentrations of PHE are blocked by propranolol, but not by phentolamine. In guinea pigs, similar results have been reported by Govier (8). In PTU fed rats, however, the potentiated positive inotropic effect of PHE could be always antagonized by phentolamine, but not by propranolol. These phenomena suggest that the positive inotropic effects mediated by alpha adrenergic receptor in the atria become more marked after PTU treatment.

Adrenergic mechanisms in the heart are generally considered purely excitatory and classified as the beta receptor type (13–15). Recently, several workers (16–19) have reported the existence of both alpha and beta receptors in the hearts of mammals. On the basis of our observations, it was concluded that alpha adrenergic receptors play some role in the inotropic effect of PHE and that PHE has both alpha and beta stimulant actions.

The interaction between alpha and beta blocking agents has been reported by many authors (20–22). In our experiments, when phentolamine and propranolol were added simultaneously to the tissue bath, no antagonism was found between both drugs and rather the combination produced a blockade of PHE response greater than that by phentolamine or propranolol alone in both control and PTU fed rats.

Privitera et al. (23) reported that PHE did not produce an oxygen wasting effect. Govier (8) suggested the possibility that activation of myocardial alpha adrenergic receptors
may be a useful means of increasing myocardial force of contraction without the increase in myocardial oxygen uptake due to a direct stimulation of myocardial metabolism. And he also suggested that an examination of a sufficiently potent and selective alpha stimulating sympathomimetic agent under conditions of cardiac failure would be of interest. While, it has been reported that the deliberate induction of hypothyroid status was useful for treatment of patients with angina pectoris or congestive heart failure (24, 25). The present results may give more informations for the treatment of congestive heart failure.

SUMMARY

1. The dose response curve for the positive inotropic action of PHE was significantly shifted to the left after PTU treatment.

2. In control rats, phentolamine inhibited partially the effect of lower concentrations of PHE and propranolol inhibited partially the effect of higher concentrations of PHE.

3. In PTU fed rats, the response to PHE was profoundly depressed by phentolamine, but not by propranolol.

4. The dose response curve of ISO was shifted to the right after PTU treatment.

The present results suggest: 1) the existence and function of myocardial alpha adrenergic receptors and 2) the cardiotimultant action of PHE is due to a direct effect on both alpha and beta adrenergic receptors, and 3) positive inotropic response mediated by alpha adrenergic receptor becomes more sensitive after PTU treatment, while that mediated by beta adrenergic receptor less sensitive.

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PHENYLEPHRINE IN HYPOTHYROID STATUS