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ENHANCEMENT BY THYROID HORMONE TREATMENT OF NOREPINEPHRINE-INDUCED PHOSPHORYLASE ACTIVATION IN THE RAT HEART*

K. R. HORNBROOK† and A. CABRAL

Department of Pharmacology, Division of Basic Health Sciences,
Emory University, Atlanta, Ga. 30322, U.S.A.

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Abstract—The effects of norepinephrine on the activity of phosphorylase *a*, the concentrations of creatine-P and ATP, and contractile amplitude were determined in isolated perfused hearts from normal rats and rats given triiodothyronine (100 μg/day) for 3 days. The hearts were perfused with oxygenated salt solution at a constant rate of flow at either 30° or 37°. Hearts of both euthyroid and hyperthyroid rats had control activities of phosphorylase *a* equal to about 10 per cent of the total enzyme activity. The concentration of creatine-P was lower in hearts of triiodothyronine-treated rats than in those of euthyroid rats whether perfusions were done at 30° or at 37°. Graded doses of norepinephrine caused a greater increase of the percentage phosphorylase *a* in hearts from hyperthyroid rats than in those from euthyroid rats at both perfusion temperatures, but the contractile amplitude response was not potentiated. After norepinephrine, the concentration of creatine-P was decreased in hearts from both euthyroid and hyperthyroid rats when perfused at 37°, but not when perfused at 30°. However, at both temperatures, there was a correlation between the concentration of creatine-P and the activation of phosphorylase produced by norepinephrine. In addition, nonperfused hearts from triiodothyronine-treated rats, which have been found previously to have elevated activities of phosphorylase *a*, had the lowest concentrations of creatine-P of the conditions tested. These relationships are consistent with the hypothesis that thyroid hormone has no special effect on the cardiac adrenergic receptor, and that the greater activation of phosphorylase produced by norepinephrine in hearts of hyperthyroid rats may be due in part to increased phosphorylase *b* kinase activity resulting from lower concentrations of creatine-P.

TREATMENT of rats with thyroid hormones produces an increased activity of phosphorylase *a* in the heart and apparent potentiation of catecholamine-induced activation of phosphorylase.[1–3] However, the mechanisms of these interactions are not clear. Neither enhanced activity of the sympathetic nervous system[4] nor increased responsiveness of adrenergic receptors appears to be an adequate explanation for these effects of thyroid hormone treatment. The myocardial concentration of cyclic 3′,5′-AMP is not elevated by thyroid hormone treatment,[5,6] and adenyl cyclase preparations from hearts of hyperthyroid rats[6] or cats[7] do not have an increased sensitivity to norepinephrine. In addition, neither the inotropic nor the chronotropic responses produced by catecholamines are potentiated by thyroid hormone treatment of cats, rats, man or dogs.[8–13] Interpretation of the phosphorylase response found in rat heart is further complicated by the findings of Hickenbottom and Mayer (cited in Mayer[14])

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that phosphorylase activation produced by norepinephrine is not enhanced in hearts of hyperthyroid dogs.

Wurtman *et al.*[15,16] have suggested that potentiation of catecholamine responses in hearts of thyroid hormone-treated rats is caused by increased delivery of the catecholamine to the heart and by decreased uptake of catecholamines by nerve endings in the hypertrophied heart. However, McNeill and Brody[3] discounted the importance of diminished neuronal uptake as a mechanism for the potentiation produced by thyroid hormone treatment, since they found that the effects of isoproterenol, an amine not actively taken up by nerve endings, were enhanced as much as those produced by norepinephrine. Their experiments, however, did not rule out increased delivery of drug as a mechanism for the apparent potentiation.

In our experiments, hearts from euthyroid and hyperthyroid rats were perfused at a constant rate of flow to eliminate possible differences in delivery of catecholamine. The activity of phosphorylase, concentrations of ATP and creatine-P and contractile amplitude were determined with and without the administration of norepinephrine to assess the possibility that changes in the concentration of high energy phosphate compounds might account for the enhanced responsiveness of phosphorylase found in hearts of hyperthyroid rats. Also, the concentrations of creatine-P and ATP were determined in hearts of euthyroid and hyperthyroid rats frozen immediately after excision and these were compared with the concentrations found in perfused hearts.

METHODS

For each experiment, male Holtzman rats weighing 225–250 g were divided into two groups. The rats in one group were injected s.c. for 3 consecutive days with 100 μg of 3′,3,5-triiodothyronine dissolved in 0·9% NaCl with NaOH. The rats in the control group were given equivalent volumes of 0·9% NaCl–NaOH solution. In initial experiments, the effectiveness of triiodothyronine treatment was determined by measuring the oxygen consumption of control and drug-treated rats by the method of Holtkamp *et al.*[17] Since the increase in oxygen consumption was always associated with a loss of body weight, only changes in body weight were used to evaluate triiodothyronine treatment in later experiments.

Animals were used on the day after the third injection of triiodothyronine or control solution. For perfusion experiments, the rats were given 2 mg heparin i.p., and were then anesthetized with ether. Hearts were excised and placed in ice-cooled perfusion solution; a glass cannula was tied into the ascending aorta, which was then connected to a temperature-controlled perfusion apparatus. The perfusion solution was delivered to the heart at a rate of 7 ml/min by means of a Holter pump. The perfusion solution was that described by Namm *et al.*[18] and the reservoir was gassed continuously with 95% O_2–5% CO_2. Depending on the experiment, the temperature of the solution at the aortic cannula was maintained at either 30° or 37°. Hearts were perfused for at least 30 min before other procedures were done.

Norepinephrine or radionuclides were injected rapidly 10 cm above the tip of the aortic cannula in 0·2 ml of perfusion solution. Apparent contractile force and the rate of perfused hearts were measured by tying a Teflon-coated string through the apex and connecting the string by a pulley to a force-displacement transducer. The resting tension on the hearts was 1 g. Heart rates were determined from these recordings. After the administration of norepinephrine to hearts perfused at 37°, the time to peak

increase in contractile force averaged 13 sec for euthyroid rats and 16 sec for triiodothyronine-treated rats; for hearts perfused at 30°, the times were 20 sec for euthyroid rats and 22 sec for hyperthyroid rats. Hearts were frozen while still attached to the perfusion system by compression between two silver blocks cooled to the temperature of liquid nitrogen. Hearts perfused at 37° were frozen 20 sec after injection. This time of sampling corresponds to the peak phosphorylase activation found after administration of catecholamines either to hearts perfused at a similar rate of flow[18] or in hearts perfused at constant pressure.[19] Hearts perfused at 30° were frozen 25 sec after injection, since the time to peak contractile force was longer at this temperature.

For experiments in which hearts were excised and frozen without perfusion, rats were anesthetized with 50 mg/kg of pentobarbital and their hearts were frozen in dichlorodifluoromethane cooled to its melting point (−158°) in liquid nitrogen.

Frozen heart samples were stored at −80°, and samples of ventricle were cut and weighed at −20° just prior to extraction for assays of phosphorylase activity or concentration of metabolites.

Biochemical methods. Phosphorylase activity was determined in the direction of glycogen synthesis by measuring production of orthophosphate from glucose 1-phosphate in the presence or absence of added 5′-AMP (0·5 mM). Homogenates of cardiac muscle were prepared and incubated as described by Diamond and Brody,[20] except that 50 mM beta-glycerophosphate-NaOH (pH 6·8) was used and the incubation temperature was 30°. The rate of inorganic phosphate production was linear with both time of incubation and concentration of tissue. Inorganic phosphate was determined spectrophotometrically by the method of Fiske and Subbarow,[21] as modified by Buell and Hansen.[22] Per cent phosphorylase *a* was calculated as 100 times the ratio of the phosphorylase activity in the absence of AMP to the activity in the presence of added AMP.

Creatine-P *and* ATP were determined in neutralized $HClO_4$ extracts of ventricular muscle by the enzymatic methods of Lowry *et al.*[23] NADPH was measured with a Farrand model A-3 fluorometer equipped with suitable filters. Enzymes and cofactors were obtained from Boehringer–Mannheim Corp. The concentrations of metabolites were expressed per gram of dry weight of heart. For hearts perfused at 37°, dry weight was 15·4 per cent of the wet weight value; for hearts perfused at 30°, dry weight was 17·4 per cent of the wet weight value; for nonperfused hearts, dry weight was 19·8 per cent of the wet weight value.

Uptake of ^{86}RbCl *and* [^{3}H]*norepinephrine by perfused hearts.* To determine whether hearts from control and triiodothyronine-treated rats received equivalent amounts of injected solutions, hearts perfused at 37° were given approximately 1 μc ^{86}RbCl with or without 2 μc (about 0·03 μg) DL-norepinephrine-7-^{3}H. Twenty sec after injection, the ventricles were cut off, rinsed in 0·9% NaCl, blotted and weighed at room temperature. A similar technique was used by Wurtman *et al.*[15,16] in intact rats to show that the proportion of cardiac output received by the heart is increased by thyroid treatment. The weighed hearts were extracted with $HClO_4$ as described by Hardman *et al.*[24] The extracts were centrifuged to remove precipitated protein, and aliquots of the supernatant fluids were used for determination of $^{86}Rb^{+}$. The remainder of the supernatant fluid was used for determination of [^{3}H]norepinephrine. Norepinephrine was adsorbed onto alumina and eluted with 0·1 N HCl.[25] Aliquots of the injected

solution and extracts were added to 15 ml of a Triton X-100–toluene counting solution[26] and radioactivity was measured in a Packard model 4321 liquid scintillation spectrometer. For [^{3}H]norepinephrine, counting efficiencies were determined by addition of internal standards. Myocardial concentrations of $^{86}Rb^+$ and [^{3}H]norepinephrine were calculated as per cent of the administered dose retained by the heart. Both ^{86}RbCl and [^{3}H]norepinephrine were obtained from New England Nuclear Corp.

Statistical analysis. Significance of differences was determined with *t*-tests for either paired or unpaired data.[27] Correlation coefficients for the relationship between the percentage phosphorylase *a* and the concentration of creatine-P were calculated by the Spearman rank correlation coefficient (r_s).[28]

TABLE 1. EFFECT OF TREATMENT WITH TRIIODOTHYRONINE ON BODY WEIGHT, OXYGEN CONSUMPTION, HEART WEIGHT AND HEART RATE

	Control	Triiodothyronine*
Body weight (g)		
Before	236 ± 9 (14)†	238 ± 9 (14)
After	242 ± 9‡	223 ± 10‡
Oxygen consumption (l./m^2/hr)		
Before	7·7 ± 0·2 (14)	7·5 ± 0·1 (14)
After	7·7 ± 0·2	10·6 ± 0·3‡
Heart weight (mg)	858 ± 20 (6)	1050 ± 20 (7)§
Heart rate after 30-min perfusion (beats/min)		
At 37°	244 ± 4 (38)	288 ± 4 (32)§
At 30°	176 ± 3 (12)	207 ± 3 (12)§

* Triiodothyronine given s.c. 100 μg/day for 3 days.

† Values are averages ± S.E. for the numbers of animals indicated in parentheses.

‡ Values after treatment with control solution or triiodothyronine different from those before treatment using *t*-test for paired samples ($P < 0.005$).

§ Values for triiodothyronine-treated rats different from those of control rats using *t*-test for unpaired samples ($P < 0.001$).

RESULTS

Effectiveness of triiodothyronine treatment. Oxygen consumption was measured on groups of normal and triiodothyronine-treated rats, and was compared with the changes in body weight which occurred during the 3-day treatment period (Table 1). Hyperthyroid rats consumed 44 per cent more oxygen than control rats, and rats given triiodothyronine decreased in body weight during the treatment period. Also, treatment with triiodothyronine resulted in a substantial increase in the weight of ventricular muscle. Heart rates were determined during perfusion for all hearts used, and hearts from triiodothyronine-treated rats had higher spontaneous rates than those from control rats at both temperatures of perfusion (Table 1).

Effect of norepinephrine on phosphorylase activity. Initial activities of phosphorylase *a* were similar in hearts from control and hyperthyroid rats whether perfusions were done at 30° or at 37° (Fig. 1). Hearts from triiodothyronine-treated rats had higher

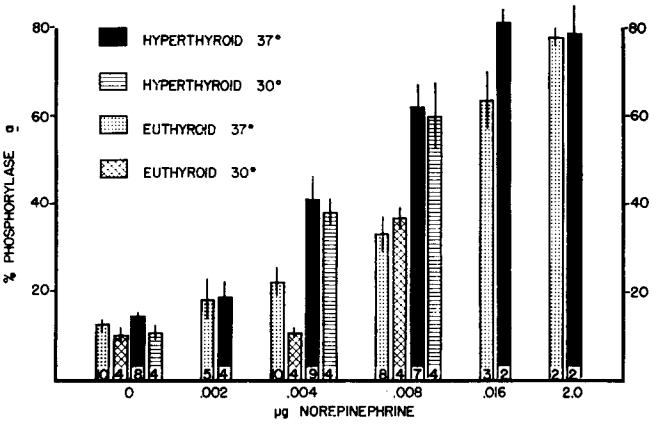

FIG. 1. Activation of myocardial phosphorylase by the administration of norepinephrine to isolated perfused hearts from euthyroid or triiodothyronine-treated rats. Phosphorylase *a* is expressed as the percentage of the total activity in the extract. Hearts were perfused at a temperature of either 37° or 30°. When norepinephrine was given in doses of 0·004 and 0·008 μg, hearts from hyperthyroid rats had higher activities of phosphorylase *a* than those from euthyroid rats ($P < 0·01$). Numbers of rats in each group are indicated at the base of each bar. Vertical lines are ± S.E.

total phosphorylase activities than those of euthyroid rats (Table 2). For hearts from either group of rats, the total phosphorylase activity was higher in hearts perfused at 37° than in those perfused at 30°. This difference may or may not be real, because the two groups were studied several months apart and, in either case, the difference is not pertinent to the present discussion. An elevated total phosphorylase activity without

TABLE 2. TOTAL PHOSPHORYLASE ACTIVITY IN ISOLATED RAT HEARTS PERFUSED AT 37° OR 30°

Rats	Total phosphorylase activity (μmoles/g dry wt./hr)	
	37°	30°
Control	112·5 ± 2·6 (38)*	78·1 ± 2·2 (12)†
Triiodothyronine (100 μg/day 3 days)	128·0 ± 3·2 (32)‡	87·1 ± 1·5 (12)†,‡

* Values are averages ± S.E. for the numbers of animals in parentheses.
‡ Values in hearts from triiodothyronine-treated rats greater than in those from control rats ($P < 0·005$).
† Values in hearts perfused at 30° less than in those perfused at 37° ($P < 0·001$).

an increased percentage phosphorylase *a* was found also in hearts of thyroxine-treated dogs by Hickenbottom and Mayer (cited in Mayer[14]).

Administration of norepinephrine increased the activity of phosphorylase *a* in hearts from both euthyroid and hyperthyroid rats and, at intermediate doses of

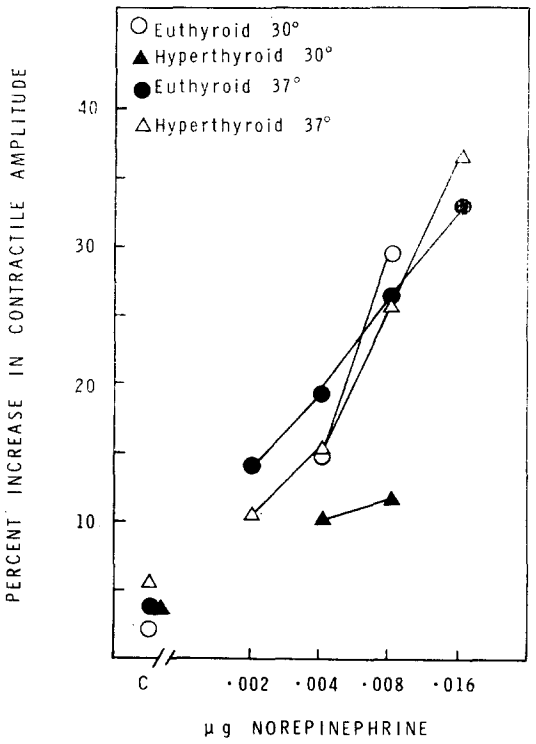

FIG. 2. Increase in contractile amplitude of perfused rat hearts produced by the administration of norepinephrine. Values are expressed as per cent increase in amplitude over that found just prior to injection. The letter C indicates the injection of 0·2 ml of perfusion medium. Hearts from triiodothyronine-treated rats had less of an increase in amplitude after norepinephrine when perfused at 30° ($P < 0·05$). The numbers of hearts per point are the same as those for the bars in Fig. 1.

norepinephrine, the response was greater in hearts from hyperthyroid rats at both temperatures of perfusion (Fig. 1).

Initial contractile amplitudes were similar for hearts of euthyroid and hyperthyroid rats. With perfusion at 37°, no differences were found between the two groups of rats in their response to the administration of norepinephrine (Fig. 2). However, when hearts were perfused at 30°, hearts from triiodothyronine-treated rats had a smaller increase in contractile force than hearts from euthyroid rats. Van der Schoot and Moran[8] have reported that treatment of animals with thyroid hormone resulted in decreased cardiac contractile force response to catecholamines. In contrast to the changes found in contractile amplitude with norepinephrine, heart rates were not increased in perfused hearts of either euthyroid or hyperthyroid rats.

Concentrations of creatine-P and ATP. Whether perfusions were done at 30° or 37°, the concentration of creatine-P was higher in hearts from euthyroid rats than in those from hyperthyroid rats (Fig. 3). The concentration of creatine-P found in hearts from control rats is comparable to those previously reported for isolated perfused hearts[29–31] and for nonperfused rat hearts.[30,32] The concentration of creatine-P has been reported to range from 20 to 24 μmoles/g dry weight, although a concentration as high as 39 μmoles/g has been found. The lower concentration of creatine-P found

in perfused hearts of hyperthyroid rats could represent either an artifact resulting from an insufficient supply of oxygen by the perfusion system coupled with the increased metabolic rate of these hearts or a physiological consequence of treatment with excess thyroid hormone. The latter possibility is supported by the findings of Skelton *et al.*[33] that the creatine-P concentration was decreased in contracting, but not in resting, papillary muscles isolated from hearts of hyperthyroid cats. They concluded that thyroid hormone treatment increased the utilization of high energy compounds by the heart, but did not affect their production. The concentrations of creatine-P found in hearts of hyperthyroid rats frozen without perfusion was markedly

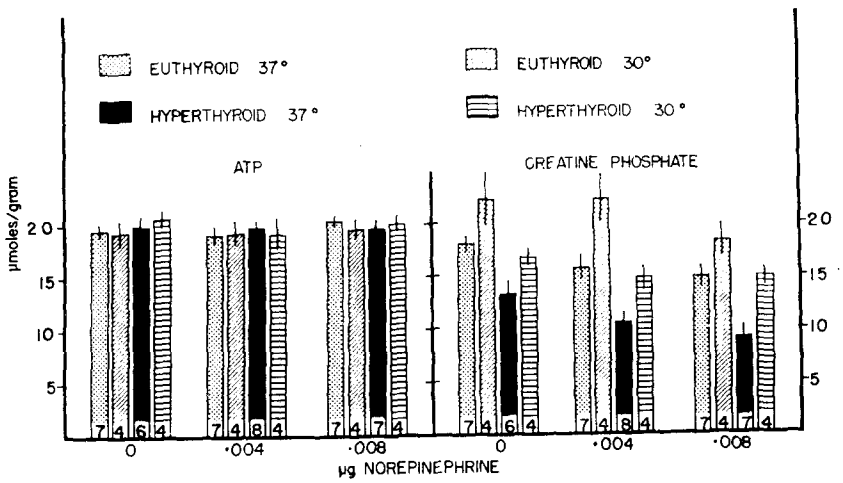

FIG. 3. Concentrations of creatine-P and of ATP in isolated perfused hearts from either euthyroid rats or triiodothyronine-treated rats with or without the administration of graded doses of norepinephrine. Values are given as micromoles per gram dry weight. Perfusions were done at a temperature of either 37° or 30°. The concentration of creatine-P was lower in hearts from hyperthyroid rats than in those from euthyroid rats ($P < 0{\cdot}01$ at 37°; $P < 0{\cdot}05$ at 30°). Numbers of rats in each group are indicated at the base of each bar. Vertical lines indicate ± S.E.

lower than those found in perfused hearts (Table 3). This difference probably reflects the greater work load imposed on the heart in the intact hyperthyroid rat.

Administration of norepinephrine decreased the concentration of creatine-P in hearts from both groups of rats when perfusions were done at 37°, but not when they were done at 30° (Fig. 3).

In contrast to creatine-P, the concentrations of ATP were similar in perfused hearts from control and hyperthyroid rats, and this nucleotide was not affected by the administration of norepinephrine (Fig. 3). However, nonperfused hearts from hyperthyroid rats had lower concentrations of ATP than those found in hearts of control rats (Table 3). This change is probably related to the marked decrease of creatine-P found in these hearts.

Uptake of $^{86}Rb^+$ *and* [^{3}H]*norepinephrine by perfused hearts.* Retention of injected $^{86}Rb^+$ and [^{3}H]norepinephrine by perfused hearts was measured to determine whether hearts of euthyroid and hyperthyroid rats received equivalent amounts of an injected solution. Hearts from hyperthyroid rats received a greater fraction of injected $^{86}Rb^+$,

TABLE 3. CONCENTRATIONS OF ATP AND CREATINE-P IN NONPERFUSED HEARTS OF CONTROL AND TRIIODOTHYRONINE-TREATED RATS

Rats	ATP (μmoles/g dry wt.)	Creatine-P (μmoles/g dry wt.)
Control (3)	24·3 ± 1·0*	20·9 ± 0·5
Triiodothyronine (3) (100 μg/day 3 days)	19·6 ± 1·4†	5·4 ± 1·4‡

* Values are averages ± S.E. for numbers of animals in parentheses. Hearts were excised from pentobarbital-anesthetized rats and frozen immediately.

† Values for hearts from triiodothyronine-treated rats lower than those for hearts from control rats ($P < 0·05$).

‡ Values for hearts from triiodothyronine-treated rats lower than those for hearts from control rats ($P < 0·001$).

but since these hearts were 22 per cent larger than hearts from euthyroid rats, the retention of $^{86}Rb^+$ per gram of heart was the same for both groups of rat hearts (Table 4). This finding indicates that delivery of an injected solution was the same for hearts of both euthyroid and hyperthyroid rats. Hearts from euthyroid and hyperthyroid rats retained equivalent amounts of [^{3}H]norepinephrine; however, the trend of the data is toward lower values in hearts from triiodothyronine-treated rats when the results are expressed per gram of heart. Wurtman *et al*[16] found less retention of norepinephrine by hearts of hyperthyroid rats *in vivo* and suggested that this resulted from hypertrophy of heart muscle without an increase in sympathetic nerve endings.

TABLE 4. EFFECT OF PRETREATMENT OF RATS WITH TRIIODOTHYRONINE ON UPTAKE OF $^{86}Rb^+$ AND [^{3}H]NOREPINEPHRINE BY PERFUSED RAT HEARTS*

Rats	$^{86}Rb^+$		[^{3}H]Norepinephrine	
	(% of dose/ heart)	(% of dose/g of heart)	(% of dose/ heart)	(% of dose/g of heart)
Control	18·4 ± 0·6† (6)	21·6 ± 0·4	3·7 ± 0·4 (3)	4·2 ± 0·4
Triiodothyronine (100 μg/day 3 days)	22·1 ± 1·0‡ (7)	21·5 ± 0·8	3·4 ± 0·2 (3)	3·3 ± 0·2

* Hearts were perfused for 30 min at 37° and then approximately 1 μc $^{86}Rb^+$ with or without 2 μc [^{3}H]norepinephrine was injected into the aortic cannula. The ventricles were removed 20 sec after injection, rinsed and the content of radioisotopes determined as described in Methods.

† Values are averages ± S.E. for the numbers of hearts indicated in parentheses.

‡ Values for hearts from rats given triiodothyronine greater than those for hearts from control rats ($P < 0·02$).

DISCUSSION

The increase in phosphorylase *a* produced by norepinephrine was larger in isolated perfused hearts from hyperthyroid rats than in those from euthyroid rats. This observation confirms previous findings[1,3] with intact hyperthyroid rats of an enhanced

responsiveness of myocardial phosphorylase to administered catecholamines. Although these data could be used to support the hypothesis of an interaction between thyroid hormone and catecholamines at the level of the adrenergic receptor,[4] other explanations are possible. Hickenbottom and Mayer (cited in Mayer[14]) did not find an enhanced activation of myocardial phosphorylase by norepinephrine in thyroxine-treated dogs. In addition, other investigators[6–13] found no potentiation of the effects of catecholamines by thyroid hormone treatment on contractile force, rate or adenyl cyclase activity in heart preparations from several species, including the rat. In this study we confirmed that thyroid hormone treatment does not enhance the contractile amplitude response to norepinephrine, yet in the same heart preparation the phosphorylase *a* response was enhanced. Thus, interactions of thyroid hormone and catecholamines, such as the potentiation of phosphorylase activation that we have observed, probably occur at a site other than the adrenergic receptor.

Wurtman *et al.*[15,16] suggested that the enhanced cardiovascular responses to catecholamines found in intact hyperthyroid rats result from increased delivery of catecholamine to the heart and altered distribution of the amines within the heart due to dilution of sympathetic nerve endings by hypertrophy. However, in our experiments the enhanced phosphorylase response cannot be due to increased delivery of norepinephrine because the isolated hearts were perfused at a constant rate of flow. Additional evidence against the hypothesis of Wurtman *et al.*[15,16] is provided by the experiments of McNeill and Brody.[3] These authors found that the phosphorylase activation produced by isoproterenol, as well as that produced by norepinephrine, was enhanced in the hearts of hyperthyroid rats and they discounted the importance of diminished uptake of amines by nerve endings as a mechanism of the apparent potentiation. Thus, neither increased delivery nor altered distribution of catecholamines can adequately explain the potentiation of phosphorylase activation found in the hearts of hyperthyroid rats.

If thyroid hormone does not affect the adrenergic receptor, and if delivery and uptake of catecholamines by the heart can be eliminated as mechanisms of the enhanced phosphorylase response found in hearts of hyperthyroid rats, then the interaction between thyroid hormone and catecholamines must occur at a site between adenyl cyclase and phosphorylase.

A possible mechanism for the enhanced phosphorylase *a* activities produced by norepinephrine in hearts from hyperthyroid rats can be derived from our findings of lower concentrations of creatine-P in hearts of hyperthyroid rats than in those of euthyroid rats. With the administration of norepinephrine, there were negative correlations between the activation of phosphorylase and the concentration of creatine-P at 37° ($r_s = 0{\cdot}66$, $P < 0{\cdot}01$) and at 30° ($r_s = 0{\cdot}55$, $P < 0{\cdot}05$). Therefore, the greater activation of phosphorylase found in hearts of hyperthyroid rats would result from activation of phosphorylase *b* kinase subsequent to an alkaline pH shift produced by the hydrolysis of creatine-P. Further, Frazer *et al.*[5] found that thyroid hormone treatment increases substantially the total phosphorylase *b* kinase activity in rat heart, a change which would enhance the phosphorylase activation resulting from the lower concentration of creatine-P.

The feasibility of this type of control of phosphorylase *a* activity in muscle has been shown by Mayer *et al.*[32,34] who found that the amount of phosphorylase activation produced in rat hearts by ischemia or anoxia was related to the concentration of

creatine-P, and by Danforth,[35] who found that the activity of phosphorylase *a* in isolated frog sartorius muscle was related to the hydrogen ion concentration of the incubation medium.

Although the above mechanism appears to account for the enhanced phosphorylase activation produced by norepinephrine in hearts from hyperthyroid rats, the lack of a simple relationship between the concentration of creatine-P and the activity of phosphorylase *a* when the two temperatures of perfusion are considered together suggests the involvement of other as yet unknown factors. One of these factors could be calcium ion, which is required for phosphorylase *b* kinase activity in heart muscle[18] and the concentration of which might be affected by thyroid hormone treatment.

An additional observation of our study was that isolated perfused hearts from hyperthyroid rats had phosphorylase *a* activities similar to those found in perfused hearts from euthyroid rats. This finding contrasts with the increased activity of phosphorylase *a* consistently observed in hearts of hyperthyroid rats frozen immediately after excision.[1-3,5,6] The difference between the two groups of hearts may be related to their concentrations of creatine-P. Creatine-P was substantially lower in hearts of hyperthyroid rats *in situ* than in those hearts which were isolated and perfused, a difference which probably reflects the greater work load imposed on the heart in the intact rat. As discussed above, the lower concentration of creatine-P should be associated with increased phosphorylase *b* kinase activity and thus a higher activity of phosphorylase *a*. It is apparent from the studies with perfused hearts that thyroid hormone does not have a direct effect on the activity of phosphorylase *a* in the heart, in contrast to its apparently direct positive effect on the contractile mechanism of heart muscle found in isolated preparations of several species.[12,13]

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