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Decreased Responsiveness of the Adenylate Cyclase System on Left Atria from Hypothyroid Rats

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SUMMARY

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On isolated electrically driven left atria (37°, 1 Hz) of rats made hypothyroid by feeding 6-propyl-2-thiouracil the β-sympathomimetic effects of isoprenaline and of phenylephrine—in the presence of 10^{-5} M yohimbine—on force of contraction and on the cyclic AMP level were compared with those obtained on atria from euthyroid rats. In hypothyroid rats the dose–response curves for the positive inotropic effects of isoprenaline and of phenylephrine were shifted to the right by about 0.4–0.5 log unit. The positive inotropic effect evoked by Ca^{2+}, however, was not affected by the hypothyroid state. On atria from euthyroid rats a submaximal effective concentration (EC_{80} for the positive inotropic effect) of isoprenaline (30 nM) increased the cyclic AMP level by about 60% after 60 sec; in hypothyroid rats, on the other hand, the same concentration of isoprenaline produced only about a 25% increase in cyclic AMP. Phenylephrine (10^{-5} M, the EC_{80} for its positive inotropic effect) in the presence of 10^{-5} M yohimbine increased in euthyroid rats the cyclic AMP level by about 25%, but failed to do so in hypothyroid rats. When used in three times higher concentrations, however, both agonists were capable of inducing increases in cyclic AMP that were comparable with the effects obtained in euthyroid rats. On atria from hypothyroid rats the basal as well as the isoprenaline- and NaF-stimulated activity of the adenylate cyclase was significantly lower than on atria from euthyroid rats. From these results it is concluded that the decreased sensitivity of cardiac β-adrenoceptors in the hypothyroid state is due to the decreased responsiveness—at least in part—of the adenylate cyclase system linked to the β-adrenoceptors. The results confirm the close correlation of the β-sympathomimetic positive inotropic effect to the cyclic AMP generating system.

INTRODUCTION

Evidence has accumulated that in addition to the well-known positive inotropic effect evoked by β-adrenoceptor stimulation a positive inotropic response can be elicited by stimulation of myocardial α-adrenoceptors (1, see here further references). The responsiveness of both types of cardiac adrenoceptors seems to be regulated by the metabolic rate (2). It has been reported that under conditions associated with high metabolic activity, as for instance in the hyperthyroid state, the sensitivity of cardiac β-adrenoceptors is increased (3, 4). An increased number of β-adrenoceptors may be, at least in part, responsible for these enhanced responses to catecholamines (5). In the hypothyroid state—a condition of low metabolic activity—on the contrary, the effects evoked by myocardial α-adrenoceptor stimulation were potentiated, but those via cardiac β-adrenoceptors were attenuated (1, 6–8).

It is now widely accepted that the positive inotropic effect induced by cardiac β-adrenoceptor stimulation is mediated by an increase of the intracellular level of cyclic AMP (9–13), while that via myocardial α-adrenoceptors is produced by a process independent of cyclic AMP (1, 11, 12, 14–16).

In order to find out whether or not the decreased sensitivity of cardiac β-adrenoceptors in the hypothyroid state is associated with a diminished activity of the adenylate cyclase system, in the present study the effects of isoprenaline, a β-mimetic agent with high intrinsic activity, and of phenylephrine, a sympathomimetic agent with low β-adrenoceptor intrinsic activity, on force of contraction and on the cyclic AMP level were investigated on isolated, electrically driven left atria from eu-

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thyroid rats and from those made hypothyroid by feeding 6-propyl-2-thiouracil (PTU)[1] (7). Since phenylephrine has been shown to elicit its positive inotropic effect through stimulation of both cardiac α- *and* β-adrenoceptors, the experiments with phenylephrine were performed in the presence of the α-adrenolytic drug yohimbine (10^{-5} M) in order to obtain a pure β-adrenoceptor stimulating effect.

In addition, we determined the isoprenaline- and NaF-stimulated adenylate cyclase activity of atria from euthyroid and hypothyroid rats in order to get more detailed information on the mechanism of decreased sensitivity of cardiac β-adrenoceptors in the hypothyroid state.

METHODS

Male Wistar rats (150–200 g) were used. The rats were divided in two groups. One group was fed a normal diet while the other one was made hypothyroid by feeding a diet containing 0.15% PTU for at least 6 weeks, as described by Nakashima *et al.* (7). This group developed dry fur, slow heart rate, and a considerable growth retardation.

The rats were killed by a blow on the head and the left atria were rapidly removed. The tissues were fixed on needle-shaped platinum electrodes and mounted in a 50 ml organ bath containing Krebs–Henseleit solution of the following composition (mM): NaCl 119; $CaCl_2$ 2.5; KCl 4.8; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 24.9; glucose 10.0, ascorbic acid 0.057 bubbled with 95% O_2 and 5% CO_2 at a temperature of 37°. They were electrically stimulated by square wave impulses at 5 msec duration and a voltage about 20% above threshold at a frequency of 1.0 Hz. The developed tension under a resting tension of 0.5 g was recorded via a strain gauge on a Hellige recorder. In all experiments atria were equilibrated for 60 min before any drug was administered.

After equilibration a submaximal effective concentration of isoprenaline (10^{-8} M) or of phenylephrine (10^{-5} M) was administered twice or three times until the successive response remained unchanged. Cumulative dose–response curves were determined by adding 0.1 ml of the drug solution thus increasing the final bath concentration in steps of 0.5 log unit. When a steady state of the developed tension was reached, the next higher concentration was applied. For all experiments with phenylephrine, atria were equilibrated in a Krebs–Henseleit solution containing yohimbine (10^{-5} M) for 60 min.

Cyclic AMP Assay. At a certain time after administration of the drugs used the atria were removed from the organ bath, blotted with filter paper and frozen immediately in liquid nitrogen. The atria were weighed and homogenized in 0.5 ml of 5% trichloroacetic acid for 30 sec by use of a microdismembrator (B. Braun, Melsungen). After addition of 10 μl 1 N HCl aliquots of 100 μl of the 2000 *g* supernatant were extracted three times with 1 ml ether, heated at 80° for 4 min to evaporate the residual ether and dried overnight at 4° *in vacuo*. For cyclic AMP determination the residues were dissolved in 50 and 100 μl of 100 mM sodium acetate buffer pH 4, respectively; the content of cyclic AMP of each atrium was calculated as the mean of three determinations, a single and duplicate, in aliquots of 50 μl. The content of cyclic AMP was determined by the protein binding method of Gilman (17) as modified by Schwabe and Ebert (18). The level of cyclic AMP was expressed as pmole/mg wet wt or as percentage increase of the control value. Chromatographic separation of cyclic AMP could be omitted since ATP in concentrations up to 0.1 mM did not interfere with the binding assay following the dilutions used in these experiments. The recovery of a known amount (400 pmole) of unlabeled cyclic AMP added to 1 ml of 5% trichloroacetic acid before the homogenization of atria pretreated with the given drugs as well as untreated control atria amounted to 94.8 ± 10.3% (N = 15); thus, corrections were not necessary.

Adenylate cyclase assay. Three to four atria were pooled and homogenized in 10 vol (based on tissue weight) of 0.05 M Tris–HCl buffer, pH 7.4, by the use of a microdismembrator for 30 sec; the homogenate was centrifuged at 12,000*g* for 2 min and the precipitate washed four times by resuspending in 0.05 M Tris–HCl buffer, pH 7.4 (original volume), and centrifuging as described above. Finally the pellets were resuspended in 0.05 M Tris–HCl buffer, pH 7.4, to give a protein concentration of about 8–10 mg/ml (for preparations from euthyroid rats) and 15–20 mg/ml (for preparations from hypothyroid rats). Adenylate cyclase activity was determined according to Schwabe *et al.* (19) using the protein binding method of Gilman (17) to determine the amount of cyclic AMP formed. The assay mixture contained 0.3 mM ATP, 10 mM $MgCl_2$, 40 mM Tris–HCl, pH 7.4, 1 mg/ml bovine serum albumin, an ATP-regenerating system consisting of 5 mM creatine phosphate and 0.1 mg/ml creatine kinase in a final volume of 100 μl. In order to inhibit phosphodiesterase activity, 0.3 mM papaverine was added to the incubation medium. The reaction was started by addition of the enzyme preparation in a volume of 20 μl (containing at least 200 μg of protein from euthyroid and 350 μg from hypothroid rats), carried out for 5 min at 37°, and terminated by transferring the tubes for 3 min to a preheated thermoblock of 95°. After cooling, 0.5 ml of distilled water was added, the samples were centrifuged at 12,000*g* for 2 min to remove protein and the amount of cyclic AMP formed was measured in duplicate 50-μl aliquots.

Measurement of cyclic AMP. The calibration curve was determined with 0.5 pmole [^{3}H]cAMP and 0.05 to 5 pmole of unlabeled cyclic AMP in a total volume of 150 μl. The calibration standards were dissolved in a mixture containing all substances of the cyclase assay except the protein prepared from the atria. The amount of cyclic AMP formed was at least 0.3 pmole at each determination, which is clearly above the detection limit of this binding assay. ATP in concentrations up to 0.3 mM did not interfere with the binding assay following the 24-fold dilution used in these series of experiments (see also (19)). Thus, separation of cyclic AMP from ATP and other nucleotides was not necessary.

Phosphodiesterase assay. Three to four atria were pooled and homogenized in 10 vol (based on tissue wet weight) of 0.25 M Tris–HCl buffer, pH 7.5, containing 15 mM magnesium acetate for 30 sec by the use of micro-

[1] The abbreviation used is: PTU, 6-propyl-2-thiouracil.

dismembrator. After centrifugation at 4000*g* for 15 min the supernatant was diluted 1:30 (w/v) with buffer solution. Phosphodiesterase activity was determined by the method of Pöch (20) at two different cyclic AMP concentrations (2×10^{-4} and 10^{-4} M). The activity of phosphodiesterase is expressed as nanomoles of cyclic AMP hydrolyzed per 30 min incubation at 37°.

Statistical methods. The significance of differences was estimated by means of Student's *t* test. *P* values smaller than 0.05 were considered to be significant. The pD_2 values were calculated as described by Van Rossum (21).

Drugs used. (±)-Isoprenaline sulfate, (−)-phenylephrine hydrochloride and yohimbine hydrochloride (Boehringer, Ingelheim); papaverine hydrochloride (Knoll, Ludwigshafen); ATP, creatine phosphate and creatine kinase from rabbit muscle, 25 units/mg (Boehringer, Mannheim); bovine serum albumin (Serva, Feinbiochemica, Heidelberg); [^{3}H]cAMP (specific activity 38.4 Ci/mmole, New England Nuclear, Dreieichenhain). All other chemicals were of analytical grade.

RESULTS

1. *Effect of the hypothyroid state on the dose-response curves for the positive inotropic effects of isoprenaline and of phenylephrine in the presence of yohimbine (10^{-5} M).* The dose-response curves for the positive inotropic effects evoked by isoprenaline and by phenylephrine in the presence of yohimbine (10^{-5} M) stimulating under these conditions predominantly cardiac β-adrenoceptors, were displaced to the right on atria from rats made hypothyroid when compared with the control curves (Fig. 1). Thus, the pD_2 values for isoprenaline and for phenylephrine were significantly decreased by about 0.4–0.5 log unit on hypothyroid rats (Table 1). The intrinsic activity of phenylephrine which is known as a weakly acting β-sympathomimetic agent (22) was small: it amounted on atria from euthyroid as well as on those from hypothyroid rats to only 60% of the activity of isoprenaline (Table 1).

2. *Influence of the hypothyroid state on the time course of the effects of isoprenaline and of phenylephrine in the presence of yohimbine (10^{-5} M) on force of contraction and on the cyclic AMP level.* On atria from euthyroid rats a submaximal effective concentration of isoprenaline—30 nM, i.e., the EC_{80} for the positive inotropic effect (cf. Fig. 1)—led to a rapid increase of the cyclic AMP level, which was maximal after 60 sec and amounted to about 60% (Fig. 2A). This cyclic AMP increase preceded that of the force of contraction, which reached its maximum after 120 sec, when the cyclic AMP level had slightly declined.

On atria from hypothyroid rats, on the contrary, the effects evoked by the same concentration of isoprenaline (30 nM) were markedly reduced. On these preparations the maximal increase of cyclic AMP amounted only to about 25% after 60 sec (Fig. 2B) and the maximal increase of the developed tension reached was significantly reduced (maximal increase in tension developed in euthyroid rats: 267 ± 28 mg; in hypothyroid rats: 177 ± 16 mg). Similar effects were obtained with a submaximal effective concentration of phenylephrine (10^{-5} M) in the presence of 10^{-5} M yohimbine—*i.e.,* also the EC_{80} for the positive inotropic effect (cf. Fig. 1). On atria from euthyroid rats this concentration of phenylephrine led to a slight, but significant, increase of the cyclic AMP level preceding that of the force of contraction, which was maximal after 30 sec and amounted to about 25% (Fig. 3A). In contrast to these effects on atria from hypothyroid rats the same concentration of phenylephrine failed to increase the cyclic AMP level (Fig. 3B). However, on these preparations the maximum of the developed tension reached was only slightly, but not significantly, reduced.

In order to reach effects on atria from hypothyroid rats comparable to those on euthyroid rats, the concentrations of isoprenaline and of phenylephrine have to be increased by three times. This is shown in Fig. 4, where the maximal tissue values for cyclic AMP (in pmole/mg wet wt) and for increases in tension developed (in mg) obtainable after administration of the amines are given. As described above, on euthyroid rats 30 nM isoprenaline increased the cyclic AMP level up to 1.20 ± 0.1 pmole/

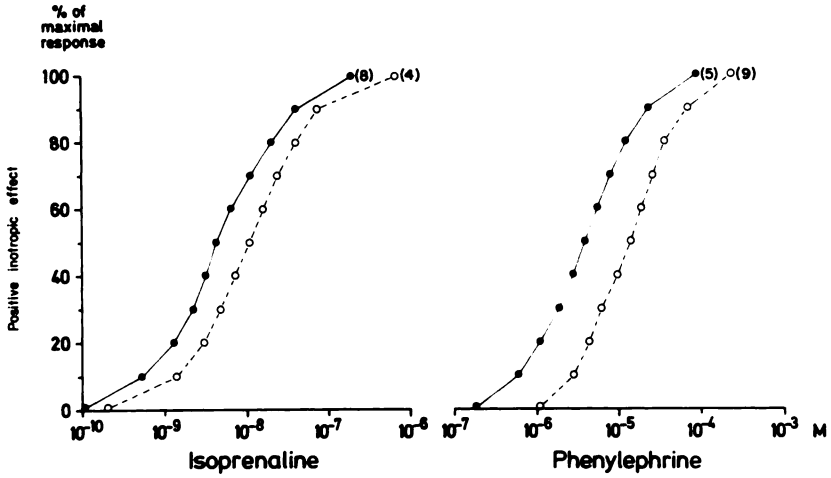

FIG. 1. *Dose-response curves for the positive inotropic effects of isoprenaline (left) and of phenylephrine in the presence of 10^{-5} M yohimbine (right) on atria from untreated* (●——●) *and PTU-fed* (○-----○) *rats*

Number of experiments in parentheses.

TABLE 1

Maximal increases in developed tension, pD_2-values and intrinsic activities (α) of isoprenaline and of phenylephrine in the presence of 10^{-5} M yohimbine on atria from untreated and PTU-fed rats

Note. Given are means ± SEM. N = Number of experiments. For α the maximal effects induced by isoprenaline were set as 1.

Drug	Untreated rats					PTU-fed rats				
	N	Basal tension (mg)	Maximal increase in tension (mg)	pD_2 values	α	N	Basal tension (mg)	Maximal increase in tension (mg)	pD_2 values	α
Isoprenaline	8	181.3 ±8.5	207.8 ±10.3	8.39 ±0.07	1	4	148.5 ±13.1	305.0 ±54.8	7.99*** ±0.10	1
Phenylephrine	5	192.5 ±30.2	126.2* ±14.5	5.44 ±0.08	0.6	9	163.9 ±26.0	175.0* ±17.5	4.93** ±0.08	0.57

* $P < 0.005$ compared with the corresponding values for isoprenaline.
** $P < 0.005$ compared with the corresponding values from untreated rats.
*** $P < 0.05$ compared with the corresponding values from untreated rats.

mg wet wt, on hypothyroid rats only up to 0.87 ± 0.07 pmole. The three times higher concentration (10^{-7} M), however, led to an increase of the cyclic AMP level in hypothyroid rats (up to 1.19 ± 0.11 pmole), which is very similar to that obtained on euthyroid rats with the three times lower concentration. The same holds true for the maximum of the developed tension reached. Isoprenaline (10^{-7} M) produced in hypothyroid rats the same maximum as that reached in euthyroid rats with the three times lower concentration (30 nM). Similar effects were obtained with phenylephrine. While 10^{-5} M phenylephrine failed to increase the cyclic AMP level in hypothyroid rats (cf. Fig. 3B), a three times higher concentration (30 μM) produced an increase of the cyclic AMP level (up to 0.84 ± 0.06 pmole) which is equal to that obtained with the three times lower concentration (10^{-5} M) in euthyroid rats (up to 0.83 ± 0.08 pmole). The same tendency can also be seen for the maxima of the developed tension evoked by phenylephrine, although the differences are only small and not significant. This may be due to an enhanced sensitivity of α-adrenoceptors in the hypothyroid state.

3. *Influence of the hypothyroid state on adenylate*

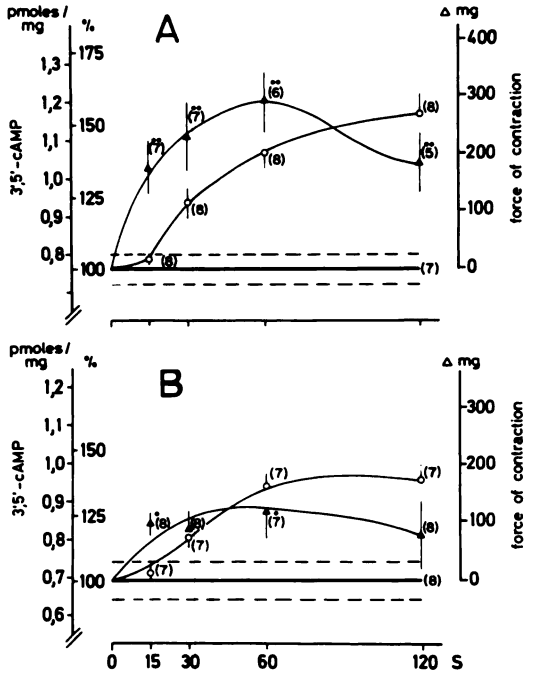

FIG. 2. *Time course of the effects of isoprenaline (3×10^{-8} M) on force of contraction and on the cyclic AMP level of atria from untreated (A) and PTU-fed rats (B)*

Abscissa: time after administration of isoprenaline in sec. Ordinate: cyclic AMP in pmole/mg wet wt or in percentage change, whereby the control values were taken as 100% (left) and force of contraction in Δmg (right). Solid horizontal lines with broken lines: control cyclic AMP values ± SEM. Given are means ± SEM. Number of experiments in parentheses. ▲——▲ cyclic AMP, ○——○ force of contraction. **$P <$ 0.005, *$P < 0.05$ vs the corresponding control.

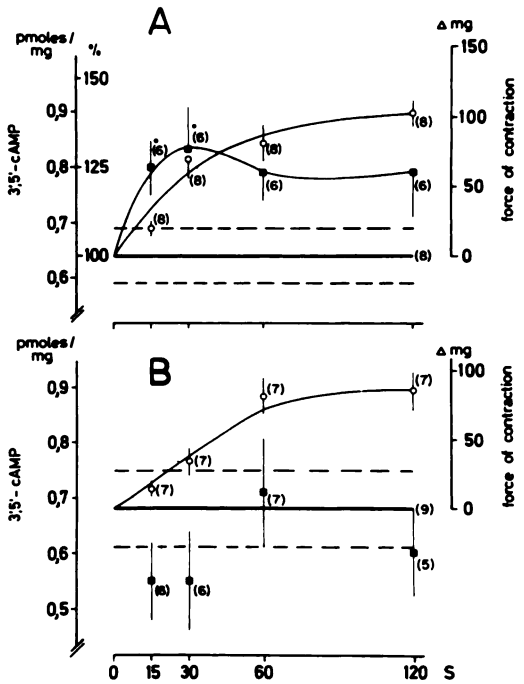

FIG. 3. *Time course of the effects of phenylephrine (10^{-5}) M in the presence of yohimbine (10^{-5} M) on force of contraction and on the cyclic AMP level of atria from untreated (A) and PTU-fed rats (B)*

Abscissa: time after the administration of phenylephrine in sec. Ordinates: cyclic AMP in pmole/mg wet wt or in percentage change, whereby the control cyclic AMP values were taken as 100% (left) and force of contraction in Δmg (right). Solid horizontal lines with broken lines: control cyclic AMP values ± SEM after 1 hr equilibration with 10^{-5} M yohimbine. Given are means ± SEM. Number of experiments in parentheses. ■——■ cyclic AMP, ○——○ force of contraction. *$P <$ 0.025 vs the corresponding control.

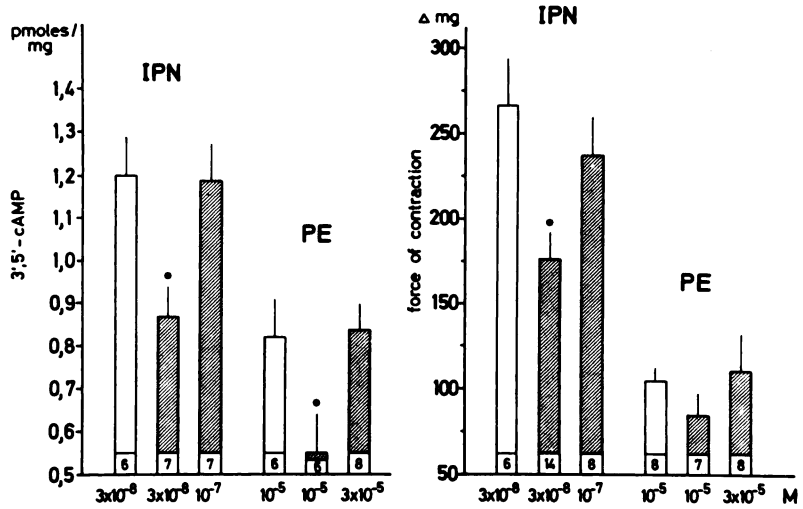

FIG. 4. *Influence of propylthiouracil treatment on the maximal effects of isoprenaline (IPN) and of phenylephrine (in the presence of 10^{-5} M yohimbine, PE) on force of contraction and on the cyclic AMP level of isolated left atria from rats*

Ordinates: cyclic AMP in pmole/mg wet wt (left), force of contraction in Δmg (right). Given are the maximally obtainable tissue content of cyclic AMP and the maximal increase of the developed tension reached after administration of the drugs used in the concentrations indicated below the columns. Cyclic AMP content was measured 60 sec after the administration of isoprenaline (cf. Fig. 2) and 30 sec after that of phenylephrine (cf. Fig. 3). Open columns: untreated rats; hatched columns: PTU-fed rats. Given are means ± SEM. $^*P < 0.05$ compared with the corresponding values in untreated animals.

cyclase and phosphodiesterase activities. On atria from rats made hypothyroid the basal activity of adenylate cyclase was significantly diminished when compared with that on atria from euthyroid rats (Table 2). The same held true for the isoprenaline (3×10^{-6}–10^{-4} M)- and NaF (5×10^{-4}–5×10^{-3} M)-stimulated adenylate cyclase activity. At each concentration used, the activity of the enzyme derived from hypothyroid rats was significantly lower than that from euthyroid rats (Table 2). In contrast to the effects on adenylate cyclase, hypothyroidism did not influence the activity of phosphodiesterase on rat atria (Table 3).

4. *Influence of the hypothyroid state on the dose-response curve for the positive inotropic effect of Ca^{2+}.* In a final series of experiments we determined the dose–response curves for the positive inotropic effect evoked by Ca^{2+} on atria from euthyroid rats and from rats made hypothyroid. As may be seen from Fig. 5, hypothyroidism did not affect the positive inotropic effect elicited by Ca^{2+}; accordingly the pD_2 values for Ca^{2+} were not changed (euthyroid: $pD_2 = 2.79 \pm 0.03$, $N = 8$; hypothyroid: $pD_2 = 2.72 \pm 0.03$, $N = 10$).

DISCUSSION

On isolated, electrically driven left atria from hypothyroid rats the dose–response curves for the positive inotropic effect of isoprenaline, a β-mimetic agent with high intrinsic activity, as well as of phenylephrine (in the presence of 10^{-5} M yohimbine), a β-mimetic agent with low intrinsic activity, were displaced to the right by about 0.4–0.5 log unit compared to the control curves. These results demonstrate that hypothyroidism decreases the sensitivity of cardiac β-adrenoceptors to β-mimetic agents independently of their intrinsic activities, since the potency ratio phenylephrine/isoprenaline was not changed in the hypothyroid state (cf. Table 1). The data presented here are in accordance with observations pre-

TABLE 2

Basal and isoprenaline- and NaF- stimulated adenylate cyclase activity on atria from untreated and PTU-fed rats

Note. Given are means ± SEM. Number of experiments in parentheses.

Drug	Concentration (M)	Adenylate cyclase activity (pmole cAMP formed/mg protein/5 min)	
		Untreated rats	PTU-fed rats
(Control)		21.28 ± 2.09 (15)	10.61 ± 1.71 (16)
Isoprenaline	3×10^{-6}	30.78 ± 3.85* (12)	13.29 ± 2.20*** (12)
Isoprenaline	10^{-5}	34.98 ± 4.81** (12)	12.81 ± 1.99*** (12)
Isoprenaline	3×10^{-5}	38.44 ± 5.08** (12)	18.22 ± 2.73**,*** (12)
Isoprenaline	10^{-4}	40.64 ± 6.33** (12)	23.64 ± 3.33**,*** (12)
NaF	5×10^{-4}	40.51 ± 4.32** (6)	22.0 ± 5.04**,*** (7)
NaF	10^{-3}	81.79 ± 9.97** (6)	44.79 ± 7.91**,*** (7)
NaF	2×10^{-3}	199.99 ±18.7** (6)	100.21 ±19.65**,*** (7)
NaF	5×10^{-3}	280.59 ±27.98** (6)	125.79 ±20.68**,*** (7)

* $P < 0.05$ when compared with the corresponding control.
** $P < 0.01$ when compared with the corresponding control.
*** $P < 0.01$ when compared with the corresponding values on untreated rats.

TABLE 3

Phosphodiesterase activity on atria from untreated and PTU-fed rats

Note. Phosphodiesterase activity is expressed as nanomoles of cyclic AMP hydrolyzed per 30 min incubation at 37°. Given are means ± SEM of *N* experiments.

Substrate concentration	*N*	Phosphodiesterase activity	
		Untreated rats	PTU-fed rats
2×10^{-4} M cAMP	6	32.7 ± 2.1	33.1 ± 1.9
10^{-4} M cAMP	6	18.2 ± 0.6	18.7 ± 0.7

viously reported by Nakashima *et al.* (6, 7) and Kunos *et al.* (8) who also showed that on atria from hypothyroid rats the sensitivity of cardiac β-adrenoceptors to β-mimetic agents was significantly lower than on euthyroid rats. However, in the present experiments isoprenaline as well as phenylephrine produced on atria from hypothyroid rats a greater maximal increase in tension developed than on control atria. This may be due to the depression of the basal developed tension (cf. Table 1) in the hypothyroid state. Similar findings have been previously reported by Nakashima *et al.* (7) who also observed that the increase in tension developed evoked by high concentrations of isoprenaline was greater on atria from PTU-fed rats than on control atria, although the dose–response curve was shifted to the right.

It is now generally accepted that the positive inotropic effect evoked by β-adrenoceptor stimulation is closely correlated to the adenylate cyclase system (9–13). Since in the hypothyroid state the sensitivity of cardiac β-adrenoceptors is reduced, one should expect that under these conditions the responses of the adenylate cyclase system to β-agonists also should be attenuated. This is indeed the case (cf. Figs. 2 and 3). On atria from euthyroid rats, isoprenaline (30 nM) increased the cyclic AMP level

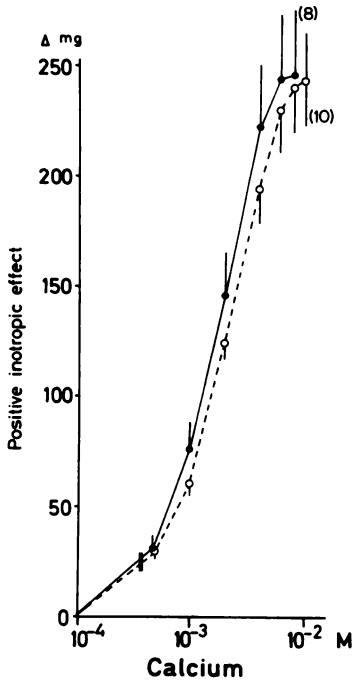

FIG. 5. *Dose–response curve for the positive inotropic effect of Ca^{2+} on atria from untreated* (●——●) *and PTU-fed rats* (O-----O)
Given are means ± SEM., Number of experiments in parentheses.

by about 60% after 60 sec; on atria from hypothyroid rats, on the contrary, the effects of the same concentration of isoprenaline on the adenylate cyclase system were markedly diminished: under these conditions the increase of the cyclic AMP content amounted only to about 25%. Similar results were obtained with phenylephrine in the presence of 10^{-5} M yohimbine stimulating under these conditions predominantly β-adrenoceptors. On atria from euthyroid rats phenylephrine (10^{-5} M) increased the cyclic AMP level by about 25%, which is in accordance with previously reported data on rabbit papillary muscle (16). On atria from hypothyroid rats, however, the same concentration of phenylephrine failed to increase the cyclic AMP content. Thus the reduced sensitivity of cardiac β-adrenoceptors is associated with a decreased responsiveness of the adenylate cyclase system. Accordingly, a three times higher concentration of isoprenaline and of phenylephrine is needed in order to reach on atria from hypothyroid rats the same effects on the adenylate cyclase system as on control rats (cf. Fig. 4). Similar results have been described by Kunos *et al.* (23), who could demonstrate that on atria from thyroidectomized rats the β-mimetic effects of isoprenaline and of phenylephrine on the adenylate cyclase system were significantly reduced.

An accelerated degradation of cyclic AMP by an enhanced activity of the phosphodiesterase cannot significantly contribute to the reduced accumulation of cyclic AMP in the hypothyroid state, since we could not find any differences in the activity of phosphodiesterase on atria from euthyroid and hypothyroid rats (cf. Table 3). Similar findings have been obtained by Levey *et al.* (24) on hearts from hypothyroid cats and by Malbon *et al.* (25) on fat cell ghosts from hypothyroid rats, who also could not demonstrate an alteration of the phosphodiesterase activity.

The decreased responsiveness of the β-adrenoceptor–adenylate cyclase system in the hypothyroid state rather may be due to a reduced activity of the adenylate cyclase system. As shown in Table 2, the basal adenylate cyclase activity was significantly lower on atria from hypothyroid rats than on those from euthyroid rats. Isoprenaline stimulated in euthyroid rats the adenylate cyclase activity in a concentration-dependent manner. Maximal stimulation (1.8-fold) occurred at 100 μM in accordance with recently reported data on guinea pig auricles (26). On atria from hypothyroid rats, however, the accumulation of cyclic AMP induced by isoprenaline was significantly smaller at each concentration used and a ten times higher concentration was needed to stimulate the enzyme. Thus, in the hypothyroid state the isoprenaline concentration–adenylate cyclase response curve was shifted to the right. Moreover, not only the β-adrenoceptor-mediated stimulation of the adenylate cyclase activity was diminished, but also that evoked by NaF. Similar results have been previously described by Levey *et al.* (24), who showed that on the particulate fraction of heart homogenates from cats made hypothyroid, the activation of adenylate cyclase by norepinephrine and NaF was significantly lower than in the control group. In the present experiments, however, stimulation of adenylate cyclase activity by NaF was in the hypothyroid state—apart from the decreased basal activity—in the same order of magnitude

at each concentration of NaF used as on atria from euthyroid rats. These observations may reflect reduced adenylate cyclase activity, but they may also reflect a decrease in the number of enzyme molecules per milligram of tissue protein without actual changes in the enzyme activity. Since the NaF concentration–adenylate cyclase response curve was not shifted to the right—apart from the lower levels due to a (perhaps nonselective) decrease in the basal activity—but the isoprenaline curve was displaced, and since NaF is believed to be capable of maximally stimulating the activity of adenylate cyclase (27), we favor the latter possibility. Thus from the present results it may be concluded that the decreased responsiveness of β-adrenoceptors in the hypothyroid state is due to a nonselective reduction of the adenylate cyclase activity concomitantly with selective changes at the receptor site. This view is supported by recent findings that the thyroid state can regulate the number of β-adrenoceptors in the rat heart. It has been demonstrated by *in vitro* binding studies using the β-adrenoceptor antagonist [^{3}H]dihydroalprenolol that in the hyperthyroid state the specific binding is significantly increased (5), while in the hypothyroid state it is markedly reduced (28, 29). Similar observations were made with the β-adrenoceptor agonist [^{3}H]norepinephrine (30). These results indicate that in the hyperthyroid state the affinity and/or number of β-adrenoceptors is increased, whereas it is decreased in the hypothyroid state. Thus, a decreased number of receptors may be responsible for the decreased responsiveness of β-adrenoceptors in the hypothyroid state.

On the other hand, the fact that hypothyroidism does not influence the positive inotropic effect evoked by Ca^{2+} (cf. Fig. 5) strongly supports the view that the alteration of the responsiveness of the heart to catecholamines in the hypothyroid state is caused by changes on the β-adrenoceptor–adenylate cyclase system and not by changes in the chain of events following the stimulation of this system.

The decreased responses of cardiac β-adrenoceptors to β-mimetic agents in the hypothyroid state are due to a concomitant decrease of the affinity and/or number of β-adrenoceptors as well as to a decrease of adenylate cyclase activity linked to the β-adrenoceptors. Since not only increases in cyclic AMP, but also those in developed tension, are reduced these results confirm the close correlation between the positive inotropic effect induced by β-adrenoceptor stimulation and the adenylate cyclase system.

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