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INCREASED HEART ADENYLCYCLASE ACTIVITY IN THE HYPOTHYROID RAT

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Abstract—Adenylcyclase activity was measured in heart homogenates and membrane fractions from normal and hypothyroid rats. Much more activity was found in hypothyroid animals than in normal controls. Thyroxine *in vitro* has no action on the enzyme activity from normal animals, and a slight depressing action on the enzyme from hypothyroid animals. Oral thyroxine treatment of thyroid deprived rats tends to lower the heart adenylcyclase activity towards normal values. Adenylcyclase increase seems to be due to activation rather than to enzyme synthesis. This activation could not be ascribed, however, to TSH increase, because TSH *in vitro* does not influence the enzyme from normal rat heart. Similarly, the activation could not be ascribed to myocardial catecholamine increase, because the β-blocking agent propranolol does not lower the enzyme activity from hypothyroid rat heart, and also because DOPAmine does not increase the adenylcyclase activity from normal rat heart. It is concluded that activation of adenylcyclase cannot account for the cardiac effects of thyroid hormones.

THE RELATIONSHIP between the sympathetic nervous system and the cardiac manifestations of hyperthyroidism has been much studied in recent years. It has been shown that the hyperthyroid state increases the vascular[1–3] and cardiac[4–6] effects of exogenous catecholamines. Brodie *et al.*[7] suggested that thyroid hormone may increase myocardial adenylcyclase synthesis, leading to enhanced cyclic 3′,5′-adenosine monophosphate (3′,5′-AMP) concentration in the heart. Recent work in this field failed, however, to support the idea of an increased 3′,5′-AMP concentration or increased adenylcyclase activity in the heart of hyperthyroid animals.[8,9] We have investigated whether heart adenylcyclase of hypothyroid rats is significantly different from that of normal euthyroid animals.

MATERIAL AND METHODS

White rats of the Wistar strain, weighing about 200–250 g, were used throughout this work. Thyroid deprivation was achieved by i.p. injection of 1 mc $^{131}I^-$ per animal 5–8 weeks before the measurements. The animals were kept at 24° on a standard laboratory diet, their drinking water being supplemented with calcium lactate. Thyroid destruction was ascertained by radioiodine uptake tests and confirmed by autopsy.

The animals were killed by a blow on the neck and bled. The heart was quickly removed and immersed in ice-cold saline. It was freed from adhering tissues, blotted, weighed and homogenized in a Potter device. Each homogenate was made with the hearts of at least three animals. The homogenates were assayed for adenylcyclase activity by a combination of the methods of Krishna *et al.*[10] and of Streeto and Reddy,[11] modified as follows.

Preparation of homogenate. The heart is homogenized with 15 ml 0·25 M sucrose per g wet tissue. Protein content is usually about 10 mg/ml.

Preparation of "membrane fraction". The homogenate is centrifuged at 12,000 *g* during 10 min in the cold. The supernatant is discarded and the pellet is suspended in the same volume of 2 mM glycylglycine buffer, pH 7·5, containing 1 mM $MgSO_4$. The mixture is centrifuged again in the same conditions, the supernatant discarded and the pellet suspended in the required amount of glycylglycine–$MgSO_4$ buffer to obtain a final protein concentration of about 10 mg/ml. If the preparation is not used immediately, it can be stored at a higher concentration (30 mg/ml) after freezing in isopentane cooled at — 180° (stored on dry ice, this preparation keeps its activity for several weeks).

Incubation mixture

0·75 ml 64 mM tris buffer, pH 7·7, containing 5·3 mM $MgSO_4$ and 16 mM theophylline (ethylenediamine complex).

0·10 ml ATP 7 mg/ml containing 2·5 μc AT^{32}P-α (The Radiochemical Centre, Amersham, Code No. PB 107), final concentration ascertained by absorption at 260 nm.

0·15 ml 40 mM phosphoenolpyruvate containing 0·2 mg/ml pyruvate kinase[11] (C. F. Boehringer, Code No. 15744 EPAU).

0·10 ml 3′,5′-AMP 5 mg/ml containing 0·5 μc 3′,5′-AMP-8-^{3}H (The Radiochemical Centre, Amersham, Code No. TRA 304).

The drugs tested *in vitro* were introduced in the homogenate or membrane fraction about 5 min prior to the initiation of the reaction. Thyroid hormones were dissolved in propylene glycol, all other substances in water.

The tubes are placed in a shaking water bath at 30° and the reaction initiated with 0·10 ml homogenate or membrane fraction (containing about 1 mg protein). The reaction is stopped after 15 min by heating the tubes in a boiling water bath for 3 min.

Separation of 3′,5′-AMP.[10] The reaction mixture is centrifuged at about 3000 *g* for 10 min. The supernatant is passed through a column of Dowex 50W-X4 (200–400 mesh) made by pouring 3·5 ml of a 50:50 suspension of the resin in water into a 90 × 10 mm tube and thoroughly washed with water. Elution is performed with water: the first fractions (8 ml) are discarded, and the three following 2-ml fractions are saved. Each fraction is treated with 0·2 ml 0·17 M $ZnSO_4$ and 0·2 ml 0·15 M $Ba(OH)_2$, shaken, centrifuged, and again treated with both reagents, shaken and centrifuged. One ml of supernatant is mixed with 10 ml scintillation mixture* and the radioactivity of both isotopes (^{32}P and ^{3}H) is measured.

Determination of adenylcyclase activity. Tritium measurement gives the yield of 3′,5′-AMP recovery and permits the calculation of the amount of 3′,5′-AM^{32}P formed. This is expressed as picomoles per minute per milligram protein (referred to as units (U) in this paper).

RESULTS

1. *Basal adenylcyclase activity.* The activity was measured in heart homogenates from 17 groups of normal rats and 11 groups of hypothyroid animals. For each of the

* 7·5 g PPO (2,5-diphenyloxazole), 0·3 g POPOP (1,4-di-[2-(5-phenyloxazolyl)]-benzene), 500 ml Triton-X-100 and 1000 ml toluene.

28 groups, a mean value was obtained from four to six identical determinations performed on the same homogenate. The results are given in Table 1.

TABLE 1. ADENYLCYCLASE ACTIVITY (U) IN HEART HOMOGENATES FROM NORMAL AND HYPOTHYROID RATS (± S.D.)

Normal	20·2 ± 8·0
Hypothyroid	45·9 ± 10·3
P < 0·001	

It is obvious that although large variations occur in the activity of different groups of normal animals, hypothyroid rats have much higher adenylcyclase activity.

2. *Total adenylcyclase activity.* Total activity was estimated by incubating the homogenates with 10 mM NaF.[12] In two groups of control rats, NaF increased the mean activity by 88·2 ± 5·6 U, and in one group of hypothyroid animals by 88·4 U (Table 2).

TABLE 2. BASAL AND FLUORIDE ACTIVATED HEART ADENYLCYCLASE ACTIVITY IN HOMOGENATES FROM ONE GROUP OF HYPOTHYROID RATS AND TWO GROUPS OF NORMAL ANIMALS

	Controls		Hypothyroid
	Pool 1	Pool 2	Pool 3
Basal activity (U) ± S.D.	35·0 ± 2·3 (4)	33·2 ± 1·1 (5)	50·0 ± 1·6 (4)
Total activity (U) ± S.D.	119·2 ± 4·9 (4)	125·3 ± 3·7 (5)	138·4 ± 4·5 (4)
Absolute activity increase (U)	84·2	92·1	88·4
Relative activity increase (%)	240	277	176

3. *Effect of thyroxine on adenylcyclase activity* in vitro. Homogenates from normal and hypothyroid rats were incubated with thyroxine as shown in Table 3. Only minor changes could be observed: thyroxine increases non significantly the enzyme from normal animals, and slightly (but significantly) decreases the activity in hypothyroid rats.

TABLE 3. EFFECT OF THYROXINE ON HEART ADENYLCYCLASE OF HOMOGENATES FROM TWO GROUPS OF NORMAL RATS AND TWO GROUPS OF HYPOTHYROID RATS

	Controls		Hypothyroid	
Basal activity (U) ± S.D.	7·7 ± 0·9 (4)	11·5 ± 2·0 (4)	53·8 ± 1·6 (5)	49·6 ± 0·5 (4
Hormone	L-	DL-	L-	L-
Hormone concentration (μM)	5	20	5 50	10
Absolute activity change (U)	+ 0·9	+ 1·8	−2·4 − 9·5	− 4·4
Relative activity change (%)	+12	+16	−4 −18	− 9
P	> 0·05	> 0·05	>0·05 < 0·001	< 0·001

Membrane preparation from normal animals did not respond at all to thyroid hormones (L-thyroxine was assayed at 50 μM and triiodothyronine at 5 μM).

4. *Effect of thyroxine treatment on adenylcyclase activity in hypothyroid rats.* Hypothyroid rats were divided into three groups. The animals of the first group received no treatment, those of the second group each received daily 5 μg L-thyroxine by stomach tube, those of the third group each 20 μg L-thyroxine, during 6 days. The hearts were removed 30 min after the last dose. Results are summarized in Table 4. It can be seen that oral thyroxine treatment of short duration tends to normalise (i.e. to decrease) the adenylcyclase activity in the heart of thyroid deprived rats.

TABLE 4. EFFECT OF 6-day THYROXINE TREATMENT ON HEART ADENYLCYCLASE ACTIVITY OF HYPOTHYROID RATS

Thyroxine (μg)	Heart adenylcyclase (U) ± S.D.
0	47·7 ± 1·8 (5)*
5	39·1 ± 2·3 (5)
20	35·0 ± 1·9 (6)

* Indicates the number of experiments made on the same homogenate.

5. *Effect of catecholamines on adenylcyclase activity.* Adrenaline or noradrenaline 10 μM was added to the homogenate 5 min prior to the initiation of the reaction (Table 5). Activity increased by about 100 per cent in the control groups and only 24 per cent in the hypothyroid ones.

6. *Effect of DOPAmine on adenylcyclase activity of normal rats.* Dihydroxyphenethylamine (DOPAmine) at 10 and 100 μM had no significant effect on the basal adenylcyclase activity of the heart membrane fraction from normal animals (Table 6).

TABLE 6. EFFECT OF TSH AND OF DOPAMINE ON ADENYLCYCLASE ACTIVITY OF NORMAL RAT HEART MEMBRANE FRACTION

	Activity (U) ± S.D.	P
Controls	17·9 ± 0·7 (8)	
TSH 10 μM	16·3 ± 0·7 (8)	< 0·001
Controls	19·3 ± 1·7 (5)	
DOPAmine 100 μM	17·9 ± 1·9 (5)	> 0·05
DOPAmine 10 μM	19·7 ± 1·4 (6)	> 0·05

7. *Effect of TSH on adenylcyclase activity of normal rats.* Thyrotropic stimulating hormone (TSH) 10 μM significantly decreased the basal adenylcyclase activity of the heart membrane fraction from normal rats. This decrease was, however, small: 9 per cent (Table 6).

TABLE 5. EFFECT OF ADRENALINE OR NORADRENALINE 10 μM ON HEART ADENYLCYCLASE ACTIVITY IN HOMOGENATES FROM NORMAL AND HYPOTHYROID RATS

	Control rats			Hypothyroid rats		
	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
Basal activity (U) ± S.D.	13·6 ± 2·4 (4)	21·9 ± 5·5 (5)	7·8 ± 0·8 (10)	29·0 ± 3·6 (4)	32·2 ± 1·9 (4)	35·1 ± 1·9 (4)
With adrenaline (U) ± S.D.	26·7 ± 3·2 (4)	—	18·7 ± 2·0 (10)	37·0 ± 0·7 (4)	39·6 ± 1·4 (4)	—
With noradrenaline (U) ± S.D.	—	41·4 ± 4·3 (5)	—	—	—	42·6 ± 3·1 (4)
Absolute activity increase (U)	13·1	19·5	10·9	8·0	7·4	7·5
Relative activity increase (%)	97	89	140	28	23	21

8. *Effect of propranolol on adenylcyclase activity of hypothyroid rats.* Propranolol 10^{-5} M had no effect *in vitro* on the basal adenylcyclase activity of the heart homogenates from hypothyroid animals [controls: 48·6 U ± 1·8 (8); propranolol: 46·7 U ± 2·6 (8)]. Heart adenylcyclase activity of hypothyroid rats increased, however, slightly when the animals were treated by 0·5 mg/kg i.p. propranolol daily (Table 7).

TABLE 7. EFFECT OF PROPRANOLOL TREATMENT (0·5 mg/kg i.p. DAILY) ON HEART ADENYLCYCLASE ACTIVITY (U) OF HYPOTHYROID RATS

Duration of treatment		11 days	13 days
Controls	(U ± S.D.)	64·3 ± 2·6 (5)	50·7 ± 4·0 (5)
Treated	(U ± S.D.)	70·8 ± 3·4 (5)	59·0 ± 2·8 (5)
		P < 0·01	P < 0·001

DISCUSSION

Although it has been suggested that thyroid hormone increases 3′,5′-AMP concentration in the heart by activation of adenylcyclase[7,13] in order to explain the cardiac manifestations of hypo- and hyperthyroidism, such an increase could not be demonstrated in thyroxine-treated animals.[8,9] The results in Table 1 clearly show that thyroid deprivation leads to a significant two-fold increase of heart adenylcyclase activity in the rat. This increase could be due to the activation of the enzyme, or to a greater enzyme concentration. The data of Table 2 indicate that total (fluoride) activity is only 13 per cent higher in hypothyroid rats, basal activity being increased by 46 per cent above controls in that particular experiment. It seems unlikely therefore that the activity increase could be due to enzyme concentration enhancement only: hypothyroid animals activate in some way their available heart adenylcyclase.

Levey and Epstein[13] observed an important activation of adenylcyclase from cat heart membrane fraction by thyroid hormone at 5 μM. Table 3 shows that thyroxine has no significant action on the basal activity of the enzyme from normal rat heart homogenates. It is also without action on the membrane preparation. These results are in agreement with those of McNeil *et al.*,[14] who observed, however, an increase of glycogen phosphorylase a by triiodothyronine treatment *in vivo*. Incubation of the homogenate from hypothyroid rats with thyroxine (Table 3) reduces slightly but significantly the adenylcyclase activity.

Oral treatment of hypothyroid rats by L-thyroxine (daily 5 or 20 μg per animal) during 6 days decreases heart adenylcyclase activity (Table 4).

Adenylcyclase activation by adrenaline and noradrenaline is a well-known phenomenon.[15] Our rat heart homogenate activity could be doubled with 10 μM adrenaline or noradrenaline (Table 5). Homogenates from hypothyroid animals responded much less to the same concentration of these catecholamines (both on an absolute (50%) and on a relative (25%) basis), but, owing to the already high basal activity, the values for the activated enzyme were still higher than those of the controls.

DOPAmine on the other hand, in spite of its established cardiac stimulating properties[16–19] had no measurable effects on the activity of normal rat heart membrane

DOPAmine on the other hand, in spite of its established cardiac stimulating properfraction at 10 and 100 μM (Table 6), whereas it has been shown to stimulate adenylcyclase activity in homogenates of rat cerebral cortex.[20]

It seems therefore unlikely that the cardiac response to catecholamines is entirely mediated by the adenylcyclase system.

In order to find some reason for the increase of adenylcyclase activity in the heart of hypothyroid rats, we measured the *in vitro* effect of thyrotropic stimulating hormone (TSH), the production of which is enhanced in the hypothyroid state. It appears that 10 μM TSH does not increase the enzyme activity, as shown earlier by Levey *et al.*[21]

It has been shown that thyroid deprivation enhances the catecholamine content of heart muscle[22–24] and such an increase could possibly account for the adenylcyclase increased activity we observed in hypothyroid rats. As was pointed out above, the increased myocardial concentration of DOPAmine[22] cannot explain the activation, because DOPAmine has no effect on heart adenylcyclase activity. In the case of adrenaline and noradrenaline, the β-blocking agent propranolol should be able to inhibit catecholamine activation and restore normal values, but the drug failed to exert such an effect in our experiments. Furthermore, *in vivo* treatment with propranolol did not decrease adenylcyclase activity of hypothyroid rats (Table 7). Impairment of calcium metabolism could also have played a role. Radiothyroidectomy, as performed on the animals used in this work, destroys the parathyroids and, although the rats were supplemented with calcium, it cannot be ascertained whether myocardial calcium content was normal. Calcium is known to inhibit heart adenylcyclase activity,[25] and a low calcium concentration could explain higher enzymatic activity. Comparative measurements in the presence of a calcium complexing agent were not performed, but Table 4 shows that a mere 6-day treatment by oral thyroxine alone actually decreases the enzymatic activity.

The sharp increase of heart adenylcyclase activity in hypothyroid rats is not in agreement with the idea that the cardiac effects of thyroid hormone are mediated by 3′,5′-AMP. Moreover, the cardiac effects of adrenaline and noradrenaline, which undoubtedly increase heart adenylcyclase activity, cannot be considered as being due principally to this activation, if the same kind of activation is reached after thyroid deprivation. It has been shown that in this case, activation is due neither to adrenaline, noradrenaline, DOPAmine nor TSH increases. Furthermore, it appears that DOPAmine, the cardiac stimulating properties of which are well known,[16–19] does not activate heart adenylcyclase *in vitro*. Thyroid hormone has no effect on adenylcyclase activity of heart homogenates or membrane fractions from normal animals *in vitro*, and decreases the activity of the enzyme from hypothyroid animals both *in vitro* and *in vivo*. The increased activity is perhaps to be related to very profound changes brought about by thyroid deprivation: it has been shown, e.g. that even the aminoacid composition of cardiac myosine is altered[26] in the hypothyroid state.

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