DECREASED SECRETIN AND GLUCAGON RESPONSIVENESS OF ADENYLATE CYCLASE IN CARDIAC MEMBRANES FROM HYPOTHYROID RATS

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1. Introduction

Thyroid hormones modulate cardiac activity and the number of adrenoceptors and muscarinic receptors in heart: in cardiac membranes from hypothyroid rats, there is a moderate decrease in β-adrenoceptors [1,2], an increase in muscarinic receptors [3], and a slight increase [4] or no change [1] in α-adrenoceptors. By contrast, hyperthyroidism is characterized by an increase in the number of β-adrenoceptors (quantified by [^{3}H]dihydroalprenolol) [1,5] whereas the number of muscarinic receptors [3] and α-adrenoceptors [1,2,6] decreases.

No information is available on the incidence of the thyroid status on cardiac receptors to peptide hormones and peptide neuro-effectors. We have characterized the presence of receptors specific for secretin and the parent peptide VIP (vasoactive intestinal peptide) in rat heart membranes by the capacity of these peptides to stimulate adenylate cyclase activity in the presence of guanine nucleotides [7]. This receptor system is altered in congenitally hypertensive (SHR) [8,9] and obese (fa/fa) Zucker rats [10]. Since one of the clinical features in obese fa/fa rats is a mild hypothyroidism [11], we investigated here whether hypothyroidism induced by propylthiouracil (PTU) or surgical thyroidectomy could alter secretin-stimulated

adenylate cyclase activity in rat heart membranes. The stimulation of the same enzyme system with glucagon, isoproterenol, NaF and guanine nucleotides served as a reference. The results showed that hypothyroidism decreased the response of adenylate cyclase to secretin and glucagon, increased the response to NaF, and was without effect on the response to isoproterenol and guanine nucleotides.

2. Materials and methods

Hypothyroidism was induced by 2 methods:
(1) A batch of 10 male Wistar albino rats weighing 80 ± 5 g and 6 weeks old was used. Five of these animals received 0.05% propylthiouracil (PTU) dissolved in drinking water for 8 weeks and the other 5 served as controls. Hypothyroidism was ascertained by reduced growth (body weight was 102 ± 12 g only in PTU-treated rats as compared to 302 ± 8 g in control animals, at the time of sacrifice) and low levels of circulating T_3 measured by a standard RIA (180 ± 30 ng/l in the serum of PTU-treated rats as compared to 750 ± 50 ng/l in control animals).
(2) A group of 10 adult male Wistar albino rats 315 ± 9 g body wt was used. Five animals were surgically thyroidectomized under ether anesthesia and sacrificed 10 days after surgery: at that time the body weight was 308 ± 7 g in thyroidectomized rats and 358 ± 4 g in control animals, and the corresponding serum T_3 values were 100 ± 20 ng/l and 750 ± 50 ng/l.

The animals were sacrificed by decapitation and the heart was carefully dissected out, rinsed at room temperature with isotonic NaCl, weighed and frozen in liquid nitrogen. A crude particulate fraction from heart

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was prepared as in [7] and adenylate cyclase activity was determined as in [12]. Protein concentration was measured by the Lowry procedure, using bovine serum albumin as a standard.

Synthetic secretin was kindly supplied by Dr W. König (Hoechst Aktiengesellschaft, Frankfurt); glucagon was supplied by Novo Industri (Etablissement Couvreur, Brussels). D,L-Isoproterenol, ATP, GTP, Gpp[NH]p and cyclic AMP were from Sigma Chemical Co. (St Louis MO); 4(6)-propyl-2-thiouracil (PTU) was from Koch-Light Labs (Colnbrook). $[\alpha\text{-}^{32}P]ATP$ (15 Ci/mmol) and cyclic $[8\text{-}^{3}H]AMP$ (27 Ci/mmol) were purchased from the Radiochemical Centre (Amersham). All other reagents were of the highest grade available.

3. Results

The hypothyroidism achieved after PTU treatment or thyroidectomy was demonstrated by the low levels of circulating T_3 (see above). The values of the parameters of adenylate cyclase activity in the 2 groups of control rats were similar so that these control data were combined in table 1 and fig.1.2.

Basal, GTP- and Gpp[NH]p-activated cardiac adenylate cyclase activities were not affected by hypothyroidism (table 1). The D_{50} for Gpp[NH]p activation was normal (fig.1). NaF stimulation (at 10 mM) was
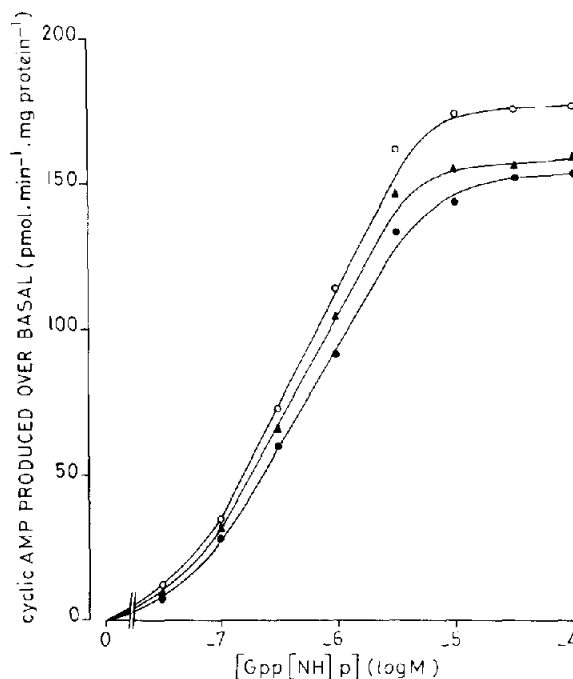

Fig.1. Dose–effect curves of adenylate cyclase activation by Gpp[NH]p in control (o—o, $n = 10$), PTU-treated (▲—▲, $n = 5$), and thyroidectomized (●—●, $n = 5$) rats. The results are expressed in pmol cyclic AMP produced over basal . min^{-1} . mg membrane protein^{-1}.

Table 1
Adenylate cyclase activity in cardiac membranes from control, PTU-treated, and thyroidectomized rats

Addition(s)	Control rats ($n = 10$)	PTU-treated rats ($n = 5$)	Thyroidectomized rats ($n = 5$)
None	29 ± 3	35 ± 2	34 ± 2
10^{-5} M GTP	36 ± 3	37 ± 3	38 ± 3
10^{-4} M Gpp[NH]p	215 ± 10	190 ± 16	221 ± 22
10^{-2} M NaF	371 ± 28	572 ± 36^a	503 ± 37^a
10^{-5} M GTP + 10^{-4} M D,L-isoproterenol	127 ± 8	144 ± 11	115 ± 6
10^{-5} M GTP + 10^{-5} M glucagon	108 ± 7	75 ± 3^a	86 ± 8
10^{-5} M GTP + 3 × 10^{-6} M secretin	68 ± 7	24 ± 2^a	21 ± 2^a

^{a} Indicates values significantly different ($p < 0.05$) from values in corresponding controls, using the Student's t-test

The data are expressed as pmol cyclic AMP formed . min^{-1} . mg protein^{-1}. Those with D,L-isoproterenol, glucagon, and secretin were obtained after subtracting the control value observed in the presence of 10^{-5} M GTP alone. Means ± SEM from 10 or 5 animals
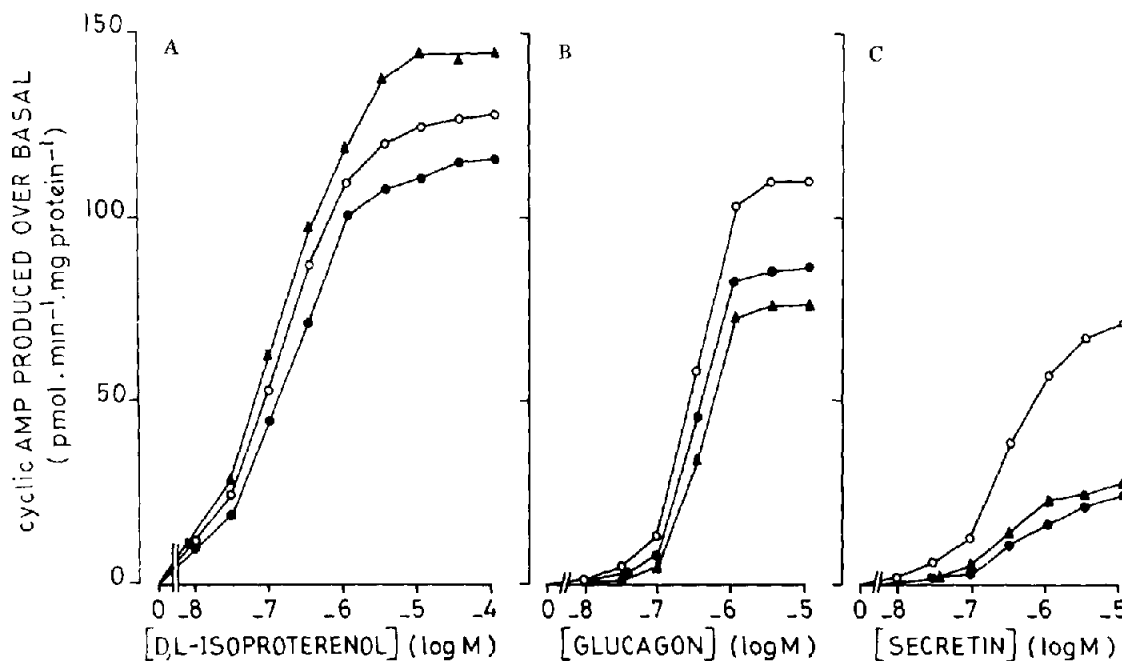
Fig.2. Dose—effect curves of adenylate cyclase activation by D,L-isoproterenol (A), glucagon (B) and secretin (C) in control ($\circ$—$\circ$, $n = 10$), PTU-treated ($\triangle$—$\triangle$, $n = 5$), and thyroidectomized ($\bullet$—$\bullet$, $n = 5$) rats. The results are expressed in pmol cyclic AMP produced min^{-1} . mg membrane $protein^{-1}$ over the unstimulated value obtained in the presence of 0.1 μM GTP.

increased by 54% in PTU-treated rats and by 36% in thyroidectomized rats (table 1).

The stimuli acting through membrane receptors were tested in the presence of 10^{-5} M GTP, since they were inefficient otherwise [7]. In both PTU-treated and thyroidectomized rats the maximal response to D,L-isoproterenol and the corresponding D_{50} of stimulation were unaffected when compared to the control group (table 1, fig.2). A significant decrease in maximal glucagon stimulation was observed in PTU-treated and thyroidectomized rats but the D_{50} for glucagon remained normal (table 1, fig.2). The maximal response to secretin was the most severely impaired by hypothyroidism (by 63% and 68%, respectively, in PTU-treated and thyroidectomized rats) with, again, no change in the D_{50} for secretin (table 1, fig.2).

4. Discussion

Two clear results emerged from our study:

(1) Hypothyroidism in male Wistar rats induced a decrease in secretin- and glucagon-stimulated cardiac

adenylate cyclase activity (fig.2). These effects were observed as early as 10 days after thyroidectomy in the second experiment. In line with these data, reduced glucagon-stimulated adenylate cyclase activity has been reported in fat cells [13] and liver membranes together with a lower density of glucagon receptors in liver membranes [14] in thyroidectomized rats. The latter result was, however, not confirmed in [15]. Here, the alterations due to hypothyroidism were even more striking for secretin than for glucagon. It is tempting, therefore, to suggest that secretin receptors in heart are especially responsive to the levels of circulating thyroid hormones. The possible relationship between the altered secretin- and glucagon-stimulated adenylate cyclase activity and various cardiac symptoms observed in hypothyroid patients, such as bradycardia and diminished cardiac output, remains to be established [16].

(2) NaF-stimulated adenylate cyclase activity increased significantly in cardiac membranes from hypothyroid rats, while Gpp[NH]p- and isoproterenol-stimulations remained normal over the entire dose—effect curves (fig.1,2). These data suggest that hypothyroidism was unable to influence the coupling mechanism involving the guanine nucleotide regulatory

protein of the adenylate cyclase system (the G-protein also known as G/F, G or N). They were in clear contradiction with those in [17] where a sharp decrease in Gpp[NH]p-stimulation of adenylate cyclase and in the number of β-adrenoceptors in the heart of rats suffering from prolonged hypothyroidism was shown. The same problem has been investigated in adipose tissue and liver from hypothyroid animals [18,19]. It was observed that several properties of the guanine nucleotide regulatory protein and the number of β-adrenoceptors remained normal in fat cells but that isoproterenol-stimulation of adenylate cyclase was sharply reduced. In liver cells, the situation was different: isoproterenol-stimulated adenylate cyclase was increased and GTP could not normally reduce the affinity of β-adrenoceptors for their ligands. Here, when the normal response of cardiac adenylate cyclase to Gpp[NH]p and D,L-isoproterenol is confronted with the high NaF sensitivity of the enzyme system, there was no indication of an effect of hypothyroidism on cardiac β-adrenergic receptors and it is clear that the situation of guanine nucleotide regulatory protein(s) warrants further study by a more direct approach.

Acknowledgements

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