ADRENORECEPTOR-INDEPENDENT ACTION OF THYROXINE AND ADRENOXYL ON MYOCARDIAL ADENYLATE CYCLASE ACTIVITY AND CYCLIC AMP LEVEL

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Structural lesions in the myocardium can be induced by injection of large doses of catecholamines. Meanwhile, in myocardial infarction [3, 6, 11] and in neurogenic lesions of the myocardium [1, 2] the tissue catecholamine reserves are exhausted and the use of precursors of catecholamines or of β-adrenoblockers prevents the development of both structural and metabolic injuries to the heart muscle [2]. Since the metabolic effects of catecholamines are mediated through β-adrenoreceptors in the myocardium drugs acting on the cyclase system in conjunction with β-adrenoblockers can evidently be used. Other hormones, their metabolites, or substances modifying the metabolism of metabolic regulators in a specific direction [5], can probably be used for this purpose. It has been shown, for instance, that thyroid hormones not only influence bioenergetic processes but can also modify the sensitivity of cardiac adrenoreceptors [8] and can raise the cyclic AMP level [10]. Thyroxine in a dose of 10^{-8} M raises, but in a dose of 10^{-4} M lowers, the cyclic AMP level in the heart and other organs [4].

Accordingly, the aim of the investigation described below was to study the effect of certain hormones and their metabolites on adenylate cyclase activity and on the cyclic AMP level in the myocardium in conjunction with β-adrenoblockers.

EXPERIMENTAL METHOD

Experiments were carried out on 24 albino rats weighing 130-150 g. β-Adrenoreceptors were blocked by propranolol in a concentration of 0.1 mg/0.25 ml of suspension of the coarse fraction of myocardial cells (1-1.5 mg protein). In some experiments animals with neurogenic lesions of the myocardium were used [1]. After completion of all the necessary experimental procedures the animals were decapitated. The animals' hearts were homogenized at 0°C in 0.25 M sucrose, pH 7.5. The coarse fraction of myocardial cells (3000g) was suspended in medium containing Tris-HCl buffer, pH 7.5, theophylline, and magnesium chloride. The cyclic AMP content was determined by competitive protein binding with the cyclic AMP assay kit (Radiochemical Centre, Amersham, England). Adenylate cyclase activity was judged from the quantity of cyclic AMP formed in incubation medium containing $2 \cdot 10^{-3}$ M ATP during 15 min at 37°C. The quantity of cyclic AMP was expressed in picomoles/gram protein. Protein was estimated by Lowry's method.

EXPERIMENTAL RESULTS

The cyclic AMP content in myocardial cells of intact animals was 293 pmoles/g. Addition of the hormones to the homogenate of the coarse fraction of myocardial cells led to an increase in the cyclic AMP content. For instance, noradrenalin almost doubled the cyclic AMP content, whereas

TABLE 1. Cyclic AMP Level in Myocardium of Intact Animals (pmoles/g protein)

Control	Thyroxine	Noradrenalin 10^{-5} M	Adrenalin 10^{-5} M	Adrenoxyl 10^{-5} M
293,0±49,8 ($n=11$)	259,0±24,9 $P>0,1$	503,5±55,3 $P<0,05$	441,5±31,8 $P<0,05$	414,6±15,6 $P<0,05$

KEY WORDS: myocardial lesions; adenylate cyclase; cyclic AMP; thyroxine; adrenoxyl.

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TABLE 2. Effect of Noradrenalin and Adrenalin on Myocardial Adenylate Cyclase Activity (in pmoles cyclic AMP/g protein/15 min)

Experimental conditions	Control	Noradrenalin		Adrenalin	
		10^{-8}M	10^{-5}M	10^{-8}M	10^{-5}M
Intact animals (n = 11)	1200,9±56,1	3527,0±77,2 $P<0,05$	5765,8±47,4 $P<0,05$	2396,0±30,7 $P<0,05$	2541,2±35,9 $P<0,05$
Blocking of β-adrenoreceptors (n = 6)	1026,4±30,3		1033,0±93,6 $P>0,1$		834,8±21,8 $P<0,05$

TABLE 3. Effect of Thyroxine and Adrenoxyl on Myocardial Adenylate Cyclase Activity (in pmoles cyclic AMP/g protein/15 min)

Experimental conditions	Control	Thyroxine		Adrenoxyl	
		10^{-8}M	10^{-5}M	10^{-8}M	10^{-5}M
Intact animals (n = 11)	1200,9±56,1	2948,5±38,6 $P<0,05$	3882,1±66,6 $P<0,05$	2949,8±66,4 $P<0,05$	3725,5±52,1 $P<0,05$
Blocking of β-adrenoreceptors (n = 6)	1026,4±30,3		2773,8±14,8 $P<0,01$		2146,2±29,5 $P<0,01$

adrenalin and adrenoxyl (a stabilized preparation of adrenochrome) increased the cyclic AMP concentration by 1.5 times (Table 1). Adenylate cyclase activity in the myocardium of intact animals was 1200.9 pmoles cyclic AMP/g protein/15 min incubation with ATP. Addition of the hormones in concentrations of 10^{-5} and 10^{-8} M led to considerable activation of this enzyme. Not only noradrenalin and adrenalin (Table 2) had an activating effect on adenylate cyclase, but also thyroxine and adrenoxyl (Table 3). Their action on the enzyme depended on the hormone concentration. For instance, noradrenalin increased adenylate cyclase activity by almost fivefold in a concentration of 10^{-5} M and threefold in a concentration of 10^{-8} M. Adrenoxyl and thyroxine had a somewhat stronger action than adrenalin on myocardial cyclase. Thyroxine in a concentration of 10^{-4} M was shown [4] not to cause changes in adenylate cyclase activity, whereas in a concentration of 10^{-5} M (Table 3) it activated the enzyme almost threefold. A single injection of thyroxine 2 h before the experiment in a concentration of 0.52 μg/g body weight also caused activation of adenylate cyclase in the heart (2847.5 ± 75.2 pmoles/g; $P < 0.01$).

Blocking the β-adrenoreceptors by propranolol prevented activation of adenylate cyclase by adrenalin and noradrenalin (Table 2) but did not abolish activation of the enzyme caused by thyroxine and adrenoxyl (Table 3).

The results confirm the view that thyroxine produces some of its metabolic effects through the adenylate cyclase system, but the mechanism of its action on cyclase differs from that of the catecholamines the action of thyroxine is independent of β-adrenoreceptors. Adrenoxyl, a metabolite of adrenalin, also activates adenylate cyclase when β-adrenoreceptors are blocked. Thyroxine and adrenoxyl can thus be used, if necessary, in conjunction with β-adrenoblockers to increase adenylate cyclase activity and to raise the cyclic AMP level in myocardial cells. The need to increase adenylate cyclase activity in the myocardium in conjunction with blocking of β-adrenoreceptors probably may arise in the presence of myocardial infarction or of neurogenic lesions of the myocardium. Investigations conducted by the present writers on animals with neurogenic lesions of the myocardium showed that under these circumstances the cyclic AMP level in the heart muscle falls sharply, on account of a deficiency of ATP required for cyclic AMP synthesis. None of the hormones used restored the cyclic AMP level because of the ATP de-

TABLE 4. Effect of Thyroxine and Adrenoxyl on Adenylate Cyclase Activity (in pmoles cyclic AMP/g protein/15 min) in Neurogenic Lesions of the Myocardium (M ± m)

Control	Thyroxine		Adrenoxyl	
	10^{-8}M	0,52 μg/g/2 h	10^{-8}M	10^{-5}M
1037,0±24,0 ($n = 7$)	1555,7±43,9 $P<0,05$	1853,8±110,1 $P<0,05$	1552,1±10,1 $P<0,05$	1812,0±10,6 $P<0,05$

ficiency in the cell. Adenylate cyclase activity also is somewhat lower in animals with neurogenic lesions of the myocardium than in intact animals (Table 4). However, an increase in cyclase activity and, consequently, in the cyclic AMP concentration in the myocardium (in the presence of ATP) can be observed not only under the influence of noradrenalin and adrenalin (injection of these hormones in neurogenic lesions of the myocardium may be either undesirable or, in combinations with β-adrenoblockers, ineffective), but also of thyroxine in close to physiological concentrations (10^{-8} M), or of thyroxine given 2 h before the experiment in a dose of 0.52 μg/g body weight and of adrenoxyl in concentrations of 10^{-5} and 10^{-8} M.

LITERATURE CITED

1. Z. I. Vedeneeva, Kardiologiya, No. 5, 56 (1964).
2. I. S. Zavodskaya, E. V. Moreva, and N. A. Novikova, Effect of Neurotropic Drugs on Neurogenic Lesions of the Heart [in Russian], Moscow (1977).
3. L. T. Malaya and V. I. Volkov, Ischemic Heart Disease in the Young [in Russian], Kiev (1978).
4. Ya. Kh. Turakulov, in: Recent Advances in Hormones and the Mechanism of Their Action [in Russian], Kiev (1977), p. 65.
5. A. M. Utevskii and V. O. Osinskaya, Ukr. Biokhim. Zh., No. 6, 683 (1975).
6. I. K. Shkhvatsabaya, Ischemic Heart Disease [in Russian], Moscow (1975).
7. A. Bertelli, C. Bianchi, and L. Beany, Eur. J. Pharmacol., 19, 130 (1972).
8. T. Ciaraldi and A. V. Marinetti, Biochem. Biophys. Res. Commun., 74, 984 (1977).
9. A. Dietze and K. D. Hepp, Biochem. Biophys. Res. Commun., 46, 262 (1972).
10. A. Levey, C. Skelton, and C. Epsteini, J. Clin. Invest., 48, 2244 (1960).
11. P. Lukomsky and R. Oganov, Am. Heart J., 83, 182 (1972).
12. S. E. Mayer, in: Metabolism of the Myocardium [in Russian], Moscow (1975), p. 268.
13. E. Sutherland and G. Robinson, Pharmacol. Rev., 18, 145 (1966).

EFFECT OF PITUITARY HORMONES ON PHOSPHODIESTERASE AND ADENYLATE CYCLASE ACTIVITY IN BRAIN TISSUE *in vitro*

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The presence of peptide and protein pituitary hormones in the CNS has recently been established by radioimmunologic, biological, and immunocytochemical methods [8]. Among the pituitary hormones found in different parts of the brain are somatotrophin (STH), corticotrophin (ACTH), and prolactin. Evidence has been obtained that substances similar to pituitary hormones can be synthesized in the CNS. In particular, liberation of immunoreactive STH into the medium from cells of the amygdaloid nucleus of the rat brain cultured *in vitro* has been demonstrated [10]. The possibility of retrograde transport of pituitary hormones into the CNS through the portal vascular system of the pituitary and through ependymal tanycytes also has been discussed in the literature [8, 10]. The question accordingly arises of the role of pituitary hormones or of substances similar to them, produced by brain cells, in brain function and the likely point of application of their action in the brain. Yet no communications on the direct effect of pituitary hormones on biochemical processes in brain tissue have hitherto been published.

In the investigation described below the effect of STH and of its biologically active fragment and of ACTH and prolactin on phosphodiesterase and adenylate cyclase activity of glial cells and synaptosomes obtained from the rat cerebral cortex was studied *in vitro*.

KEY WORDS: pituitary hormones; glia; synaptosomes; adenylate cyclase; phosphodiesterase.

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