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.

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

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UDC 612.822.1.015.1.014.46:615.357.814.32:577.175.32

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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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 4, pp. 429-431, April, 1981. Original article submitted October 8, 1980.

0007-4888/81/9104- 0461$07.50

TABLE 1. Effect of Pituitary Hormones on Phosphodiesterase Activity (in nmoles AMP/min/mg protein) of Rat Brain Glia and Synaptosomes (M ± m)

Experimental conditions	Glia	Synaptosomes
Control	4,18±0,22 (100)	20,83±1,04 (100)
STH	6,23±0,28 (149,0)	36,80±0,64 (176,7)
STH fragment	23,22±1,06 (555,5)	36,25±3,12 (174,1)
Prolactin	5,44±0,08 (130,1)	28,46±0,74 (136,6)
ACTH	20,55±1,65 (491,6)	46,48±0,90 (223,1)

TABLE 2. Effect of Pituitary Hormones on Adenylate Cyclase Activity (in pmoles cyclic AMP/min/mg protein) of Rat Brain Glia and Synaptosomes (M ± m)

Experimental conditions	Glia	Synaptosomes
Control	6,15±0,27 (100)	12,65±0,78 (100)
STH	7,49±0,73 (121,8)	10,68±0,59 (84,4)
STH fragment	6,16±0,02 (100,2)	13,23±1,21 (104,6)
Prolactin	7,15±0,43 (116,3)	13,87±0,74 (109,6)
ACTH	5,89±0,37 (95,7)	7,01±0,05 (55,4)

These two enzymes are known to determine the tissue level of cyclic 3',5'-AMP, which plays a key role in regulatory processes, including in nerve tissue.

EXPERIMENTAL METHOD*

Highly purified preparations of STH and prolactin, isolated from sei whale pituitary glands, a preparation of hog ACTH (1-39) from Serva (West Germany), and also a 31-member peptide with phenylalanine at the N-end, obtained by hydrolysis of whale STH with trypsin, and corresponding to region 77-107 of the amino acid sequence of the hormone, were used. The method of obtaining the STH and its characteristics are given in [1, 2], those of the STH fragment are given below, and the method of isolation of prolactin was described in [3]. The preparations of STH, its fragment, and prolactin were all homogeneous, as shown by analysis of the N-terminal amino acids.

Fractions of glial cells and synaptosomes were obtained from the rat cerebral cortex by a method modified by the authors. The cortex was passed through a sieve with pore diameter 1000 and 500 μ in 10 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA and 0.32 M sucrose, and centrifuged for 15 min at 250g. Synaptosomes were obtained from the supernatant by the method in [12], using a 0.8, 1.0, and 1.2 M sucrose density gradient. Glial cells were isolated from the residue. They were treated with 20% Ficoll and the suspension was forced through nylon gauze with a mesh of 500, 250, 120, and 80 μ, and then layered by the method in [7] at the middle of a Ficoll concentration gradient (10, 15, 20, 30, and 41%) and centrifuged at 56,000g for 1 h. The resulting fractions of glial cells and synaptosomes were washed with 0.32 M sucrose and kept at −20°C. The purity of the fractions was verified by phase-contrast and electron microscopy. To determine adenylate cyclase and phosphodiesterase activity, 10-μl samples of homogenate were taken from each fraction (0.7-0.9 μg protein) and introduced together with 10 μl of a solution of the test hormone in 20 mM Tris-HCl buffer, pH 8.3, into the corresponding medium used to determine enzyme activity. The final volume of the samples was 50 μl and the concentration of hormones in the sample 30 μg/ml.

Phosphodiesterase activity was determined by the method of [5] in medium containing 20 mM Tris-HCl buffer, pH 8.3, 5 mM $MgCl_2$, 10 μM 3',5'-AMP, and 0.3 μCi ^{3}H-3',5'-AMP. The samples were incubated for 10 min at 37°C, after which 10 μl of the incubation mixture was applied to Silufol UV-254 plates (Czechoslovakia) and the nucleotides were separated in an isopropanol–water–ammonia (7:2:1) system. Phosphodiesterase activity was expressed in nanomoles AMP per minute per milligram protein.

To determine adenylate cyclase activity a modified method [6] was used. The incubation medium contained 50 mM Tris-HCl buffer, pH 7.5, 10 mM creatine phosphate, 0.2 mg/ml creatine phosphatase, 5 mM theophylline, 1 mM 3',5'-AMP, and 0.5 μCi ^{32}P-ATP. The samples were incubated for 20 min at 30°C. The reaction was stopped by the addition of 200 μl 0.5 N HCl. After heating for a short time (90°C, 15 min) the incubation mixture was neutralized with 1.5 M imidazole and transferred to columns with alumina. The ^{32}P-3',5'-AMP was eluted from the column with 4 ml water. Adenylate cyclase activity was expressed as the quantity of cyclic

*The authors are grateful to Academician M. E. Severin, Head of the Department of Biochemistry, Moscow University, and to Senior Scientist V. A. Tkachuk for valuable advice and technical help with this investigation.

AMP formed in picomoles per minute per milligram protein.

The radioactivity of ^{3}H-AMP and of ^{32}P-3',5'-AMP was measured on an SL-4000 counter (France). Protein was determined by Lowry's method [9].

EXPERIMENTAL RESULTS

Table 1 shows that phosphodiesterase activity of the synaptosomes and glial cells increased in the presence of pituitary hormones. However, the increase in enzyme activity in the synaptosomes and glial cells under the influence of different hormones varies substantially. ACTH caused an increase in enzyme activity of the glial cells by 392%, and of the synaptosomes by 123% compared with the control, whereas STH caused increases of 49 and 77%, respectively. Prolactin had rather weak stimulating activity. STH fragment 77-107 was a powerful phosphodiesterase stimulator in the brain tissue. By its action on phosphodiesterase of glial cells this 31-member peptide was the most active of all agents tested in the experiments. Under its influence the increase in enzyme activity amounted to 455% of the control, and was several times greater than the increase in enzyme activity in the presence of STH. This result indicates that the action of STH on phosphodiesterase of brain glial cells can be enhanced as a result of specific restricted proteolysis.

Fragments 77-107 of whale STH, accounting for 16% of the amino acid sequence of the whole hormone, exhibited the characteristic growth activity of STH in the tibia test on hypophysectomized rats. However, by contrast to its effect on phosphodiesterase, the growth-stimulating effect of the fragment in the brain was much weaker than the effect of the hormone itself.

A study of the effect of pituitary hormones on adenylate cyclase of brain glial cells and synaptosomes showed that the activity of this enzyme was modified significantly only by ACTH and only in synaptosomes (Table 2). Activity of synaptosomal adenylate cyclase was reduced by almost 50% in the presence of ACTH. The cyclic AMP level in the synaptosomes thus ought to fall sharply under the influence of ACTH, both on account of activation of phosphodiesterase, hydrolyzing the cyclic nucleotide, and also through inhibition of adenylate cyclase, catalyzing cyclic AMP formation. Fragment 77-107 of STH, a highly effective stimulator of phosphodiesterase in brain tissue, was inactive against adenylate cyclase. The action of STH on adenylate cyclase was weak and opposite in direction in glial cells and synaptosomes. Prolactin left adenylate cyclase activity virtually unchanged.

On the whole, the results are evidence that some pituitary hormones, primarily ACTH and STH, evidently have their own receptors in the brain and can perform the role of peptidergic neuromodulators in particular brain structures by lowering the cyclic AMP level in them, activating phosphodiesterase (STH and ACTH), and suppressing adenylate cyclase activity (ACTH in synaptosomes).

The steroidogenic action of ACTH in the adrenals is known to be mediated through the directly opposite effect on the cyclic AMP level, namely an increase on account of activation of adenylate cyclase [4]. This means that the mechanism of action of ACTH in the brain and adrenals differs, although it extends to the same processes of synthesis and breakdown of cyclic AMP.

The stimulating action of STH on brain phosphodiesterase evidently coincides with the action of this hormone in the diaphragm. Payne and Kostyo [11] suggested that the action of STH on transport of amino acids and their incorporation into protein in the diaphragm may be connected with lowering of the tissue cyclic AMP level. This hypothesis was based on complete blocking of the action of STH on the above-mentioned processes in the diaphragm by theophylline, a phosphodiesterase inhibitor, *in vitro*.

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EFFECT OF LOW TEMPERATURE ON ACTIVITY AND SUBSTRATE SPECIFICITY OF MONOAMINE OXIDASES IN RAT BRAIN MITOCHONDRIA

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UDC 612.58:612.822.1.015.1:577.152.143

Modification of the activity and properties of enzymes is one of the more important components of adaptive changes to low temperature at the molecular level. The leading role of neurohumoral mechanisms of regulation of adaptation to cold [11, 14] explains the current importance of the study of the effect of low temperatures on mitochondrial monoamine oxidase [monoamine:oxygen-oxidoreductase (deaminating) — MAO], which is of fundamental inportance to metabolism of the monoamines and the performance of their mediator functions. Two forms of the enzyme are distinguished: type A MAO, inhibited by chlorgyline, substrates of which are serotonin and noradrenalin, and type B MAO, inhibited by deprenil, substrates of which are β-phenylethylamine and benzylamine [15].

The object of this investigation was to study the activity and substrate specificity of MAO of types A and B under conditions of cold stress.

EXPERIMENTAL METHOD

Albino rats weighing 150-180 g were kept in a cold room at 2°C for 3 days. Under these circumstances the animals' rectal temperature remained unchanged. Keeping the animals at low temperatures for 3 days induced marked manifestations of a stress reaction in them the outflow of noradrenalin from the hypothalamus and sympathetic nerve endings, the secretion of catecholamines and corticosteroids from the adrenals, and so on, were increased [1, 9, 14]. Rats of the same age and body weight, kept in the animal house at 20-22°C, served as the control. The animals were decapitated in the cold room and all subsequent operations were performed in the cold. The brain was homogenized in 0.25 Msucrose solution made up in0.02 M phosphate buffer, pH 7.45. Mitochondria were isolated by differential centrifugation [10]. Deamination of serotonin, noradrenalin, glucosamine, AMP, putrescein, and γ-aminobutyric acid (GABA) was judged from the liberation of ammonia after incubation of the suspension of mitochondria with one of the substrates in saturating concentration. Incubation was carried out in a medium of air at 37.5°C and pH 7.45 for 30 min. The ammonia content was determined by the phenol and hypochlorite method after isothermic distillation [4]. When p-nitrophenylethylamine was used as the substrate, MAO activity was judged from the intensity of the color which developed as a result of condensation of the aldehyde, formed under the influence of the enzyme, with excess of substrate [10]. Protein was determined by Lowry's method. When the effect of chloryline and deprenil on MAO activity was studied the suspension of mitochondria was preincubated in one of the inhibitors for 15 min at 20°C. The work was done in January-March, which is important in connection with data showing seasonal differences in the response of animals to cold [13].

EXPERIMENTAL RESULTS

As Table 1 shows, keeping the animals for 3 days at 2°C led to a significant fall in type A MAO activity: The intensity of deamination of serotonin was reduced by 32% and of noradrenalin by 29%. The decrease in MAO activity with natural substrates was accompanied by a

KEY WORDS: cooling; brain; monoamine oxidase.

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 0007-4888/81/9104-0464$07.50