CYCLIC AMP CONTENT IN SOME STRUCTURES OF THE LIMBIC SYSTEM IN RATS TREATED WITH CORTICOSTEROIDS

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The effect of hydrocortisone and deoxycorticosterone acetate (DOCA) on the content of cyclic AMP in the hypothalamus, hippocampus, and corpus striatum was studied in rats. Single (determination after 1 and 24 h) and repeated (7 days) injection of hydrocortisone in a dose of 5 mg/100 g body weight was followed by an increase in the cyclic AMP concentration in these brain structures. After a single injection of DOCA in a dose of 0.5 mg/100 g body weight no change was found in the cyclic AMP level in structures of the limbic system in rats, but an increase in the cyclic AMP concentration was found after a dose of 2.5 mg. Prolonged administration of the hormone in these doses caused no changes in the cyclic AMP level in the brain structures tested. Only in the hippocampus was an increase in the cyclic AMP concentration by 210% observed after injection of 0.5 mg DOCA.

KEY WORDS; cyclic AMP; limbic system; hydrocortisone; deoxycorticosterone.

Neurophysiological investigations have shown that one pathway for the regulatory effect of adrenocortical hormones on the CNS is through a change in functional relations between the limbic structures of the brain [3, 8]. These structures are known to play an important role in memory processes and in the formation of emotional and behavioral responses, the degree of manifestation of which, in turn, is substantially influenced by steroid hormones [1, 13].

To study the possible mechanism of these neurophysiological responses it is important to examine the effect of adrenocortical hormones on the cyclic AMP concentration in structures of the limbic system for cyclic nucleotides play a direct role in neurochemical processes [10].

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male Wistar rats weighing 150-200 g. The animals were decapitated and the brain removed, after which the structures of the limbic system – the hypothalamus, hippocampus, and corpus striatum – were isolated at 0-4°C. The cyclic AMP concentration was determined by competitive radioisotope binding [9], using standard kits from the Radiochemical Center, Amersham, England. Cyclic AMP was extracted from the brain tissues by the method of Albans and Barnes [6]; 50 μl of the extract was transferred to test tubes with 0.05 ml cyclic AMP-^{3}H and 0.1 ml binding protein. After incubation (2 h, 2-4°C) 100 μl of a suspension of charcoal was added to the mixture, which was centrifuged, and 200-μl samples were transferred from each tube to flasks containing scintillation fluid, and investigated radiometrically. The cyclic AMP concentration was expressed in picomoles/g weight of tissue.

Hydrocortisone (from Gedeon Richter (Hungary) was injected intramuscularly in a dose of 5 mg/100 g body weight. The cyclic AMP level was determined 1, 4, and 24 h after injection of the hormone. During repeated injection of hydrocortisone the same dose was given over a period of 7 days.

A Soviet preparation of deoxycorticosterone acetate (DOCA) was injected intramuscularly in a dose of 0.5 and 2.5 mg/100 g body weight. The concentration of the nucleotide was determined 5 h after injection of the hormone. DOCA was injected repeatedly (over a period of 7 days) in these same doses.

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TABLE 1. Cyclic AMP Concentration (in pmoles/g wet weight of tissue) in Structures of Limbic System of Rats Receiving Corticosteroids (M ± m, n = 6)

Conditions	Repeatedly (7 days)	Time of testing after administration	Hypothalamus	Hippocampus
Control		1034±116	673±82	745±21
Hydrocortisone 5 mg/100 g body weight				
	1 h	1800±159* (74%)	1748±91* (160%)	1774±342* (138%)
	4 h	1296±220	717±99	1032±161
	24 h	2622±221* (154%)	1491±141* (122%)	2102±687* (+182%)
	7 days	4889±306* (+373%)	3389±221* (+404%)	4457±702* (504%)
DOCA singly:				
0,5 mg/100 g body weight	5 h	992±96	775±290	801±153
2,5 mg/100 g body weight	5 h	3021±422* (192%)	3870±111* (+210%)	5105±579* (585%)
Repeatedly (7 days):				
0,5 mg/100 g body weight	7 days	1471±295 (42%)	2086±244* (210%)	1358±239 (86%)
2,5 mg/100 g body weight	7 days	1021±69	754±146	684±74

Legend. 1) * $P < 0.05$ compared with control. 2) Changes in percent of control given in parentheses.

EXPERIMENTAL RESULTS

The experimental results given in Table 1 show that the highest cyclic AMP concentration was found in the hypothalamus, followed by the corpus striatum and hippocampus (1034, 745, and 673 pmoles/g wet weight of tissue respectively). Table 1 also shows that the cyclic AMP concentration in the rat brain structures studied was increased after single (when determined 1 and 24 h later) and repeated injections of hydrocortisone in a dose of 5 mg/100 g body weight. The cyclic AMP level was within normal limits 4 h after a single injection of the hormone.

A single injection of DOCA in a dose of 0.5 mg/100 g body weight did not change the cyclic AMP concentration in structures of the limbic system of the rat brain, but a dose of 2.5 mg increased the nucleotide concentration. Prolonged administration of the hormone in these doses caused no changes in the cyclic AMP level in the brain structures tested. Only in the hippocampus was an increase in the cyclic AMP concentration by 210% observed after injection of 0.5 mg DOCA. After injection of this dose of the hormone a tendency for the nucleotide concentration to rise was observed in the hypothalamus and corpus striatum.

The results reflecting the effect of hydrocortisone on the cyclic AMP level in the limbic structures of the brain described above agree with observations by other workers [4, 5, 12]. However, these workers found an increase in the cyclic AMP concentration in other tissues also, in human lymphocytes [12], in mouse blood, adipose tissue, heart, and skeletal muscles [4], and in blood plasma from patients with Cushing's disease [5]. As regards the action of DOCA on the cyclic AMP concentration, according to the authors of one report the cyclic AMP level in rat urine rises after prolonged administration of DOCA [7].

Consequently, data in the literature [4, 5, 7, 11, 12, 14] and the results of our previous [2] and present investigations do not rule out the possibility that cyclic nucleotides may play an essential role in the common path of biochemical reactions in the brain determined by adrenocortical hormones.

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REGIONAL DIFFERENCES IN THE NORADRENERGIC AND CHOLINERGIC INNERVATION OF THE PIAL ARTERIES OF THE BRAIN

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Considerable regional differences in the noradrenergic innervation of the pial arteries of rats were found histochemically (by the condensation reaction with glyoxal). The distal portions of the arteries (arterial bordering zones) were not innervated or were much less richly innervated then the proximal portions (the zones of supply of the individual vessels). Fibers detected by the reaction for acetylcholinesterase were more uniformly distributed. It is suggested that insufficiency of the noradrenergic innervation may be one factor responsible for the lower resistance of vessels of the arterial bordering zones to an acute rise of arterial pressure.

KEY WORDS: pial arteries; arterial bordering zones; noradrenergic innervation.

Experiments on animals showed that during an acute rise of arterial pressure compensatory constriction of the pial arteries does not arise everywhere: Some arteries are passively dilated and show signs of failure of the response of autoregulation of the cerebral blood flow. According to data obtained by Gannushkina and Shafranova [9], failure of the response of autoregulation of the cerebral blood flow followed by damage to the blood–brain barrier (BBB) and the brain substance, takes place chiefly and preferentially in zones of anastomosis between distal branches of the main arteries of the brain, which are known as arterial bordering zones [2]. The hypothetical sites for these pathological reactions are vessels running a straight course, vessels branching off at an acute angle, and end-to-end anastomoses [8]. To explain this uneven response the hypothesis of the role of the "geometry" of the arteries, which may determine the hemodynamics and so affect the intravascular pressure [1], has been suggested. Other conditions being the same, this may probably be the leading factor. But are other conditions the same? In particular, is there no difference in the innervation of those portions of the arterial system which differ in their responses? Publications in which regional differences in the adrenergic innervation of the pial and intracerebral vessels are described [4, 7, 12] do not give the answer to this question.

In this investigation the innervation of arteries was compared in those parts of the vascular system in which a normal autoregulatory response predominates and in those parts where the response of autoregulation of the cerebral blood flow is disturbed, i.e., in arterial bordering zones. Systems of nerve fibers revealed by staining for catecholamines and for acetylcholinesterase (AChE) were studied.

EXPERIMENTAL METHOD

The innervation of the pial arteries was studied in two-dimensional preparations of the pia mater taken from 20 noninbred albino rats. Biogenic amines were detected by the condensation reaction with glyoxal [13] in the writer's modification for membranous tissue. The rat brain was perfused through the left ventricle with

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