EFFECT OF CHANGES IN THE CORTICOSTEROID SUPPLY ON CYCLIC AMP LEVELS IN STRUCTURES OF THE LIMBIC SYSTEM IN RATS

V. P. Komissarenko, V. Ya. Kononenko, and N. M. Kosmina

UDC 612.453.018.2:612.8.014:612.825.1

KEY WORDS: cyclic AMP; limbic system; adrenalectomy; hydrocortisone, deoxycorticosterone acetate.

It was reported previously [2] that the corticosteroids hydrocortisone and deoxycorticosterone acetate (DOCA) have a definite influence on the cyclic AMP (cAMP) content in structures of the limbic system of rats such as the hippocampus, hypothalamus, and corpus striatum.

The object of the present investigation was to study the action of adrenalectomy and administration of corticosteroids (hydrocortisone and DOCA) after preliminary adrenalectomy on the cAMP level in the above-mentioned structures of the limbic system.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male Wistar rats weighing 150-200 g. The animals were decapitated, the brains removed, and the following structures of the limbic system were isolated at 0-4°C: hypothalamus, hippocampus, and corpus striatum. The cAMP concentration was determined by competitive radioisotope binding [7], using standard kits from Amersham Corp. (England); cAMP was extracted from brain tissues by the method in [4]. The extract was transferred in a volume of 50 µl to tubes containing 0.05 ml N-cAMP and 0.1 ml binding protein. After incubation (2 h, 2-4°C) 100 µl of charcoal suspension was added to the mixture, which was centrifuged (2500g), and 200-µl samples were transferred from each tube to flasks containing scintillation fluid for radiometric investigation. The cAMP concentration was expressed in pmoles/g wet weight of tissue.

Rats undergoing bilateral adrenalectomy which was performed under ether anesthesia in one stage were used in the experiments 7 days after the operation. During this period the animals received 0.9% common salt solution instead of drinking water. The controls of these experiments consisted of animals undergoing mock operations. In two series of experiments the effect of replacement therapy was studied on the cAMP concentration in the structures of the limbic system mentioned above. For this purpose hydrocortisone (from Gedeon Richter, Hungary) was given to the adrenalectomized animals of one group (starting from the day of adrenalectomy) for 7 days in a dose of 5 mg/100 g body weight, whereas rats of the other group received DOCA (from Rostov Chemical Factory) in a dose of 0.5 mg/100 g body weight.

EXPERIMENTAL RESULTS

The cAMP concentration in structures of the limbic system of animals undergoing mock operations was virtually unchanged compared with the controls (Table 1). Adrenalectomy in rats was accompanied by a significant fall in concentrations of the nucleotide in all structures investigated: by 89.5% in the hypothalamus, and by 30.3 and 30.5% in the hippocampus and corpus striatum, respectively. The use of replacement therapy (hydrocortisone in a dose of 5 mg/100 g) in fact prevented the decrease in the cyclic AMP concentration observed in the adrenalectomized rats, and it remained at about the same level as in intact animals. DOCA was ineffective from this standpoint.

No information could be found in the literature on the effect of differences in the corticosteroid supply of the body on the brain cAMP level. Only Makino et al. [9], in their ex-

Laboratory of Neurochemistry, Kiev Research Institute of Endocrinology and Metabolism, Ministry of Health of the Ukrainian SSR. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 6, pp. 56-57, June, 1982. Original article submitted October 12, 1981.

0007-4888/82/9306-0759$07.50

TABLE 1. Effect of Adrenalectomy and Replacement Therapy on cAMP Concentration (in pmoles/g wet weight of tissue) in Structures of Rat Limbic System (M ± m, n = 5 or 6)

Conditions	Hypothalamus	Hippocampus	Corpus striatum
control	1405 ± 71	1090 ± 44	1139 ± 144
Mock operation			
Adrenalectomy	1381 ± 75	1041 ± 75	1059 ± 86
Adrenalectomy + hydrocortisone (5 mg/100 g daily—7 days)	145 ± 6* (—89,5 %)	726 ± 92* (—30,3 %)	735 ± 60* (—30,5%)
	1232 ± 203	940 ± 157	970 ± 147
Adrenalectomy + DOCA (0,5 mg/100 g daily—7 days)	789 ± 66* (—44 %)	756 ± 107* (—27,5 %)	642 ± 77* (—39,2%)

*P < 0.05 compared with control. Note: Changes relative to control shown in % in parentheses.

periments, observed an increase in the cAMP concentration in the adenohypophysis of adrenalectomized rats. After adrenalectomy no changes were found in the cAMP in rat liver [6, 14], in mouse skeletal muscles, liver, and kidneys [13], or in rat blood plasma [8]. A decrease in the cAMP concentration was found in the liver and lungs of adrenalectomized rats [3].

Data in the literature on the effect of adrenalectomy on activity of enzymes concerned in cAMP synthesis and breakdown — adenylate cyclase (ADC) and phosphodiesterase (PDE) — are rather contradictory. Nakagawa and Kuriyama [10], for instance, reported that bilateral adrenalectomy in adult rats lowers ADC activity in brain slices obtained 3 and 7 days after the operation. PDE activity was unchanged under these circumstances. No changes likewise were observed in the activity of these enzymes in rat liver after adrenalectomy by Thompson et al. [14]. An increase in PDE activity in adrenalectomized rats was found in structures of the limbic system [1] and in homogenates of isolated fat cells [12].

When explaining the mechanism of action of corticosteroids on neurochemical processes in the cell some recent writers [11, 14] have postulated that one such mechanism is regulation of cAMP metabolism through the direct action of the hormones on PDE activity, whereas others [5, 6] postulate a permissive action of corticosteroids on the cyclic nucleotide system. It must be pointed out, however, that the mechanism of the permissive action of adrenocortical hormones on this system is not yet settled, and it is still undecided whether it can be explained by separate modulation of the level or activity of the specific enzymes or by a finer adjustment of ion (especially Ca^{++}) exchange.

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DNA DAMAGE AND ITS PREVENTION IN THE NONISCHEMIZED AREA OF HEART MUSCLE IN RATS WITH EXPERIMENTAL MYOCARDIAL INFARCTION

F. Z. Meerson, V. K. Vasil'ev, and R. S. Dosmagambetova

UDC 616.127-005.8-092.9-07:616.127-008.939.633.2-074

KEY WORDS: nonischemized zone; heart muscle; experimental myocardial infarction.

Marked disturbances of metabolism and function regularly develop in the nonischemized zones of the heart in infarction, and often they are explained by the presence of some degree of hypoxia in the zone surrounding the infarct. This explanation has recently been questioned because it has been shown that the demarcation line between the zone of ischemia and the nonischemized zone, based on parameters sensitive to hypoxia such as the ATP, acid phosphatase, glycogen, and lactate concentrations, is very sharp in experimental infarction: There is no gradual transition [9]. Similar results have been obtained by other workers [10] and it accordingly seems likely that it is not hypoxia or, at least, it is not only hypoxia that causes damage to the nonischemized zones of the heart in infarction. We have suggested that one factor damaging these zones is emotional-painful stress, which develops in myocardial infarction and exposes the myocardium to the action of an excess of catecholamines. It was shown previously that in animals with emotional-painful stress evoked by a certain environmental situation, lipid peroxidation (LPO) is activated in the myocardium and damage to DNA develops, simultaneously, in the form of a fall in its degree of polymerization [5, 4], but this can be prevented by administration of LPO inhibitors (antioxidants). In experimental myocardial infarction LPO activation has been proved and has been found to be well marked in nonischemized zones of the heart [2]. Accordingly the writers have postulated that damage and subsequent repair to DNA take place in nonischemized zones of the heart in infarction.

The aim of this investigation was to study injuries and subsequent repair to DNA in nonischemized zones of the heart in experimental myocardial infarction and to study the possibility of preventing these injuries by preliminary administration of the β-blocker inderal (propranolol) and the LPO inhibitor ionol.

EXPERIMENTAL METHOD

Male Wistar rats weighing 190-210 g were used. An experimental myocardial infarct was induced by ligation of the descending branch of the left coronary artery by Selye's method. A mock operation, thoracotomy under general anesthesia but without ligation of the coronary artery, was performed on control animals. Nuclei were isolated in the usual way [15], but magnesium was omitted from the medium and isolation carried out in the presence of EDTA [12] to inhibit intranuclear nucleases. Lysates of the nuclei were sedimented in an alkaline sucrose gradient by the method in [14] in the writers' own modification [4]. To evaluate reparative DNA synthesis, 2 h before sacrifice the animals were injected with hydroxyurea in 0.15 M NaCl solution, pH 7.4 (the remaining compounds also were injected in this solution), in a dose of 50 mg/100 g body weight, sufficient to inhibit replicative synthesis by 98%. Ten minutes after receiving hydroxyurea the animals were injected with thymidine-^{3}H (55 Ci/mmole, USSR) in a dose of 200 μCi/100 g body weight. Ionol [2,6-di(tert-butyl-4-methylphenol)], ground up beforehand with a small volume of Tween-60, was injected 96, 48, and 24 h before creation of the experimental infarct and immediately thereafter in a dose of 5 mg/100

Laboratory of Pathophysiology of the Heart, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 6, pp. 57-60, June, 1982. Original article submitted January 7, 1982.

