and under these circumstances an adequate response of the splenocyte is observed to con A, whereas subfractions C.3 and F.1 act in the opposite direction. During the first minutes the "microviscosity" is not reduced, as is usually the case, but increased.

The investigation showed that thymectomy leads to a disturbance of the structure of mouse splenocyte membranes. Under the influence of tactivin the "microviscosity" of the membranes is restored. Moreover, subfractions of tactivin possess the opposite kind of action, confirming the hypothesis of functional heterogeneity of tactivin, postulated previously [2].

LITERATURE CITED

1. V. Ya. Arion, I. V. Sanina, Yu. N. Breusov, et al., Chemistry and Biology of Immunoregulators [in Russian], Riga (1985), pp. 39-44.
2. V. Ya. Arion, Byull. Éksp. Biol. Med., **104**, No. 9, 332 (1987).
3. V. Ya. Arion, Zh. Mikrobiol., No. 4, 98 (1987).
4. V. Ya. Arion, A. V. Khromenkov, A. K. Tagirova, et al., Vopr. Med. Khim., **33**, No. 6, 56 (1987).
5. V. S. Kozhevnikov, V. Ya. Arion, I. V. Sanina, et al., Immunologiya, No. 4, 34 (1985).
6. R. V. Petrov, Progress in Science and Technology. Series: Immunology [in Russian], Vol. 26, Moscow (1988), p.3.
7. A. A. Yarilin, Problems and Perspectives in Modern Immunology [in Russian], Novosibirsk (1988), pp. 234-247.
8. J. F. Bach and G. Goldstein, Thymus, **2**, 1 (1980).
9. C. C. Curtain, F. D. Looney, J. J. Marchalonis, et al., J. Membr. Biol., **44**, 211 (1978).
10. C. C. Curtain, F. D. Looney, and J. A. Smelstorius, Biochim. Biophys. Acta, **596**, No. 1, 43 (1980).
11. C. C. Curtain, Biomembranes, **12**, 603 (1984).

EFFECT OF DEOXYCORTICOSTERONE ON 5′-NUCLEOTIDASE AND ADENOSINE DEAMINASE ACTIVITY IN THE RAT HYPOTHALAMUS AND HIPPOCAMPUS

V. P. Komissarenko, V. Ya. Kononenko, N. M. Kosmina, and L. I. Pil'kevich

UDC 616.357:577.175.533].015.4:612.826.4.015.1

KEY WORDS: DOCA, 5′-nucleotidase, adenosine deaminase, hypothalamus, hippocampus

Most hormones, including corticosteroids, have a considerable influence on the state of brain function, and modify metabolism in its functionally different structures. Corticosteroids affect synaptic transmission, by modifying reception and release, biosynthesis, and conversion of mediators and modulators actually in the neuron or in its synaptic endings. The study of the character of changes in metabolism of synaptic mediators is particularly important in the limbic system of the brain, whose individual structures are responsible, on the one hand, for antagonistic control of biosynthesis and secretion of hormones of the hypophyseoadrenal system, and on the other hand, for the realization of hormonal influences on functions of the CNS. An important place in the mechanism of these neuroendocrine relations is occupied by biogenic amines: catecholamines, serotonin, etc. [3]. Meanwhile the functional role of other possible chemical mediators of neurotransmission, such as adenosine, in neuroendocrine responses of limbic structures has not been adequately studied.

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. 110, No. 7, pp. 56-58, July, 1990. Original article submitted August 4, 1989.

TABLE 1. Effect of Single and Chronic Injections of DOCA on 5′-Nucleotidase Activity (μmoles phosphorus/h/mg protein) in Rat Hypothalamus and Hippocampus (n = 5-8)

Experimental conditions	Hypothalamus		Hippocampus	
	homogenate	synaptosomes	homogenate	synaptosomes
Oil, 5 h	2,75±0,17	1,46±0,09	8,69±2,49	2,06±0,08
DOCA, 5 h	4,09±0,58*	1,66±0,15	7,33±1,79	2,36±0,14*
Oil, 7 days	2,52±0,09	1,54±0,09	3,05±0,15	2,19±0,01
DOCA, 7 days	2,86±0,10*	1,51±0,06	2,98±0,11	2,04±0,09

TABLE 2. Effect of Single and Chronic Injections of DOCA on Adenosine Deaminase Activity (nanomoles/min/mg protein) in Rat Hypothalamus and Hippocampus (n = 5-8)

Experimental conditions	Hypothalamus			Hippocampus		
	homogenate	soluble fraction	synapto-somes	homogenate	soluble fraction	synapto-somes
Oil, 5 h	49,23±4,76	119,8±28,09	16,7±1,93	56,69±15,68	58,94±18,09	6,72±0,67
DOCA, 5 h	78,01±15,19	225,9±72,05	17,88±1,09	27,38±7,40	34,38±4,77	5,23±0,45*
Oil, 7 days	91,58±10,50	55,89±13,51	14,62±2,63	44,21±3,05	54,24±4,26	9,10±1,34
DOCA, 7 days	105,54±2,18	121,3±9,41*	24,79±1,16*	36,50±0,94*	41,97±1,23*	5,31±0,28*

Legend. Here and in Table 1, asterisk indicates statistically significant difference between control and experiment.

According to the most recent findings, adenosine occupies an important place in neurophysiology, for it performs the functions of neurotransmitter or neuromodulator [12], of a factor inhibiting excitation of cortical neurons [15] and synaptic transmission [14]. It has a marked influence on the secretion of various neurotransmitters into the synaptic space [12], and modifies the level of such an important secondary messenger as *c*AMP in nerve cells [11], etc. These and other facts have led to adenosine being regarded as a unique kind of integrator of functions of the CNS and autonomic nervous system.

With the above considerations in mind, we studied the effect of deoxycorticosterone acetate (DOCA) on activity of enzymes of adenosine synthesis and conversion [5′-nucleotidase (EC 3.1.3.5) and adenosine deaminase (EC 3.5.4.4)] in the hypothalamus and hippocampus of rats.

EXPERIMENTAL METHOD

Experiments were carried out on mature male Wistar rats weighing 150-200 g. The animals were decapitated, the brain removed, and the hypothalamus and hippocampus were separated at 0-4°C, and cooled from three animals. Subcellular fractionation was carried out by the method in [8]. 5′-nucleotidase activity was determined by the method in [10]. The reaction mixture contained, in 1 ml, 40 μmoles of Tris-HCl (pH 7.45), 12 μmoles $MgSO_4$, 5 μmoles AMF, and 0.1 ml of homogenate, or of the subcellular fraction suspended in 0.32 M sucrose solution. After incubation for 1 h at 37°C the reaction was stopped by the addition of 1.5 ml TCA, followed by centrifugation. To determine phosphorus, 0.1-0.5 ml of the supernatant was taken. Enzyme activity was expressed in μmoles phosphorus/mg protein/h of incubation.

Adenosine deaminase activity was determined with the aid of (8-^{14}C)-adenosine ($3.7 \cdot 10^8$ Bq/mmole) and chromatography of the reaction products on paper [1]. The volume of the incubation mixture was 40 μl. The reaction was carried out at 30°C and stopped by immersing the sample in boiling water for 3 min. After centrifugation 10 μl of supernatant was applied to chromatography paper and ascending chromatography carried out in 0.05% ammonia solution for 3 h. Regions visible in ultraviolet light (254 nm) were cut out and immersed in vessels containing ZhS-107 scintillation fluid, after which radioactivity was measured on a "Mark 3" counter (USA). Adenosine deaminase activity was estimated from the total radioactivity of inosine and hypoxanthine. The incubation time and protein concentration were chosen so that activity of the enzyme was determined under initial reaction velocity conditions. Enzyme activity was expressed in nanomoles/mg protein/min. The protein concentration was determined by Lowry's method in Harty's modification. The results were subjected to statistical analysis by Student's and the Wilcoxon—Mann—Whitney test.

Soviet DOCA (from Rostov chemical factory) was injected intramuscularly in a dose of 2.5 mg/100g body weight. Enzyme activity was determined 5 h after a single injection of the hormone. In the case of chronic administration (for 7 days) of the hormone the rats were killed 24 h after the last injection. Animals receiving injections of the corresponding amounts of the solvent (oil) served as the control.

EXPERIMENTAL RESULTS

The experiments showed that after a single injection of DOCA, 5′-nucleotidase activity increased relative to the control in homogenate of the hypothalamus and in synaptosomes of the hippocampus (Tables 1 and 2). Repeated injections of this hormone led to an increase in enzyme activity in the homogenate of the hypothalamus. No significant changes were observed in the hippocampus in this case.

Adenosine deaminase activity after a single injection of DOCA was increased in the homogenate of the hypothalamus and reduced in synaptosomes of the hippocampus. More definitely opposite changes in the activity of this enzyme in the hypothalamus and hippocampus were found in the case of chronic administration of the hormone. Adenosine deaminase activity was increased under these circumstances in the soluble fraction and in synaptosomes of the hypothalamus, but in the hippocampus, on the other hand, it was reduced in the homogenate, the soluble fraction, and synaptosomes.

It can thus be concluded from these results that DOCA can modify activity of key enzymes of adenosine metabolism. Under these circumstances activity of both 5′-nucleotidase and adenosine deaminase was increased in the hypothalamus. This suggests that administration of DOCA is accompanied by increased turnover of adenosine in this brain structure in rats.

In the hippocampus, however, increased 5′-nucleotidase activity in the synaptosomes after a single injection of DOCA and reduced activity of adenosine deaminase in synaptosomes after a single injection and also in the homogenate, soluble fraction, and synaptosomes after chronic injections of DOCA are evidence that the neuromodulator may accumulate in this structure and, consequently, that inhibitory processes are intensified, in agreement with data in the literature. It has been shown [13] that endogenous adenosine (released independently of Ca^{2+}) causes tonic inhibition of pyramidal cell activity and performs the role of chemical mediator in the negative feedback mechanism between the metabolic state and electrical activity of the nerve tissue.

It is difficult to explain unequivocally the changes we found in 5′-nucleotidase and adenosine deaminase activity, and the increased adenosine concentration in the rat hippocampus apparently resulting from it. We showed previously [4] that repeated injections of DOCA into rats are accompanied by an increase in ATP and creatine phosphate concentrations and a decrease in the inorganic phosphorus concentration in the brain. Considering that nucleotides (ATP and, in particular, ADP) and also creatine phosphate are powerful inhibitors of 5′-nucleotidase activity [9], it becomes evident that the observed activation of this enzyme and the presumed increase in the adenosine concentration in the hippocampus are unlikely. On the other hand, since DOCA causes considerable inhibition of electron transport along the respiratory chain [5] and strongly inhibits the respiration rate [6], the observed increase in the ATP concentration evidently takes place partly as a result of activation of glycolysis by this hormone [6], and also, evidently, partly through activation of the myokinase reaction, in the course of which the concentration of ATP, a powerful 5′-nucleotidase inhibitor, falls. In our view it is an interesting fact also that Mg^{2+}, an activator of this enzyme [9], forms a complex with creatine phosphate or with ATP + ADP, and reduces inhibition of 5′-nucleotidase by these compounds. The observed decrease in adenosine deaminase activity, aimed at increasing the adenosine concentration, is probably further proof of the activation of various compensatory processes and reactions by the body under the influence of pharmacological doses of DOCA.

Yet another possible mechanism can be put forward to explain the results of this investigation. We showed previously that single and chronic injections of DOCA into rats are accompanied by an increase in the hippocampal *c*AMP concentration and an increase in phosphodiesterase activity [2], on the one hand, and the functional connection discovered by Sharova et al. [7] between phosphodiesterase and 5′-nucleotidase, manifested as effective acceleration of the combined reaction, catalyzed by these enzymes: *c*AMP — 5′-AMP — adenosine, on the other hand, indicate that elevation of the adenosine level may be the result of stimulation of *c*AMP formation and of activation of phosphodiesterase and of 5′-nucleotidase by the hormone. The adenylic acid thus formed can evidently be utilized not only for adenosine synthesis, but also in the myokinase reaction to synthesize ATP. The changes in enzyme activity studied in the synaptosomal fraction point to the possibility that DOCA may have a direct influence on this part of the neuron.

As a whole the results are evidence that the adenosine system may be one point of application of functional involvement of mineralocorticoids in the brain, but the mechanisms of these influences require further study.

LITERATURE CITED

1. N. P. Dmitrienko, S. V. Komissarenko, and V. Yu. Umanskii, Dokl. Akad. Nauk. Ukr. SSR, Ser. Biol., 68 (1980).
2. V. P. Komissparenko, V. Ya. Kononenko, and N. M. Kosmina, Seventh Belorussian Republican Conference of Endocrinologists [in Russian], Brest (1979), p. 236.
3. V. Ya. Kononenko, Endocrinology Today [in Russian], Kiev (1982), p. 65.
4. N. M. Kosmina, Third Biochemical Conference of Belorussian, Latvian, Lithuanian, and Estonian SSR [in Russian], Minsk (1968), p. 134.
5. T. T. Podvigina, Ukr. Biokhim. Zh., **52**, 36 (1980).
6. T. T. Podvigina, T. S. Bogdanova, and M. I. Mityushov, Neirokhimiya, **2**, 189 (1983).
7. N. P. Sharova, B. Ya. Gurvich, and A. A. Galoyan, Neirokhimiya, **7**, 217 (1988).
8. V. V. Shevtsov, O. M. Pozdnyakov, I. M. Musin, and R. N. Glebov, Byull. Éksp. Biol. Med., No. 1, 94 (1972).
9. R. M. Berne, Circulat. Res., **47**, 807 (1980).
10. H. C. Buniatian, Handbook of Neurochemistry, New York (1970), p. 399.
11. J. W. Daly, Biochem. Pharmacol., **24**, 159 (1975).
12. B. B. Fredholm and P. Hedquist, Biochem. Pharmacol., **29**, 1635 (1980).
13. H. L. Haas and R. W. Greene, Naunyn-Schmiedeberg's Arch. Pharmacol., **337**, 561 (1988).
14. Y. Kuroda and H. McIlwain, J. Neurochem., **22**, 691 (1974).
15. E. Phillips and E. A. Newsholme, J. Neurochem., **33**, 553 (1974).