

# Effect of Corticosterone on the Macroergic Pool and Membrane Permeability in Sections of Rat Hippocampus

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The development of effective therapy of hypoxic (and ischemic) damage to the brain is of great importance in pathophysiology of the nervous system and in neuropharmacology. There are a few published reports of preventive *in vivo* correction of bioenergetic disturbances in brain hypoxia and ischemia with the use of glucocorticoid steroid hormones, although in some cases their therapeutic effect may become transformed into the opposite effect [4,9]. The action of glucocorticoids in the brain is primarily associated with the hippocampus, where the receptors of these steroid hormones are mostly grouped [9]. On the other hand, in accordance with some studies, during hypoxia and ischemia, the hippocampus is one of the most vulnerable brain structures [8,12]. In this connection, a study of the role of glucocorticoid hormones, whose mechanism of action on neurons in health and pathology is largely unclear, in the pathogenesis of hypoxia (ischemia) is of fundamental importance, as is a study of the possibility of using them as antihypoxic agents.

A solution to these problems was the objective of the present research, in which the antihypoxic

effects of corticosterone acetate were investigated *in vitro* using sections of rat hippocampus. In view of the fact that energy exchange is a target for hypoxia, some parameters of the macroergic pool and membrane permeability were assessed.

## MATERIALS AND METHODS

The experiments were carried out on male non-pedigree albino rats weighing 200-250 g, kept under standard conditions in the vivarium. The rats were decapitated; hippocampal sections 500  $\mu$  thick were prepared on an MNT-84 microtome. The hippocampus was taken from 6-7 animals for one experiment. Preparation of the sections lasted 40-50 min. During this period the sections were stored at 0°C in the incubation medium (Krebs-Ringer medium (mM): NaCl 120, KCl 4.8,  $\text{KH}_2\text{PO}_4$  1.0,  $\text{NaHCO}_3$  24,  $\text{MgCl}_2$  1.2,  $\text{CaCl}_2$  1.0, HEPES 10, glucose 5; equilibrated with 95%  $\text{O}_2$  + 5%  $\text{CO}_2$  (carbogen); 37°C; pH 7.4). The sections were preincubated in this medium for 60 min at 37°C. Within this period the tissue level of ATP attained the maximal values and was maintained on a steady-state level for 90 min (Fig. 1). From 9 to 10 sections were then transferred to cylindrical glass cells (20 ml) for incubation, each cell containing 2 ml of Krebs-Ringer

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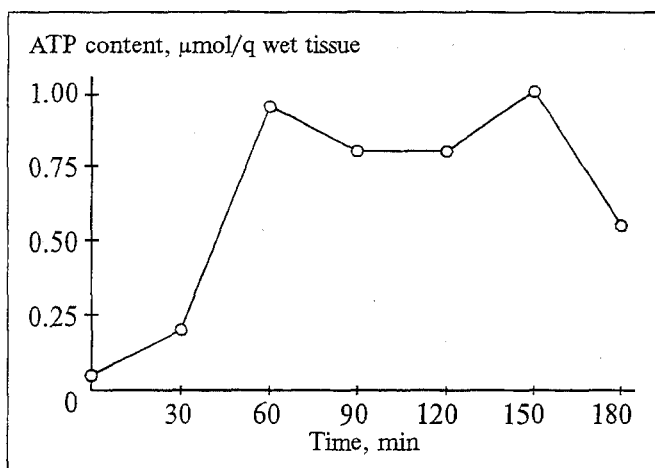


Fig. 1. ATP content in hippocampal sections as a function of time of incubation in carbogen-containing medium.

medium equilibrated with gas mixture of the corresponding composition.

The conditions created by the carbogen-equilibrated medium (900  $\mu\text{M}$   $\text{O}_2$ ) were regarded as normoxic; in such a medium the neurons in the sections preserved a spontaneous impulse activity and the ability to change it for a reduction of the  $\text{O}_2$  concentration [2-6]. In other cells the  $\text{O}_2$  concentration in the incubation medium was 700, 500, 200, and 100  $\mu\text{M}$ . Under such conditions the sections were incubated with continuous orbital stirring at a frequency of 120 oscillations/min for 30 min at 37°C. Corticosterone acetate (CSA) was dissolved in dimethylsulfoxide, and, directly before the start of hypoxia, 20  $\mu\text{l}$  of this solution were added to 2 ml of the incubation medium in a final concentration of 150  $\mu\text{M}$ . Sections incubated in medium containing 20  $\mu\text{l}$  dimethylsulfoxide served as the control.

Before the incubation was completed, aliquots were taken from the cells for measurements of the

creatine phosphokinase (CPK) activity.  $\text{HClO}_4$  was then added in a final concentration of 4%, and the cells were promptly cooled to the temperature of liquid nitrogen. The subsequent crushing of the sections and extraction of metabolites were performed at 0°C. The sections together with the liquid phase were transferred to conical plastic tubes (1.5 ml), in which they were homogenized and then centrifuged (5000 g, 10-15 min). The supernatant was separated from the pellet, neutralized with  $\text{K}_2\text{CO}_3$  to attain a pH of 7.0, and stored at -20°C prior to the measurements. The liquid was thoroughly removed from the tube with the pellet; weighing was performed, and the weight of the pellet was regarded as the wet weight of the section. The aliquots with CPK were centrifuged (5000 g, 10 min), and the upper portion of the supernatant was decanted and stored for no more than 5 days at 20°C before the measurements were performed.

The content of ATP, ADP, and creatine phosphate (CP) was determined in the tissue extracts, and the activity of CPK was measured in the incubation medium. All measurements were performed by luciferin-luciferase methods [1].

## RESULTS

As is seen from Table 1 and Fig. 2, the content of ATP, ADP, and CP in the hippocampal sections did not significantly differ for 900-700  $\mu\text{M}$   $\text{O}_2$ . However, it progressively decreased for a reduction of the  $\text{O}_2$  content from 500 to 100  $\mu\text{M}$ . The ATP level dropped most drastically in the range of 200-100  $\mu\text{M}$   $\text{O}_2$ . At 100  $\mu\text{M}$   $\text{O}_2$  the level of CP, ATP, and ADP decreased 5-fold, 4-fold, and 1.4-fold, respectively, as compared to that at 900  $\mu\text{M}$   $\text{O}_2$ . The revealed regularities of reduction

TABLE 1. Effect of CSA (150  $\mu\text{M}$ ) on the Level of Metabolites and CPK Solubilization in Sections of Rat Hippocampus for Diverse  $\text{O}_2$  Concentrations in the Incubation Medium ( $M \pm m$ ,  $n = 10 - 11$ )

Parameter	CSA	95% $\text{O}_2$ , 900 $\mu\text{M}$ $\text{O}_2$ , normoxia	70% $\text{O}_2$ , 700 $\mu\text{M}$ $\text{O}_2$	50% $\text{O}_2$ , 500 $\mu\text{M}$ $\text{O}_2$	20% $\text{O}_2$ , 200 $\mu\text{M}$ $\text{O}_2$	10% $\text{O}_2$ , 100 $\mu\text{M}$ $\text{O}_2$
ATP, $\mu\text{mol/g}$ wet tissue	—	0.85 $\pm$ 0.10	0.88 $\pm$ 0.1	0.78 $\pm$ 0.09	0.42 $\pm$ 0.05	0.24 $\pm$ 0.04
	+	0.89 $\pm$ 0.11	0.91 $\pm$ 0.11	0.99 $\pm$ 0.12*	0.63 $\pm$ 0.06*	0.47 $\pm$ 0.07*
ADP, $\mu\text{mol/g}$ wet tissue	—	0.17 $\pm$ 0.03	0.18 $\pm$ 0.02	0.17 $\pm$ 0.02	0.16 $\pm$ 0.02	0.12 $\pm$ 0.02
	+	0.18 $\pm$ 0.02	0.19 $\pm$ 0.02	0.21 $\pm$ 0.02	0.20 $\pm$ 0.03	0.17 $\pm$ 0.02
ATP/ADP	—	5.69 $\pm$ 0.55	5.71 $\pm$ 0.62	4.54 $\pm$ 0.38	2.95 $\pm$ 0.37	1.94 $\pm$ 0.41
	+	4.99 $\pm$ 0.59	5.28 $\pm$ 0.68	5.24 $\pm$ 0.76	3.48 $\pm$ 0.39	2.93 $\pm$ 0.35
CP, $\mu\text{mol/g}$ wet tissue	—	0.78 $\pm$ 0.15	0.71 $\pm$ 0.17	0.49 $\pm$ 0.05	0.29 $\pm$ 0.03	0.16 $\pm$ 0.01
	+	0.71 $\pm$ 0.16	0.77 $\pm$ 0.17	0.60 $\pm$ 0.09	0.30 $\pm$ 0.02	0.24 $\pm$ 0.03*
CPK, arb. units/min $\times$ g wet tissue	—	20.2 $\pm$ 4.6	13.7 $\pm$ 2.8	21.6 $\pm$ 4.6	59.6 $\pm$ 11.1	55.3 $\pm$ 15.0
	+	32.2 $\pm$ 6.9	12.1 $\pm$ 5.4	28.3 $\pm$ 8.2	67.2 $\pm$ 12.2	99.4 $\pm$ 20.1

Note. An asterisk indicates  $p < 0.05$  vs. the corresponding control. Minus and plus, respectively, denote absence or presence of CSA.

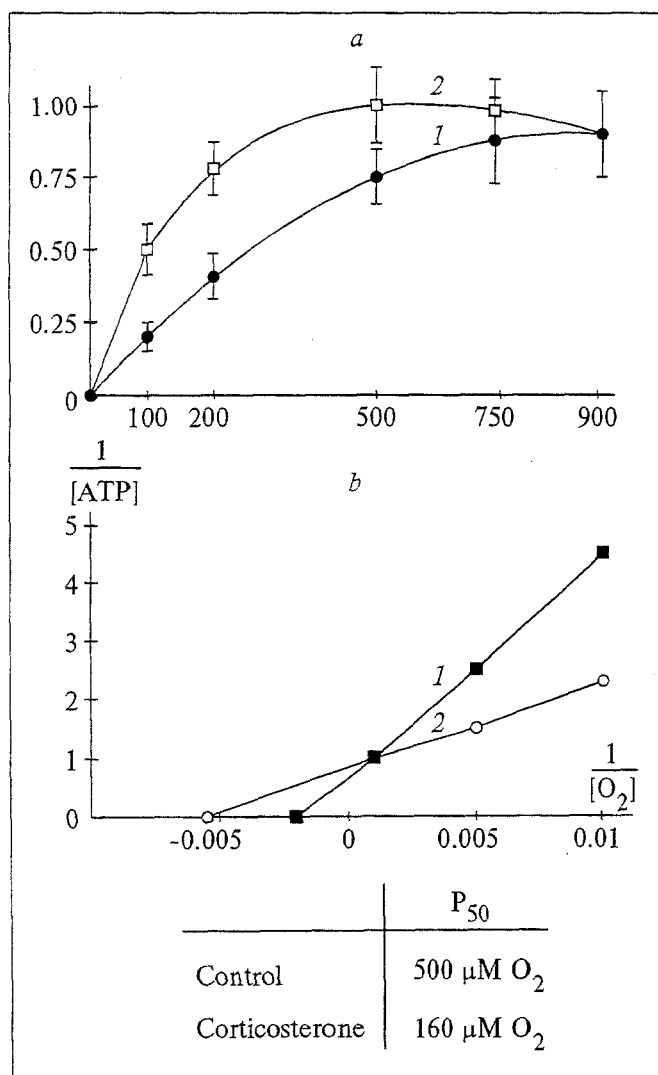


Fig. 2. Effect of O<sub>2</sub> concentration in medium on ATP content in rat hippocampus. a) abscissa: O<sub>2</sub> concentration, μM; ordinate: ATP content, μmol/g wet tissue. b) linear transformation of the same function in Lineweaver-Burke coordinates. 1) in the absence of CSA; 2) in the presence of CSA.

of the macroergic content during hypoxia are in accord with published data [3,5-7]. Thus, under the modeled conditions the energy deficiency which is characteristic of hypoxia manifests itself only when the O<sub>2</sub> content is reduced to 20% of the normoxic one or lower.

CSA (150 μM) did not significantly alter the macroergic level in the hippocampus for 900-500 μM O<sub>2</sub> (Fig. 2, a; Table 1). However, at 200 μM O<sub>2</sub> it started to exhibit an antihypoxic effect aimed at normalization of the hypoxia-lowered ATP level ( $p < 0.05$ ). In the presence of CSA this effect was enhanced at 100 μM O<sub>2</sub>, when the content of ATP, CP, and ADP, respectively, was 1.9, 1.5, and 1.4 times higher than in the absence of CSA.

The effect of CSA on the hyperbolic dependence of the ATP concentration on pO<sub>2</sub> (Fig. 2,

a) is clearly evident when this function is transformed in double inverse coordinates after Lineweaver-Burke (Fig. 2, b). Such a method makes it possible to assess the O<sub>2</sub> concentration for which the ATP content in the hippocampal sections is reduced by 50% (p<sub>50</sub>). In the absence of CSA this value was 500 μM O<sub>2</sub>; in its presence it dropped to 160 μM, i.e., 3-fold.

Thus, CSA lowers the sensitivity of the hippocampal sections to O<sub>2</sub> deficiency in the range of low pO<sub>2</sub> values, i.e., it exhibits an antihypoxic effect, this being attended by markedly smaller disturbances in the macroergic content.

Table 1 and Fig. 3 also show that as the O<sub>2</sub> concentration in the incubation medium decreases, the rate of release of the cytosol enzyme CPK from the tissue cells is altered. CPK solubilization is always higher at 900 μM O<sub>2</sub> than at 700 μM O<sub>2</sub>. Since in this case the ATP content in the hippocampal sections virtually is unchanged, it may be assumed that the higher membrane lability at 900 μM O<sub>2</sub> as compared with that at 700 μM O<sub>2</sub> is a result of hyperoxygenation. It is quite possible that in our experiments the conditions which were more appropriate for vital activity of the sections (and which could be regarded as the normoxic ones) were created not by the carbogen-containing medium, but by the medium with a somewhat lower O<sub>2</sub> concentration (700 μM).

When the content of O<sub>2</sub> in the medium falls to 500 μM or lower, the release of CPK again increases: at first, to the initial values and then to higher ones. At 100 μM O<sub>2</sub> the release of CPK is 2.5 and 4 times as high as that at 900 and 700

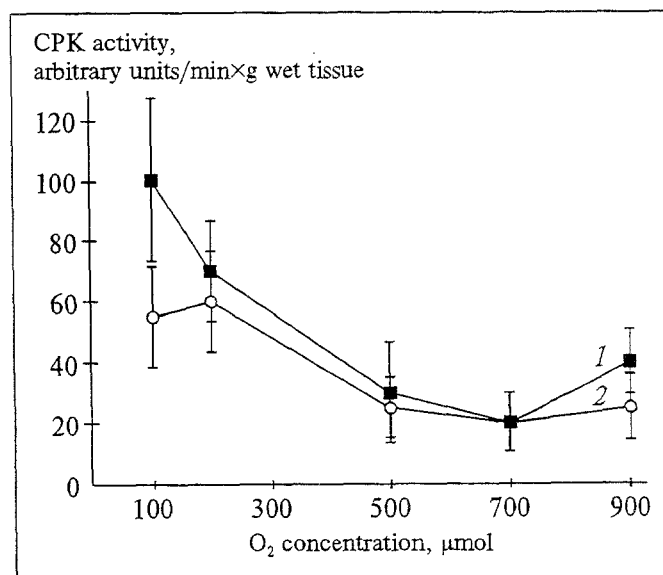


Fig. 3. Effect of O<sub>2</sub> concentration on CPK activity in incubation medium for sections of rat hippocampus. 1) in the absence of CSA; 2) in the presence of CSA (150 μM).

$\mu\text{M O}_2$ , respectively. This indicates that a reduction of the oxygen content in the medium causes a sharp increase of membrane permeability, which is highest at  $100 \mu\text{M O}_2$ , this being in line with the trend toward a decrease of the macroergic content (especially of the CP and ATP content) in the hippocampal cells.

CSA in the indicated concentration ( $150 \mu\text{M}$ ) had no effect on the CPK solubilization only in the case of  $700 \mu\text{M O}_2$ . In all other cases the corticosteroid increased it. However, reliable changes were not shown except for  $900$  and  $100 \mu\text{M O}_2$ , when CSA enhanced labilization of the cell membranes in the hippocampal sections 1.6 and 1.8-fold, respectively.

Thus, under conditions of severe hypoxia ( $100 \mu\text{M O}_2$ ) a protective antihypoxic effect of high CSA concentrations is exhibited with respect to the macroergic pool against the background of rapid labilization of membranes during hypoxia. Therefore, the latter does not reflect a neurotoxic effect of the substance and is of positive significance for the cells. The death of hippocampal neurons, triggered, for example, by hyperactivation of the glutamate receptors, is accelerated in the presence of CSA, this evidently being associated with the system of glucose transport and utilization, as well as with a reduced ATP level [9-11]. At the same time, there is evidence [11] that this CSA phenomenon is mediated by the glucocorticoid receptors. Conversely, our findings on the effect of CSA in a high concentration during acute hypoxia ( $100 \mu\text{M O}_2$ ) (Fig. 2) may be evidence that the membranotropic effect of the hormone is not realized via receptor interactions. It seems plausible that during hypoxia the demonstrated membrane labilization facilitates penetration of glucose into

the cell, this being followed by activation of glycolysis and ATP resynthesis.

Glucocorticoid hormones are also known to enhance aerobic processes in the brain by activating the utilization of amino acids as energy substrates [4]. It is still unclear whether this pathway can be used during hypoxia.

Thus, CSA in the high concentration used in our experiments ( $150 \mu\text{M}$ ): 1) exerted an antihypoxic effect, preventing a decrease of macroergic content in the hippocampus during acute hypoxia; and 2) raised the membrane permeability. In our experiments CSA did not exhibit a membranotropic effect and did not affect the macroergic pool under normoxic conditions.

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