ATP CONTENT IN THE RAT BRAIN DURING STRESS

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The total corticosterone content in the peripheral blood and ATP content in the brain of albino rats were studied after laparotomy and immobilization for various periods. The corticosterone concentration rose sharply in response to stress and reached its maximum before the appearance of the first signs of Selye's triad. The ATP content in the brain fell considerably during both types of stress in winter; in spring the change in the ATP level was very small and occurred only in response to more powerful stimulation.

The energy metabolism of the brain is aimed almost entirely at providing for specific nervous activity [2]. Indices of energy metabolism and, in particular, the ATP content [10] can therefore characterize the functional state of the CNS. However, data on changes in the ATP content in the brain in various physiological [6, 10, 11] and pathological [1, 6-9] states are difficult to interpret because of the lack of objective characteristics of the animal's physiological state.

Stress can be strictly quantified by its morphological features [5] and also by changes in the corticosteroid level in the peripheral blood [3]. In this investigation the corticosterone concentration in the peripheral blood and ATP content in the brain were compared in rats exposed to different types of stress and at different seasons of the year.

EXPERIMENTAL METHOD

Altogether 96 male noninbred albino rats weighing 160-180 g were used. Before the immobilization experiments the animals were kept separately in cages for at least 3 days. For the experiments with laparotomy animals kept in common cages were used. Laparotomy was performed without anesthesia, the animal having first been fixed in the supine position. In the immobilization experiments the animals were fixed in the prone position on a dissecting table. Not more than 40 sec elapsed between decapitation of the rats and freezing of the brain in liquid nitrogen. After decapitation the blood was collected and the cells were separated by centrifuging. The plasma was kept at $-10°C$ for 30 days. The brain was ground with liquid nitrogen to a thin powder, 0.5 N $HClO_4$ was then added at 0°C at the rate of 11 ml per brain, and the grinding continued until a homogeneous mass was obtained. The residue was separated by centrifuging at 20,000 g for 10 min at 0°C and was washed twice with 3 ml 0.5 N $HClO_4$. The cooled supernatants were quickly neutralized with KOH. The ATP concentration was determined enzymatically [12] on the same day. The corticosterone concentration was determined by a modified method of saturation analysis [4].

EXPERIMENTAL RESULTS

Because of the diurnal rhythm of corticosterone concentration in the peripheral blood of rats [3] the level of this hormone in the intact animals was determined at the same times of day as those at which all

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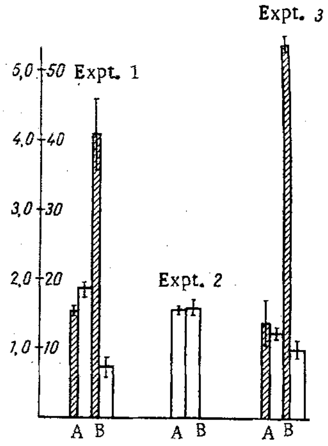

Fig. 1

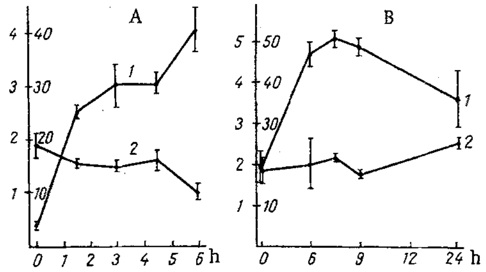

Fig. 2

Fig. 1. Total blood corticosterone concentration (shaded columns) and ATP concentration in the brain (unshaded columns) of rats before (A) and after (B) laparotomy (M ± m; n = 3). Explanation in text. Here and in Fig. 2, ordinate: on the left – ATP concentration (in μ-moles/g wet weight of brain); on the right – corticosterone concentration (in ng/0.1 ml plasma).

Fig. 2. Blood corticosterone concentration (1) and brain ATP content (2) in rats during immobilization in winter (A) and spring (B) (M ± m; n = 3).

the experiments were performed, except immobilization for 24 h. The determinations showed that fluctuations in the blood corticosterone level in the interval from noon to 6 p.m. did not exceed 20% of the mean value (measurements taken every 90 min, n = 3).

The results given in Fig. 1 show that in the winter period 15 min after laparotomy (experiment 1) the corticosterone level in the peripheral blood was 2.7 times higher; the ATP concentration in the brain at this time was reduced by 60%. However, in the same experiment but performed in the spring no change in the brain ATP level was found (experiment 2). With an increase in the intensity of stress (45 min after laparotomy; experiment 3) the blood corticosterone level in spring was increased by 4.4 times while the ATP content in the brain was reduced only very slightly. Meanwhile the state of the gastric mucosa and the relative weight of the adrenals remained within normal limits in both winter and spring.

The results of the immobilization experiment carried out in the winter (Fig. 2A) showed that after immobilization for 1.5 h the blood corticosterone concentration was increased by 6.2 times and the ATP content in the brain was reduced ($P < 0.05$). Increasing the duration of immobilization to 4.5 h caused no further change in these values, but immobilization for 6 h led to a larger increase (tenfold) in the corticosterone concentration and to a decrease in the ATP content (by 48% of its initial level).

In the analogous experiment conducted in the spring no change in the brain ATP content was found (Fig. 2B), whereas the corticosterone concentration rose to a maximum after 9 h and then fell appreciably.

Solitary ulcers in the gastric mucosa were found in some animals only after immobilization for 9 and 12 h; the relative weight of the adrenals was unchanged at this time. After immobilization for 24 h all the animals had numerous ulcers and foci of necrosis of the mucosa; the relative weight of the adrenals was increased.

An increase in the blood corticosterone level was thus discovered much sooner than the other evidence of stress.

In spring the blood corticosterone concentration of intact rats kept in single cages was 4.5 times higher than in winter, but in rats kept in communal cages no such difference was observed.

The results of these experiments show that the ATP concentration – an index of brain energy metabolism – changes in rats during stress but the degree of this change depends both on the type of stress and on the season of the year. Despite considerable changes in the ATP content in the brain during stress in winter, it can be concluded that the stability of the brain energy processes is very high and that it rises particularly high in spring.

LITERATURE CITED

1. Ya. M. Veksler, Pat. Fiziol., No. 1, 27 (1959).
2. G. E. Vladimirov, in: Extended Abstract of Proceedings of Symposia of the 9th Congress of the All-Union Society of Physiologists, Biochemists, and Pharmacologists [in Russian], Vol. 3, Moscow–Minsk (1959), p. 183.
3. N. G. Kolpakov, É. M. Kazin, G. G. Avdeev, et al., Byull. Éksperim. Biol. i Med., No. 10, 15 (1972).
4. V. B. Rozen, A. G. Volchek, I. G. Strokova, et al., Probl. Éndokrinol., No. 2, 58 (1973).
5. H. Selye, The Story of the Adaptation Syndrome [Russian translation], Moscow (1960).
6. I. A. Sytinskii, Biokhimiya, No. 3, 359 (1956).
7. R. A. Tigranyan, in: Problems in Biochemistry of the Brain [in Russian], Part 4, Erevan (1968), p. 203.
8. L. Dosseva and S. Tencheva Dimov, Dokl. Bolgarsk. Akad. Nauk, 18, 283 (1965).
9. L. Dosseva and S. Tencheva Dimov, Dokl. Bolgarsk. Akad. Nauk, 19, No. 4, 313 (1966).
10. S. Van den Noorth and K. Brine, Am. J. Physiol., 218, 1434 (1970).
11. R. Reich, S. J. Geyer, and M. L. Karnovsky, J. Neurochem., 19, 487 (1972).
12. H. Suzuki et al., Biochim. Biophys. Acta, 268, 381 (1972).