STATE OF PERIPHERAL CATECHOLAMINERGIC SYSTEMS DURING PHARMACOLOGIC CORRECTION OF IMMOBILIZATION STRESS BY SODIUM HYDROXYBUTYRATE

E. B. Khaisman, L. A. Malikova, and V. A. Arefolov

UDC 612.863-02:612.766.2]-07:616.432+616.452+616.839]-02:615.31:547.473.2

KEY WORDS: immobilization stress; catecholamines; adrenergic innervation; sodium hydroxybutyrate.

Investigations have shown that administration of sodium hydroxybutyrate (the sodium salt of γ-hydroxybutyric acid — GHBA) to stressed animals reduces activation of the pituitary-sympathicoadrenal system and limits the development of stress-induced lesions in various internal organs [6, 14]. In the existing view, the antistressor effect of this compound is exerted at the level of higher autonomic centers of the brain, and consists of activation of the inhibitory GABA-ergic system; this, in turn, prevents excessive excitation of the stress-realizing systems of the body, including the catecholaminergic system [2, 8].

In view of the leading role of the latter system in the mechanism of onset and development of the general adaptation syndrome [1, 4, 13], the writers have made a histochemical and biochemical study of the neurotransmitter and hormonal activity of its peripheral components during pharmacologic correction of immobilization stress with the aid of sodium hydroxybutyrate.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-230 g. Immobilization stress was created by fixing the animals securely in special frames for 1, 4, 24, and 48 h. These experimental times were chosen to allow for the duration of the main stages of the general adaptation syndrome, established previously by the writers on the basis of somatic and hormonal parameters of immobilization stress [5]. Material for the investigation consisted of: the adrenergic innervation of the dura mater, adrenergic nerves of the vas deferens, and the adrenal medulla. Adrenergic nerves in the dura mater were revealed by the Falck—Hillarp technique of fluorescence microscopy of catecholamines, in the writers' own modification [10]. To determine quantitative characteristics of noradrenalin luminescence, the photosensitive PEU-19 attachment to the ML-2 luminescence microscope was used. Concentrations of catecholamines in the vas deferens and adrenal medulla were determined spectrofluorometrically [12]. Sodium hydroxybutyrate was injected intraperitoneally in a tranquilizing dose of 40 mg/kg body weight [7, 11] 30 min before fixation of the rats and again immediately before fixation. In the case of prolonged immobilization, the injections were repeated every 4 h.

EXPERIMENTAL RESULTS

The study of the state of the peripheral catecholaminergic systems in rats whose immobilization was combined with treatment with sodium hydroxybutyrate showed (Table 1) that the intensity of noradrenalin luminescence of adrenergic nerves of the dura mater in experiments with control immobilization at the anxiety stage (1 h) was reduced by 40-50%, and not until the adaptation stage (4-24 h) did it increase again up to 80% of its level in intact animals. In experiments with administration of sodium hydroxybutyrate the fall in the total intensity of luminescence did not exceed 30%. Under these conditions the higher level of luminescence of adrenergic nerves also was observed between the 4th and 16th hours of immobilization. With the change into the stage of exhaustion (24-48 h) the intensity of noradrenalin luminescence in preparations of the dura mater decreased significantly and was virtually the same as the corresponding values in experiments without pharmacologic correction (Fig. 1).

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 9, pp. 317-319, September, 1985. Original article submitted January 18, 1985.

 0007-4888/85/0009-1222$09.50

TABLE 1. Concentration of Adrenergic Transmitter in Nerves of Dura Mater and of Vas Deferens and in Adrenal Medulla of Rats during Pharmacologic Correction of Immobilization Stress by Sodium Hydroxybutyrate (M ± m)

Test object	Parameter measured	Duration of immobilization, h				
		0	1	4	24	48
Dura mater	Intensity of luminescence, conventional units	20,5±1,8 21,1±2,2	12,0±1,4 16,9±1,9	15,1±1,6 18,1±1,7	16,9±1,8 17,6±1,8	13,2±1,5 15,4±1,6
Vas deferens	Noradrenalin concentration, μg/g	17,9±1,7 18,3±2,0	12,4±1,7 16,0±1,9	16,2±1,9 15,3±1,9	11,0±1,5 14,6±2,3	6,5±1,1 6,7±1,5
Adrenal	The same	643±90 712±97	625±105 670±118	556±86 527±101	239±33 252±39	132±23 137±19
Adrenal	Adrenalin concentration, μg/g	837±100 855±126	715±128 734±110	378±59 331±58	207±31 238±43	95±22 89±14

Legend. Luminescence microscopy by Falck–Hillarp method, 350×. Numerator gives value obtained during control immobilization, denominator — during action of sodium hydroxybutyrate.

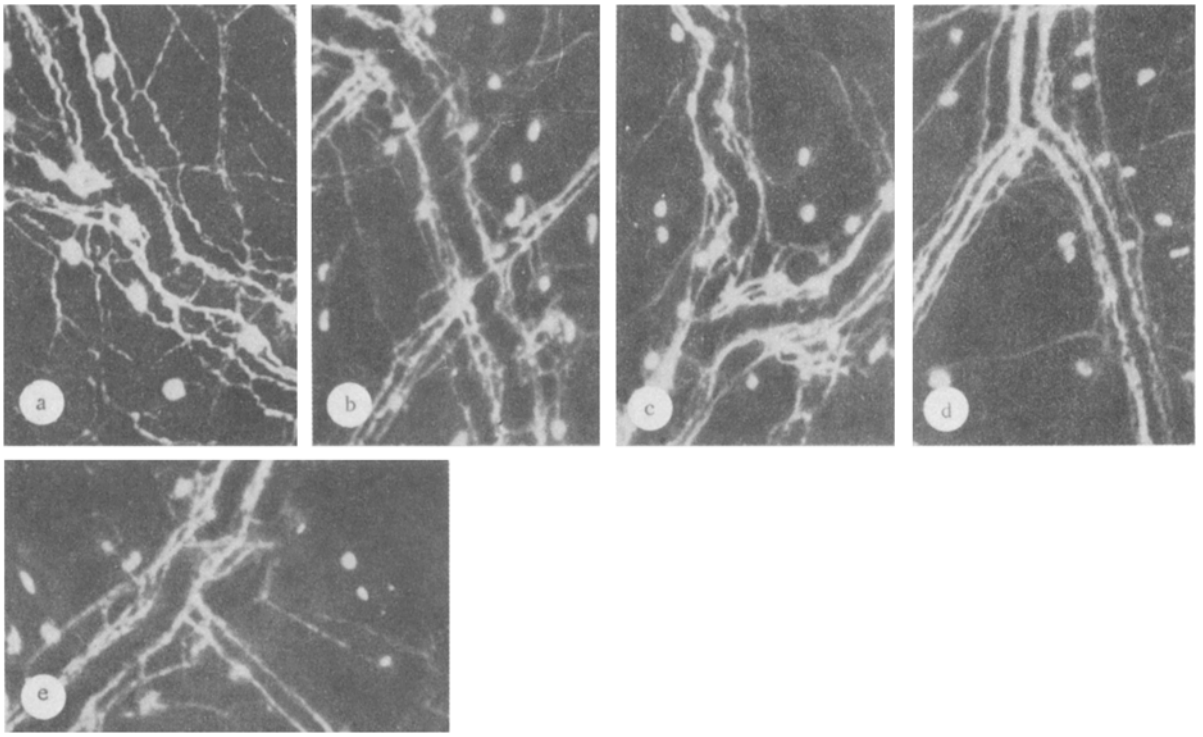

Fig. 1. Adrenergic innervation of dura mater of immobilized rats under treatment with sodium hydroxybutyrate: a) control, b) immobilization for 1 h, c) for 4 h, d) for 24 h, e) for 48 h.

A similar trend of the catecholamine level was observed in adrenergic nerves of the vas deferens. A combination of immobilization stress with the action of sodium hydroxybutyrate restored the noradrenalin concentration in these nerves largely back to normal in the anxiety stage. By contrast with control immobilization, which led to a 30% fall in the noradrenalin concentration, during treatment with the compound the fall in the concentration of the transmitter was only 10-15%. Some weakening of transmitter activity was a feature of the initial period of adaptation (4 h), but during the latest periods of this stage (until 24 h) a close to normal increase in the noradrenalin concentration was observed. In the stage of exhaustion transmitter activity of the nerves of the vas deferens decreased to the level observed during control immobilization, when it was 50-60% of the value in intact animals.

Analysis of the adrenalin and noradrenalin concentrations in the adrenal medulla of rats exposed to immobilization and treated with sodium hydroxybutyrate shows that the time course of the catecholamine levels in this organ was not affected by pharmacologic correction. The fall in the catecholamine concentration observed at different stages of the general adaptation syndrome was proportional to the duration of stress and corresponded to their trend during control immobilization.

The results of this investigation thus show that tranquilizing doses of sodium hydroxybutyrate affect the state of peripheral catecholaminergic systems during immobilization stress. The action of the compound is exhibited particularly clearly on the anxiety stage of the general adaptation syndrome, and it is reflected primarily at the level of transmitter activity of adrenergic nervous structures. Prevention of increased release of noradrenalin during the first hours of the stress reaction and preservation of large depots of neurotransmitter during adaptation are evidence that sodium hydroxybutyrate has a marked protective action against stress on an important component of the sympathicoadrenal system, namely the adrenergic peripheral innervation. Processes of tissue metabolism and adaptive activity of the body, both under normal conditions and during exposure to harmful factors [3, 4, 9], are directly linked with its trophic and effector function. It can accordingly be postulated that the positive trend, observed by the writers previously, in somatic manifestations of the stress reaction in immobilized rats receiving sodium hydroxybutyrate [5] is due to the protective effect of the compound on transmitter activity of adrenergic nerves. This effect of sodium hydroxybutyrate may be mediated through the GABA-ergic system and may also be the result of its direct action as inhibitory metabolite on central and peripheral transmitter processes [6, 8, 14].

During prolonged periods of immobilization, corresponding to the stage of exhaustion of the general adaptation syndrome, components of peripheral catecholaminergic systems which we studied did not exhibit such a marked dependence on the corrective influence of sodium hydroxybutyrate. The evident reason for this is that the disadaptation of the animal arising under these conditions must inevitably be accompanied not only by functional exhaustion of protective neurohumoral mechanisms, but also by weakening of activity of the inhibitory GABA-ergic system.

LITERATURE CITED

1. I. S. Zavodskaya and E. V. Moreva, Pharmacologic Analysis of the Mechanism of Stress and Its Consequences [in Russian], Leningrad (1981).
2. V. D. Ignatov, G. É. Galust'yan, and B. V. Andreev, in: Neurochemical Basis of the Psychotropic Effect [in Russian], Moscow (1982), p. 118.
3. O. I. Kirillov, Cellular Mechanisms of Stress [in Russian], Vladivostok (1973).
4. T. Cox, Stress [Russian translation], Moscow (1981).
5. L. A. Malikova and V. A. Arefolov, Byull. Éksp. Biol. Med., No. 10, 63 (1982).
6. F. Z. Meerson, Adaptation, Stress, and Prophylaxis [in Russian], Moscow (1981).
7. R. U. Ostrovskaya, Byull. Éksp. Biol. Med., No. 2, 42 (1982).
8. I. A. Sytinskii, Gamma-Aminobutyric Acid — Mediator of Inhibition [in Russian], Leningrad (1977).
9. T. M. Turpaev and B. I. Manukhin, in: Mechanisms of Hormonal Regulations and Role of Feedback in Phenomena of Development and Homeostasis [in Russian], Moscow (1981), p. 151.
10. E. B. Khaisman, Byull, Éksp. Biol. Med., No. 1, 101 (1982).
11. G. A. Shifrin, V. Ya. Shnol', and A. A. Khizhnyak, Farmakol. Toksikol., No. 6, 739 (1976).
12. U. S. Von Euler and F. Lishajko, Acta Physiol. Scand., 51, 348 (1961).
13. R. Kvetnansky, in: International Symposium on Catecholamines and Stress. Abstracts, Smolenice, Czechoslovakia (1979), p. 1.
14. M. Matsuda and M. Hoshima, Jikeikai Med. J., 23, 189 (1976).