CHANGES IN BLOOD AND HYPOTHALAMIC SUBSTANCE P LEVELS IN EXPERIMENTAL EMOTIONAL STRESS

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The writers showed previously that substance P (SP) is an endogenous peptide factor of resistance to emotional stress [2-4]. A single intraperitoneal injection of SP increases the survival rate of rats exposed to chronic emotional stress, reduces the stress-induced increase in weight of the adrenals, and depresses spontaneous fluctuations of blood pressure. It has also been shown that SP can relieve neurotic states, normalize sleep, and improve memory and learning [1]. These findings led Oehme et al. [10] to regard SP as a modulator of physiological and pathological processes. At the same time, we know that SP is an endogenous peptide, present in different parts of the brain; it is present, moreover, in high concentrations in the emotiogenic structures of the brain and, in particular, in hypothalamic nuclei [5-7], which participate in central mechanisms of formation of emotional stress.

The above remarks suggest that changes in the SP concentration may take place in the blood and hypothalamus during short-term and chronic stress and also after intraperitoneal injection of SP, increasing the resistance of animals to chronic stress. The aim of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male Wistar rats weighing 250-300 g. Intact animals (group 1), into which physiological saline was injected intraperitoneally, served as the control. Rats of group 2 were given SP intraperitoneally in a dose of 250 µg/kg, and their SP levels in the blood and hypothalamus also were determined. Animals of group 3 were exposed to stress in accordance with one of the following schemes: a single immobilization for 24 h; immobilization for 2.5 h daily for 4 days; immobilization for 20 h on alternate days for 2 weeks. Rats of group 4 were given SP in a dose of 250 µg/kg before the beginning of immobilization by one of the above schemes.

In the series of experiments with a single 24-hourly exposure to immobilization stress with immobilization for 20 h on alternate days for 2 weeks, SP was injected once, immediately before the beginning of the first immobilization. In the series of experiments in which immobilization was given for 2.5 h daily for 4 days, SP was injected in the same dose daily before the beginning of each immobilization.

To determine blood and hypothalamic SP levels animals of the experimental and control groups were killed simultaneously by rapid decapitation, blood (5-6 ml) and the brain were immediately removed, and the latter was quickly frozen and the hypothalamus separated from it. To obtain plasma each sample was centrifuged and frozen separately. Each hypothalamus, taken from each separate brain, was homogenized for 1 min in 3 ml of 2N acetic acid. Extraction for 2 h at 4°C followed, after which the homogenate was centrifuged for 10 min at 1500g. The supernatant was collected and 2 ml 2N acetic acid added to the residue, after which centrifugation was repeated for 10 min at 1500g. From the total volume of pooled supernatant (5 ml) a sample of 1 ml was taken and lyophilized. Before radioimmunoassay the sample was dissolved in 1 ml of RIA buffer, pH 8.6.

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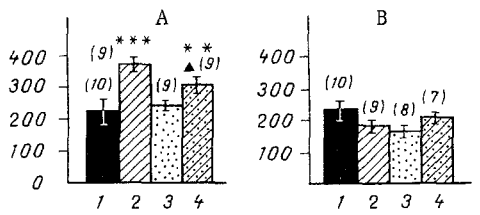

Fig. 1

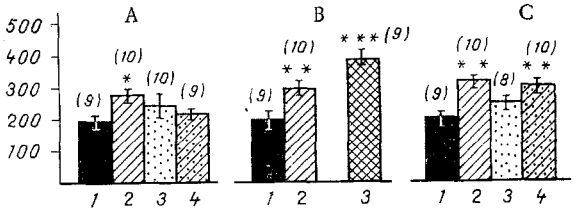

Fig. 2

Fig. 1. Blood SP level (in pg/ml) in rats after intraperitoneal injection of SP in a dose of 250 μg/kg, immobilization stress, and a combination of SP with immobilization stress. A) Immobilization for 2.5 h daily for 4 days. B) Immobilization for 20 h on alternate days for 2 weeks. 1) Control, 2) injection of SP, 3) immobilization stress, 4) stress combined with preliminary injection of SP. Number of animals in group shown in parentheses. *P < 0.05, **P < 0.01, ***P < 0.001 compared with control. Triangle) P < 0.01 compared with group 3.

Fig. 2. Hypothalamic SP level (in ng/g) in rats after intraperitoneal injection of SP in a dose of 250 μg/kg, after immobilization stress, and after a combination of SP injection and immobilization stress. A) Immobilization for 2.5 h daily for 4 days, B) single immobilization for 24 h, C) immobilization for 20 h on alternate days for 2 weeks. Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

After intraperitoneal injection of SP (in a dose of 250 μg/kg daily for 4 days) an increase in its blood concentration was found (Fig. 1A). Four exposures of 2.5 h to immobilization stress caused no change in the blood SP level. A combination of immobilization stress with injection of SP caused a significant rise in the blood SP level, which was evidently due to the SP itself but not to the development of immobilization stress. However, the increase in the SP concentration in this case was less than in rats receiving SP and not exposed to immobilization stress.

No changes were discovered in the blood SP level 2 h after a single intraperitoneal injection of SP, after immobilization stress for 2 weeks, and after stress preceded by injection of SP in all these animals (Fig. 1B).

A significant increase in the SP concentration was observed in the hypothalamus of rats receiving four intraperitoneal injections of SP. A significant rise in the hypothalamic SP level also was found 24 h and 2 weeks after a single injection (Fig. 2).

Four exposures of 2.5 h to immobilization stress caused no significant changes in the hypothalamic SP concentration (Fig. 2A), although a tendency appeared for the level to rise, with highest dispersion of individual values. A single exposure of 24 h to immobilization stress caused a significant increase in the hypothalamic SP concentration (Fig. 2B). After chronic immobilization stress for 2 weeks no significant increase could be found in the SP concentration in the hypothalamus (Fig. 2B). After chronic immobilization stress for 2 weeks no significant rise in the hypothalamic SP level could be found. After combined exposure—injection of SP and four sessions of immobilization each for 2.5 h, no change in the hypothalamic SP concentration was found (Fig. 2A). In rats exposed to immobilization stress for 2 weeks preceded by injection of SP, a significant rise in its hypothalamic level was found (Fig. 2C).

The experiments showed that intraperitoneal injections of SP, including single injections, can raise the hypothalamic SP level, and this effect may be long-term in character and may be demonstrable as long as 2 weeks after injection, i.e., it may correspond in duration to the period of increased resistance to emotional stress after injection of SP.

The question of how the injected SP passes across the blood-brain barrier still remains unanswered [11]: Is it built into the structure of the hypothalamus or does it somehow trigger synthesis of endogenous ST in the brain? Despite data on the distribution of SP in various macro- and microstructures of the brain [8, 9], the question of changes in its brain concentrations during emotional stress has not yet received adequate study.

The investigations thus showed that injection of SP leads to an increase in its hypothalamic concentration, and this may perhaps be responsible for increased resistance to emotional stress.

LITERATURE CITED

1. K. Hecht, M. G. Airapetyants, P. Oehme, et al., in: Mechanisms of Integrative Activity of the Brain [in Russian], Moscow (1981), pp. 185-192.
2. K. V. Sudakov, K. Hecht, Yu. G. Skotselyas, et al., in: Proceedings of the 8th All-Union Conference on Biochemistry of the Nervous System [in Russian], Minsk (1980), pp. 196-197.
3. E. A. Yumatov, K. Hecht, Yu. G. Skotselyas, et al., in: Vasoactive Peptides [in Russian], Sofia (1980), pp. 44-46.
4. E. A. Yumatov, K. Hecht, and Yu. G. Skotselyas, Zh. Vyssh. Nerv. Deyat., No. 4, 771 (1984).
5. M. Q. Brownstein, E. A. Mroz, Y. S. Kizer, et al., Brain Res., 116, 299 (1976).
6. P. E. Cooper, M. H. Fernstrom, O. P. Rorstad, et al., Brin Res., 218, 219 (1981).
7. L. L. Iversen, J. Jessel, and J. Kanarawa, Nature, 264, 81 (1976).
8. N. Mayer, A. Seria, and F. Lembeck, in: Neuropeptides and Neural Transmission, ed. C. A. Marsan and W. Z. Traczyk, New York (1980), pp. 19-29.
9. Y. Nakata, Y. Kusaka, T. Segawa, et al., Life Sci., 22, 259 (1978).
10. P. Oehme, K. Hecht, L. Piesche, et al., in: Neuropeptides and Neutral Transmission ed. C. A. Marsan and W. Z. Traczyk, New York (1980), pp. 73-84.
11. W. M. Pardridge, Annu. Rev. Physiol., 45, 73 (1983).

CHARACTER OF CHANGES IN CYTOSTATIC AND CYTOTOXIC FUNCTIONS OF MOUSE SPLENOCYTES AFTER IMMOBILIZATION STRESS

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Intensive stress has been shown [1-4] to lead to a substantial decrease in activity of the system of natural cell-mediated resistance of the organism in the early period after stress. In view of recent evidence of the widespread biological significance of normal, or natural, killer cells (antitumor resistance, elimination of cells contaminated with viruses and bacteria, participation in the control of proliferation and differentiation of cells of the hematopoietic system, etc.), a very varied spectrum of undesirable after-effects for the body can be postulated as a result of this poststress modulation of the natural killer system.

Meanwhile exposure to stress can modify functions of other populations of immunocompetent cells. Investigations demonstrating the essential role of cytostatic interaction (by which is meant interaction between effector cells and target cells, leading not to death of the latter, but to restriction of their proliferation), both during tumor growth and during metastasization [8], and also, possibly, during regeneration, have recently been published. For instance, the cytostatic action of activated macrophages on tumor cells has been demonstrated [14], and evidence has been obtained of antigen-specific cytostasis of suppressor cells, generated by immunization of C57BL/6 mice by allogeneic P-815 cells [6]. It has been suggested that normal killer cells can also exert a cytostatic action on various target cells [5]. The place and role of the system of cell-mediated cytostatic activity (CA) in the normal organism, during tumor growth, and also in noncancer diseases (for example, in stress states), have not yet been explained.

It was accordingly decided to study the effect of stress action on the intensity of the cytostatic function of splenocytes and to compare it with the dynamics of normal killer cells

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