NEUOROENDOCRINE MECHANISMS OF INFLUENCE OF THE NUCLEI RAPHE ON DEVELOPMENT OF HYPERTENSIVE REACTIONS IN EMOTIONAL STRESS

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KEY WORDS: stress; arterial pressure; corticosterone; nuclei raphe.

The writers showed previously the great importance of activating structures of the mesencephalic reticular formation (MRF) in the formation of neuroendocrine and autonomic (hypertensive) reactions in emotional stress. Besides structures providing the noradrenergic innervation of the brain, the region of the brain-stem reticular formation also contains the nuclei raphe, which are morphologically similar to the brain-stem reticular formation and are the main source of serotoninergic innervation [11]. Therefore, when the role of the brain-stem reticular formation in the neuroendocrine regulation of hypertensive reactions is investigated, allowance must be made for the heterochemical nature of its neurons. There is evidence of reciprocal relations within the brain-stem reticular formation [6]. It has been shown that stimulation of the mesencephalic nuclei raphe inhibits activity of MRF cells [8]. The serotoninergic system of the brain stem is regarded by some workers as antistressor [5, 13]. Meanwhile it has been found that changes in the brain serotonin level do not significantly affect the ACTH level, either under ordinary conditions [3] or during exposure to stress [1]. Meanwhile addition of serotonin to an incubation medium containing hypothalamic tissue increased synthesis and secretion of corticotrophin releasing factor [4]. Under the influence of intraventricular injection of serotonin the blood levels of ACTH and corticosteroids rose [10]. Contradictory data also have been obtained on the role of central serotonin nuclei in blood pressure regulation. According to some workers [14], activation of brain serotonin structures, like intraventricular injection of serotonin into intact cats, causes the blood pressure to fall. Other workers found that stimulation of n. dorsalis raphe in cats raises the blood pressure [12]. No final conclusion regarding the role of the nuclei raphe in the regulation of neuroendocrine and autonomic functions under normal conditions and, in particular, during stress, can be drawn from data in the literature.

The aim of this investigation was to study the effect of injury to the nuclei raphe at the brain stem level on formation of neuroendocrine mechanisms of hypertensive reactions in emotional stress.

EXPERIMENTAL METHOD

Experiments were carried out on nine adult Chinchilla rabbits weighing 2.0-2.5 kg and five cats weighing 3 kg, subjected to immobilization stress combined with unavoidable electric shocks to the feed (ESF). During immobilization of the animals for 4-5 h, seven or eight series of ESF were applied periodically to the hind limbs, each lasting 10 min and consisting of 10 stimulations. A single ESF (40-50 V, 60 Hz) lasted 2-3 sec. The blood pressure (BP) was recorded in the common carotid artery by means of a BP transducer (Elema Schönander, Sweden), introduced through a catheter inserted beforehand. Blood for determination of corticosterone was taken from the marginal vein of the rabbits' ear before the experiment, 5-10, 30, and 60 min after the end of the first series of ESF, and at the same time intervals before and after the last series of ESF. The animals were kept on a constant diet. Blood was taken before feeding. The experiments took place from 10 a.m. to 4 p.m. Corticosterone was determined by radioimmunoassay in serum kept at −20°C [7]. Electrical activity of the brain was recorded by a monopolar technique on a 17-channel polygraph (Nihon Kohden, Japan). The EEG was subjected to frequency analysis by means of a two-channel wide-band integrator from the same firm. Electrolytic coagulation of the mesencephalic nuclei raphe was carried out with an anodal current of 1-1.5 mA for 1 min under pentobarbital (40 mg/kg)

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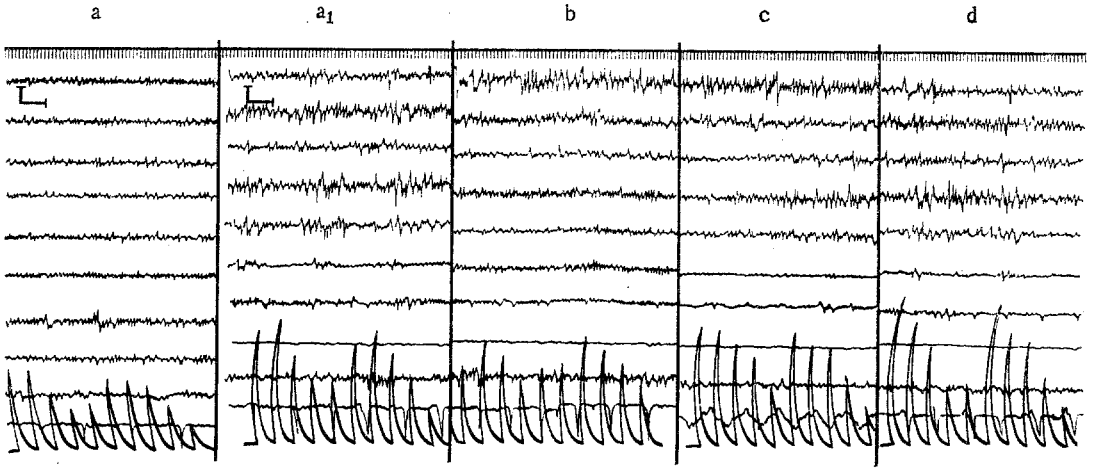

Fig. 1. Time course of changes in cat EEG during combined stress after coagulation of nuclei raphe: a) background trace from intact animal; a_1) background trace after operation; b) 1 h after beginning of experiment; c) 4 h after beginning of experiment; d) 1 h after end of exposure to stress. From top to bottom: time marker 0.1 sec, dorsal hippocampus (right), mesencephalic reticular formation (right), medial nuclei of amygdala (right), posterior hypothalamic nucleus (right), ventromedial hypothalamic nucleus (right), sensomotor cortex, locus coeruleus (right), nuclei raphe, mesencephalic reticular formation (left), respiratory movements, spectral analysis of EEG recorded from posterior and ventromedial hypothalamic nuclei. Calibration: 50 μV, 1 sec.

anesthesia. The electrodes were inserted by means of a stereotaxic apparatus, using coordinates taken from the atlas [2]. The dynamics of the EEG and BP and the corticosterone concentration were investigated again 2 weeks after the operation. After the end of the experiments the site of electrolytic destruction of brain tissue was verified morphologically. The lesions were found to be in the region of both the dorsal and the superior central nuclei in the cats and in the region of the mesencephalic nuclei raphe in the rabbits. The results were subjected to statistical analysis with estimation of the significance of differences by the Student–Fisher test.

EXPERIMENTAL RESULTS

Analysis of the EEG of the unrestrained animals before and after coagulation of the nuclei raphe showed a high degree of activation of cortical and deep brain structures after the operation (Fig. 1a, a_1). The frequency analysis curve of the EEG of the posterior and ventromedial hypothalamus revealed a marked increase in power of all rhythms, with predomination of the theta-rhythm. The appearance of pointed, spike-like waves with high amplitude also was observed in the hippocampus. The animals' locomotor activity was appreciably increased. Previously quiet animals exhibited aggressiveness relative to other animals after the operation. Under these conditions no significant increase in the basal corticosteroid and BP levels could be detected.

Under stress conditions seizure activity developed in the EEG of the dorsal hippocampus. Against this background electrical activity of the reticular formation and hypothalamic structures became relatively "quiet" in character (Fig. 1b, c). At the end of stress the reticulo-hypothalamic system was activated again. The power of the theta-rhythm in the hippocampus diminished (Fig. 1d). All this is evidence that when the postoperative animals were exposed to stress, inhibitory influences of the hippocampus on reticulo-hypothalamic mechanisms are activated.

The study of endocrine (corticosteroids) and vascular responses revealed the following patterns. Under the influence of emotional stress the response of the adrenal cortex in both intact and postoperative animals was significantly raised throughout the experiment. However, the lower hormonal response in the postoperative animals will be noted, and it was particularly marked in the late stages of stress (after the last series of ESF). Meanwhile blood hormone levels showed a sharp increase in the intact animals, whereas the hormone levels in the post-

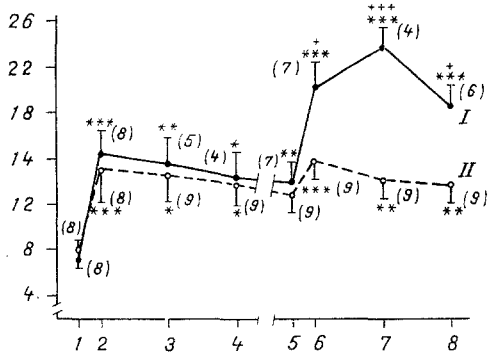

Fig. 2

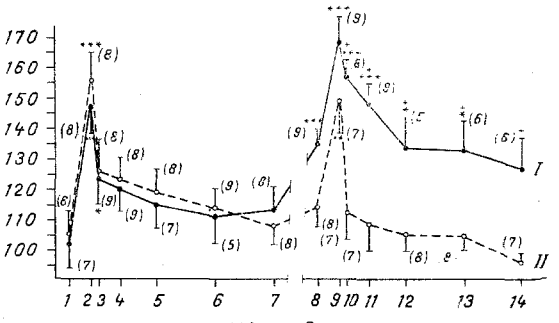

Fig. 3

Fig. 2. Dynamics of plasma corticosterone concentration in rabbits exposed to combined stress before and after coagulation of nuclei raphe. Abscissa, times of taking blood samples: 1) background; 2) 5 min; 3) 30 min; 4) 60 min after end of first series of ESF; 5) hormone level immediately before last series of ESF; 6) 5 min; 7) 30 min; 8) 60 min after end of last series of ESF. Ordinate, corticosterone concentration (in μg %). I) Before; II) after coagulation of nuclei raphe. Number of animals shown in parentheses. Significance of difference from background value: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Significance of difference of response of animals before and after coagulation of nuclei raphe: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Fig. 3. Dynamics of changes in ABP of rabbits during combined stress, before and after coagulation of nuclei raphe. Abscissa, periods of recording ABP: 1) background; 2) mean value of ABP in response to 10 ESF of first series; 3) sec; 4) 1 min; 5) 5 min; 6) 30 min; 7) 60 min after end of first series of ESF; 8) ABP immediately before last series of ESF; 9) average value of ABP in response to 10 ESF of last series, after 4 h of experiment; 10) 15 sec; 11) 1 min; 12) 5 min; 13) 30 min; 14) 60 min after end of last series of ESF. Ordinate, ABP (in mm Hg). Remainder of legend as to Fig. 2.

operative animals were stable. During this period a significant difference was noted between responses of intact and postoperative animals (Fig. 2).

Vascular responses in the postoperative animals showed the same monotonous pattern throughout the investigation. The magnitude of their response to the first and last series of ESF was virtually identical, whereas the average BP (ABP) level fell immediately after the end of exposure, to approach the background value. Meanwhile in intact animals vascular responses to ESF increased and the ABP level remained significantly high until the end of the experiment (Fig. 3).

Consequently, the functional state of reticulo-hypothalamic formations is depressed in postoperative animals during exposure to stress, and hippocampal inhibitory mechanisms participate in this effect. Accordingly, the insufficiency of the vascular and endocrine responses of these animals, mentioned above, must be regarded first of all as a phenomenon arising secondarily as the result of primary changes in the activity of brain structures that determines the level of endocrine and vascular responses.

Under stress conditions the activating influence of the brain stem are thus realized through the participation of both serotoninergic and also of certain noradrenergic neuronal formations.

LITERATURE CITED

1. M. S. Sapronov, Fiziol. Zh. SSSR, No. 6, 920 (1979).
2. J. Bureš, M. Petrań, and J. Zachar, Electrophysiological Methods in Biological Research, Prague (1960).
3. W. F. Ganong, Fed. Proc., 39, 2923 (1980).
4. M. Jones, E. Millhouse, and J. C. Burden, Endocrinology, 69, 1 (1976).
5. M. Karteszi, M. Palkovits, J. Z. Kiss, et al., Neuroendocrinology, 32, 7 (1981).
6. M. Mancia, A. Grantyn, G. Broggi, and M. Margueli, Brain Res., 33, 491 (1971).
7. B. Murphy, J. Clin. Endocrinol., 27, 973 (1967).

8. S. Nakamura, Brain Res., 93, 140 (1975).
9. Z. Olson and K. Fuxe, Brain Res., 43, 289 (1972).
10. J. C. Rose and W. F. Ganong, Current Developments in Psychopharmacology, No. 4, New York (1976), p. 87.
11. U. Ungerstedt, Acta Physiol. Scand., Suppl. 367, 1 (1971).
12. P. Vanhoutte, Trends Pharm. Sci., 3, 370 (1982).
13. J. Vernikos-Danellis, K. Kellar, D. Kent, et al., Ann. N.Y. Acad. Sci., 297, 518 (1977).
14. T. Yao, S. Andersson, and P. Thoren, Brain Res., 244, 295 (1982).

EFFECT OF PROTEOLYSIS OF LOW-DENSITY SERUM LIPOPROTEINS ON THEIR INTERACTION WITH MACROPHAGES

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The writers previously postulated, on the basis of changes observed in the structural stability of low-density lipoproteins (LDL) during treatment with pepsin or aortic cathepsin D, that enzymatic modifications may lead to potentiation of the atherogenic properties of LDL [1, 2]. We know that treatment of LDL with trypsin causes an increase in their binding with aortic glycosaminoglycans [5] and to an increase in degradation by fibroblasts of patients with hereditary hypercholesterolemia [7]. Limited proteolysis of LDL with pepsin facilitated their binding with fibronectin [4]. In recent years, to assess the atherogenicity of lipoproteins, their interaction with macrophages has been studied [6]. Popov [3], who used this model, found more rapid uptake of trypsin-treated LDL by macrophages.

In the present investigation uptake and degradation of LDL by macrophages were studied after their limited hydrolysis by pepsin — an analog of tissue cathepsin D.

EXPERIMENTAL METHOD

LDL ($1.019 < d < 1.063$ g/ml) were isolated from serum of healthy blood donors by ultracentrifugation [10]. Iodination of LDL with ^{125}I was carried out by the iodine monochloride method [14] and the reaction products were removed by dialysis. Specific radioactivity of the resulting preparations was 80-150 cpm/ng protein. Hydrolysis of native and radioiodinated LDL was carried out as described previously [1]. Low-molecular-weight products of proteolysis of LDL by pepsin were removed by gel-filtration on a column with Sephadex G-75. The efficiency of removal of low-molecular-weight products was estimated by precipitation with 10% TCA and measurement of radioactivity and of protein in the material dissolved in TCA. Protein in the samples was determined by Lowry's method [13]. To obtain human lipoprotein-deficient serum (LDS) preparative ultracentrifugation at a density of 1.21 g/ml was used [11]. The isolated LDS was dialyzed against 0.15 M NaCl in 0.02 M Na-phosphate buffer, pH 7.4, sterilized by filtration through a filter with pore diameter of 0.22 μ, and kept at −20°C.

To obtain macrophages, 5 ml of a 3% solution of peptone in Hanks' medium was injected into noninbred male albino mice. The peritoneal cavity of the mice was flushed out 2 days later with 7 ml of medium A (Eagle's medium with 10% bovine serum, 290 μg/ml of L-glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin), containing 85 U/ml of heparin. The peritoneal cells were collected by centrifugation of the washings (900 rpm, 5 min, 4°C) and the cells were washed once with medium A, resuspended in the same medium, and diluted to a density of 10^6-$2 \cdot 10^6$ cells/ml. Aliquots of the suspension (2 ml) were poured into plastic Petri dishes (40 × 10 mm) and incubated in an atmosphere of 5% CO_2 and 95% O_2 at 37°C. Each dish was washed twice after 2 h with 2 ml of warm medium A and incubated overnight at 37°C in an atmosphere of 5% CO_2 and 95% O_2.

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