AN EMOTIONAL STRESS RESISTANCE FACTOR IN RAT BRAIN

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KEY WORDS: emotional stress; transfer factor; brain.

Scores of peptides possessing marked physiological activity in extremely low concentrations, and with an influence on memory, learning, and behavior, have now been discovered in the brain and other organs [2, 13]. Modern views on the central organization of negative emotional reactions [1, 6] led us previously to postulate that neuropeptides play a role in the central neurochemical mechanisms of emotional stress, a dominant negative emotional state [7-9]. As our earlier experiments showed, among the genetic strains of rats tested some (Wistar) were found to be resistant and others (August and noninbred rats) predisposed to emotional stress [11].

This paper gives the results of investigations devoted to the isolation of fractions containing a factor evoking increased resistance to stress in recipient rats predisposed to the development of stress, from the brain of donor rats resistant to emotional stress.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar and August rats and also on noninbred rats weighing about 200 g. Resistance to emotional stress was estimated by determining survival of the animals during exposure to chronic emotional stress due to immobilization [8]. Wistar donor rats, resistant to emotional stress, were decapitated and their brain quickly removed and frozen in a bath containing acetone with dry ice. All subsequent procedures were carried out at 0-3°C. The tissue was homogenized in a glass homogenizer with Teflon pestle in deionized water, the pH of which was adjusted to 7.0 with ammonia; the ratio of the weight of the brain to the volume of water was 1:1. The brain homogenate was fractionated by various methods (A-D).

Method A. 1) The homogenate was dialyzed for 18-20 h with stirring against ten volumes of deionized water, the pH of which was adjusted to 7.0 with ammonia (the dialysate was fraction 1); 2) the dialyzed homogenate was centrifuged for 1 h at 105,000g (the supernatant was fraction 2); 3) the residue was extracted with 80% methanol for 20 min with stirring and the suspension was centrifuged at 10,000g for 30 min (the extract was fraction 3).

Method B. 1) The homogenate was heated to 65-70°C for 5 min; 2) the suspension was centrifuged at 20,000g for 30 min; 3) the supernatant was dialyzed against ten volumes of deionized water, the pH of which was adjusted to 7.0 with ammonia, for 18-20 h (the dialysate was fraction 4 and the undialyzed material fraction 5).

Method C. 1) The homogenate was heated at 65-70°C for 3 min; 2) the suspension was centrifuged at 30,000g; 3) the supernatant was freeze-dried and the residue dissolved in the minimal volume of deionized water; 4) gel filtration was carried out on a column measuring 2 × 100 cm with Sephadex G-15, equilibrated with deionized water; the column was washed with deionized water at the rate of 9 ml/h (fraction 6, the protein fraction, was eluted together with the solvent front, fraction 7 consisted of substances of low molecular weight).

Method D. 1) The acid precipitation, glacial acetic acid, was added drop by drop with stirring and the pH of the homogenate adjusted to 4.0. The subsequent procedures were all as

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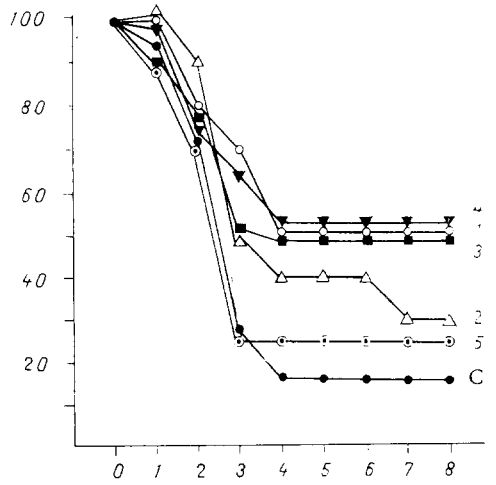

Fig. 1. Time course of death of August rats during chronic emotional stress due to immobilization for 8 weeks. Abscissa, time (in weeks); ordinate, number (in percent). 1-5) Groups of rats receiving the corresponding fraction of donors' brain, (K) control animals (n = 18) receiving physiological saline. 1-3) n = 10, 4-5) n = 8.

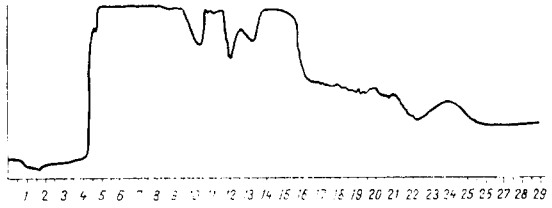

Fig. 2. Chromatography of brain homogenate fraction on column with Sephadex G-15. Abscissa, nos. of subfractions; ordinate, absorption at 280 nm (in relative units). Column measured 2 × 100 cm, elution with deionized water, rate of elution 9 ml/h. Subfractions 4-10) high-molecular-weight substances, subfractions 10-28) low-molecular-weight substances.

in Method C (fraction 8 consisted of low-molecular-weight substances obtained after acid precipitation and gel filtration).

All fractions were freeze-dried and kept when necessary at −10°C; immediately before injection into the recipient animals they were dissolved in the minimal volume of sterile physiological saline (material from 50 animals in approximately 10 ml). The solution or suspension of each fraction was injected intraperitoneally before the beginning of the chronic experiment as a single dose into August or noninbred rats predisposed to emotional stress, at the rate of material from 3 donors into 1 recipient. Animals of the control group were given an intraperitoneal injection of physiological saline. The number of groups in each series was determined by the number of fractions for testing. Homogeneous groups were formed beforehand from the recipient animals on the basis of prediction of differences in their individual resistance to emotional stress [10]. After injection of brain fractions from the donor animals, the recipient rats were periodically immobilized for 8-10 weeks in constraining cages for 20 h on alternate days. The time course of death of the recipient rats was recorded during emotional stress due to immobilization for several weeks.

Protein was determined by Lowry's method [14]. The significance of differences was determined by the Student–Fisher method.

EXPERIMENTAL RESULTS

In the experiments of series I the brain homogenate from the Wistar donor rats was fractionated by methods A and B. The recipient August rats were subjected to chronic emotional

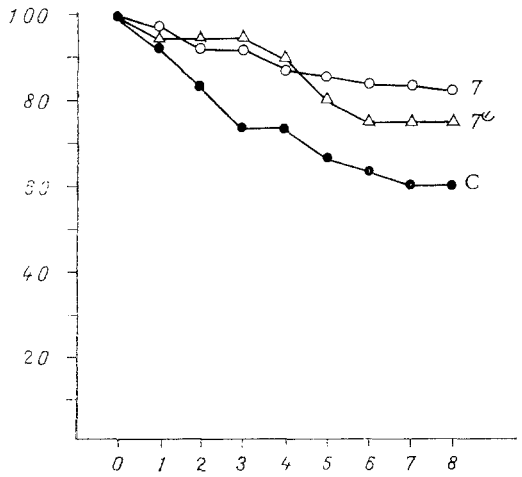

Fig. 3. Time course of death of noninbred rats during chronic emotional stress due to immobilization for 8 weeks. Abscissa, time (in weeks); ordinate, surviving animals (in percent). 7 and 7') Groups of rats receiving brain fraction from donor rats respectively. (K) control animals (n = 48) receiving physiological saline. 7) n = 50, 7') n = 16.

stress due to immobilization for 8 weeks, as described above. Rats of the control group died soonest (Fig. 1). By the 4th week of immobilization more than 80% of the control animals had died, compared with only 50% of the rats receiving fractions 1, 3, and 4. Before death characteristic types of hemodynamic disturbances, which could be accompanied by cardiovascular failure, arrhythmia, extrasystoles, and fibrillation [11] were recorded in animals predisposed to emotional stress. At autopsy on the rats which died hemorrhages in the lungs and hemothorax, ulcers or hemorrhages in the stomach wall, enlargement of the adrenals, changes in the lymphatic system, myocardial degeneration and, in some cases, necrosis of the myocardium could be observed. In the animals which died in the early period of immobilization, no morphological changes were found at autopsy. In these cases death was regarded as the result of acute cardiovascular failure.

Although the difference between the control and animals receiving fractions 1 and 4 in this series was not statistically significant, it was postulated that the biologically active material probably had a low molecular weight and was resistant to heat treatment.

On the basis of these suppositions the experiments of series II were carried out, in which methods C and D were used for purification. The high-molecular-weight fraction eluted during chromatography on a column with Sephadex G-15 together with the solvent front (fraction 6), just as in the experiments of series I, when tested for physiological action proved to be inactive. The results of chromatography on a column with Sephadex G-15 are given in Fig. 2. Fraction 8 also was inactive. The results of the experiments of series II are given in Fig. 3. Testing the effect of fractions on resistance to emotional stress was carried out on noninbred rats exposed to chronic immobilization stress for 8 weeks. Starting with the 5th week of immobilization a significant ($P < 0.05$) increase in survival of the experimental rats receiving fraction 7 was observed compared with the control.

Fraction 7' (Fig. 3) was treated with pronase. For this purpose the material of fraction 7 was freeze-dried, dissolved in 0.05 M ammonium bicarbonate containing 0.01 M $CaCl_2$, and incubated (20 h, 37°C) with pronase (0.1 mg approximately to 3 mg protein of the fraction, from Calbiochem, USA) [12].

Pronase was removed on a column with Sephadex G-50 (fine) measuring 2 × 30 cm, equilibrated with deionized water. The fraction treated in this way was freeze-dried and then injected into the animals like all the other fractions. Activity of the fraction treated with pronase did not differ significantly from activity of fraction 7.

By multistage fractionation followed by systematic testing of the physiological action of the different fractions thus is revealed a factor with low molecular weight that is relatively thermostable and is resistant to hydrolysis by pronase. On injection of the factor into recipient animals a significant increase in their resistance to emotional stress caused by immobilization is observed.

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CORRELATIONS BETWEEN THE FUNCTIONAL STATE OF THE CNS AND THYROID ACTIVITY IN CHRONIC EMOTIONAL STRESS

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Cortical and subcortical electrical activity was recorded during chronic emotional stress in cats and the state of thyroid function determined. Correlation was found between the functional state of the CNS and the level of thyroxine secretion. The trigger role of the posterior hypothalamic nucleus in the genesis of hypersynchronized activity in stress was demonstrated.

KEY WORDS: thyroid gland; stress; central nervous system.

The study of correlation between the functional state of the CNS and of the glands of internal secretion has not been undertaken during chronic emotional stress. There have been only a few isolated investigations [2, 4, 5] under acute experimental conditions. However, we know [3, 7] that it is the duration and repetition of negative emotions that play an important role in the conversion of protective reactions into a phase of overstrain, the starting point of formation of pathological syndromes. They become a background facilitating the development of certain neurogenic diseases such as essential hypertension, myocardial infarction, etc. Several workers have shown by neurophysiological analysis of emotional stress of varied genesis [6, 8-10] that the electrical activity of the brain during emotional stress reflects considerable phase shifts in relations between the cortex and deep brain structures. Analysis of the results of these investigations shows that although the authors cited noted that deep brain structures are activated before the cortex, the question of which deep brain formations perform the function of triggering mechanism, or in other words, where electrical responses to stress are formed initially and what is the time course of consecutive activation of the various hypothalamic nuclei, reticular formation, and structures of the limbic system, still remains unanswered. Yet another important question has not yet been studied: What changes take place in brain electrical activity in animals exposed repeatedly to stress for several days?

The object of the present investigation was to study correletion between the functional state of the CNS and peripheral glands of internal secretion with particular reference to activity under conditions of chronic emotional stress.

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