CHANGES IN ADRENAL OPIOID PEPTIDE LEVELS IN RATS DURING IMMOBILIZATION STRESS

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It has recently been shown that catecholamines (CA) of the adrenal adrenocytes and noradrenocytes are stored in vesicles together with opioid peptides of Leu- and Met-enkephalin type [5] and are released from the vesicles simultaneously as they empty.

In a previous study of immobilization stress in rats [2] the writers showed a changing pattern of the stages of the stress reaction, affecting the duration of the course of each stage and the character of changes in some somatic parameters of the animals during these periods. In each of the above stages exhaustion of CA in the adrenals [2] was studied for it may play an important role in the development of the response of the organism to stress [3].

It was interesting to study changes in opioid peptide levels in the adrenals in the different stages of development of the stress reaction and to compare these results with data obtained previously on exhaustion of the CA reserves [2].

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-220 g. A model of stress was produced by immobilizing the animals in special frames, so that the rats were fixed in the cervical and lumbar regions by metal brackets. The CA concentrations in the adrenals were determined spectrofluorometrically [2]. Concentrations of Leu- and Met-enkephalins were studied by radioimmunoassay. For this purpose adrenal tissue was treated at 90°C for 15 min in 0.1 M HCl and homogenized. The extracts were centrifuged (5000g, 0°C, 30 min) and the residue discarded. The supernatant was neutralized (to pH 7.0) by 1 M NaOH with 0.2 M Na_2HPO_4. The residue was removed by centrifugation and the supernatant used for radioimmunoassay of opioid peptides. Components of the incubation mixture were dissolved in 0.05 M sodium-phosphate buffer, pH 7.5, with 0.15 M NaCl and 0.2% solution of bovine serum albumin and 0.01% of sodium azide. The mixture, in a final volume of 1 ml, contained the corresponding ^{125}I-peptide (10^4 cmp in 0.1 ml), antiserum (0.1 ml), a standard quantity of peptide (for calibration) of 0.1 ml or the unknown sample 0.2 ml, and RIO buffer to a final volume of 1 ml. The technique of iodination of the peptides and preparation and use of the antisera were described previously [1]. Antiserum against Leu-enkephalin had crossed immunoreactivity with Met-enkephalin of 0.93%, and with α-, β-, and γ-endorphins and β-lipoprotein, of under 0.01%. Antiserum against Met-enkephalin had crossed immunoreactivity with Leu-enkephalin of 5.8% and with endorphins and with β-lipoprotein of under 0.1%. To determine Leu- and Met-enkephalins the samples were incubated for 18 h at 4°C. To separate "free" and "bound" ^{125}I-peptide neutral rabbit serum (0.1 ml, 1:30) donkey antibodies (0.1 ml) against rabbit immunoglobulins in a precipitating concentration, and 0.6 ml of 8% polyethylene-glycol (mol. wt. 6000 daltons) were added to the samples. The samples were centrifuged (1500g, 0°C, 30 min) and the supernatants removed. Radioactivity in the residues was determined on a Beckman 4000 Gamma-Counter.

In some experiments, to exhaust endogenous CA reserves in the adrenals, reserpine was given to the animals beforehand (5 mg/kg four times in the course of 48 h). These same rats were then subjected to immobilization.

EXPERIMENTAL RESULTS

The graph in Fig. 1 shows exhaustion of CA and was plotted from the results of previous investigations [2]. The data reflected in the graph show that during continuing stress the

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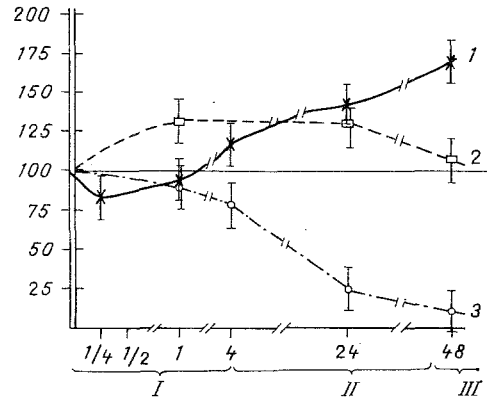

Fig. 1. Changes in concentrations of Leu-enkephalins and CA in different stages of immobilization stress in rats. Ordinate, concentrations of CA and enkephalins (in %); abscissa, time (in h); 1) concentration of enkephalins; 2) concentration of enkephalins after injection of reserpine; 3) concentration of CA. I) Anxiety, II) adaptation, III) exhaustion.

CA concentration in the adrenals falls gradually and the course of the changes is as follows. In the stage of anxiety, characterized by a tendency for the weight of the adrenals to fall and by a decrease in weight of the lymphoid organs (thymus and spleen) to 60-64%, the CA concentration falls only a little, and toward the end of this stage (the 4th hour) and the beginning of the adaptation stage, it is about 85% of the control level. In the adaptation stage, in which the above parameters of somatic manifestations return to their values in intact animals, with the exception of the hypertrophied adrenals, the CA concentration continued to fall, and by the 24th hour it had fallen to 25%. By the 48th hour of immobilization (beginning of the exhaustion stage, characterized by a decrease in body weight and the weight of the lymphoid organs, ulceration of the gastric mucosa, hypertrophy of the adrenals, culminating in death) the CA concentration falls to the limits of possible determination. This time course corresponds to data in the literature on CA release from the depots in stress [3].

A completely different picture was seen during stress in the system producing opioid peptides. The results of a study of the Leu-enkephalin concentration are shown in Fig. 1. The Met-enkephalin concentration changed in a similar manner. In the stage of anxiety, i.e., during the first hours of stress, the opioid peptide level fell a little, possibly indicating their release into the blood stream from their depots in vesicles of the adrenal cells (Fig. 1). Later, toward the end of the anxiety stage and the beginning of the adaptation stage, their concentrations began to rise, and by the 4th hour they were about 20% higher than usual. By 24 h (the adaptation stage) the opioid peptide levels had risen to 142%, and the rise continued until 48 h after the beginning of stress (beginning of the exhaustion stage) to reach 172%.

In experiments with animals receiving reserpine beforehand to exhaust the CA reserves an antistressor effect of the drug was found, as reflected in a decrease in the number of ulcers and hemorrhages in the gastric mucosa (in the stages of adaptation and exhaustion). Further confirmation was given by the results of a study of the weight of the lymphoid organs and adrenals. At the same time, despite the almost complete exhaustion of the CA reserves, the opioid peptide levels continued to rise. Even in the anxiety stage (1-4 h) the CA level in the adrenals rose to about 130% (Fig. 1).

When the facts observed are discussed, the following points can thus be distinguished: 1) the importance of the fall in the CA level and rise in the opioid peptide levels in the development of the stress reaction, 2) the possible regulatory role of CA release in the mechanism of triggering of peptide synthesis, 3) the cellular mechanisms of simultaneous storage of CA and peptides and their simultaneous co-secretion from the vesicles.

It follows from the results that the system synthesizing CA in the adrenals is unable to replenish the CA reserves in their depots during stress. There may be various reasons for this: 1) inhibition of activity of the key enzymes of CA synthesis; 2) interference with CA

storage in the vesicles; 3) activation of CA release from the vesicles. Each of these causes must be investigated additionally.

The system responsible for production of opioid peptides, on the other hand, is activated during stress, in the adrenal tissues. The physiological role of the rise of the opioid peptide level in stress is not completely understood. It may perhaps be connected with their analgesic effect in stress.

The possible regulatory role of CA in the mechanism triggering opioid peptide synthesis in the cell has been discussed in the literature [4]. It has been claimed that the release of very small quantities of CA (15%) from the adrenal may be the trigger mechanism for opioid peptide production. In the present investigation, experiments with maximal exhaustion of the CA reserves by reserpine revealed an increase in the opioid peptide concentrations from the very beginning of development of the stress reaction. This means that the system of enkephalin synthesis is activated in stress and continues to produce peptides, even though the main reserves of CA in the chromaffin cells of the adrenal are absent. Moreover, the opioid peptide concentrations rise actually more rapidly to begin with after reserpinization. However, these facts do not necessarily contradict the view [4] that small quantities of CA may be released to trigger peptide synthesis. The fact is that even after almost total exhaustion of the CA reserves by adrenalin a small quantity of CA evidently still remains in the adrenals and may be released during stress, when it is sufficient to trigger peptide synthesis.

We know that CA and enkephalins are stored in the same vesicles and are co-secreted simultaneously by exocytosis [4, 5]. If co-secretion proves to be a reliably established fact, parallel release not only of CA but also of enkephalins must take place during stress. The rise in the peptide levels in the adrenal tissues is evidently linked with marked activation of their synthesis during stress. In that case the newly synthesized enkephalins may probably accumulate outside the vesicles.

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