Significance of Hormonal Feedback Regulation for Hypothalamic Nonapeptidergic Center Response to Short-Term Immobilization Stress

L. S. Voropanova, I. A. Krasnovskaya, T. V. Sheibak, E. Sikerska, and A. L. Polenov

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The accumulated published data provide clear evidence of the involvement of the hypothalamic neurohormones vasopressin (VP) and oxytocin (OT) in the organism is response to stress. In most cases an elevation of VP and OT blood levels is registered for short-term nonspecific stress influences [4,7]. As conventionally accepted, an increase in the blood concentration of ACTH and corticosteroids is a typical characteristic of the stress response. However, the role of feedback relations between the anterior pituitary and the peripheral endocrine glands, in particular, the adrenal cortex and thyroid gland, in the response of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus to nonspecific short-term stress remains unclear.

There are indications in the literature concerning feedback correlations between the blood concentration of corticosteroids, on the one hand, and the functional state of corticoliberin- and VP-secreting cells of PVN, on the other, for long-term changes in corticoid blood level [3,8,10]. In addition, as demonstrated in experiments with functional impairment of the pituitary-thyroid system, there is an interdependence between the functional state of OT-ergic neurosecretory cells (NSC) from PVN and the TTH level in the blood [1,2]. However, morphofunctional analysis of OT- and VP-ergic NSC of the hypothalamic centers in short-term stress immobilization has been performed so far in few studies and has not been supported by quantitative evaluation [9].

In the present study, we used a morphometric evaluation of OT- and VP-ergic NSC activity to compare the stress response of the neurosecretory hypothalamic centers in rats with the intact pituitary and after hypophysectomy. The changes in the hormonal status in hypophysectomized animals would enable us to elucidate whether the fluctuations in the blood concentration of adenohypophyseal hormones and the functional state of the peripheral endocrine glands influence SON and PVN response to stress.

MATERIALS AND METHODS

Four groups of male Wistar rats with a body weight of 150-160 g (4-6 animals in each group) were included in the experiment performed in the fall-winter season. Group 1 consisted of intact nonoperated animals (control); group 2 included hypophysectomized rats 7 days after the operation; group 3 comprised in-

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St.Petersburg

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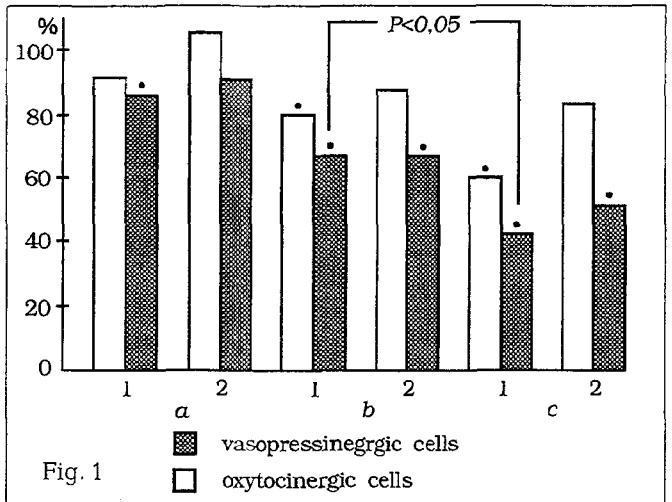

Fig. 1

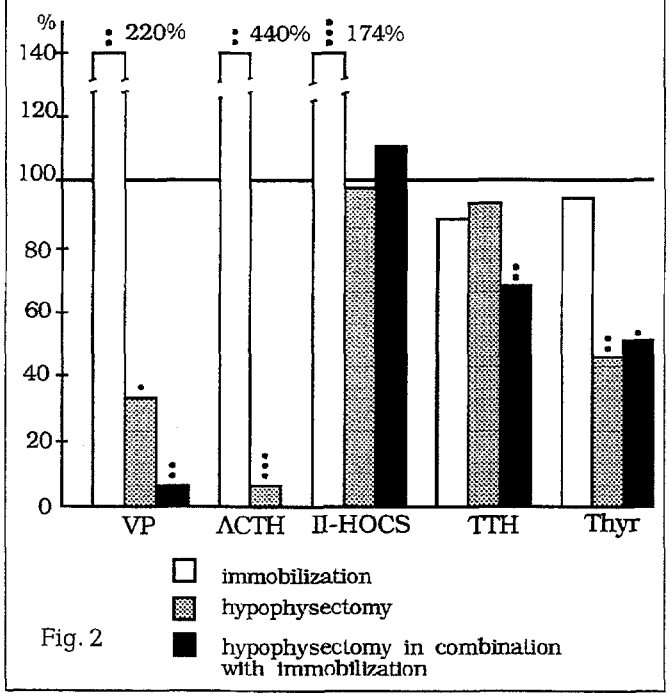

Fig. 2

Fig. 1. Nucleolar volume of neurosecretory cells from paraventricular (1) and supraoptic (2) nuclei of hypothalamus. *a*) immobilization of animals with intact pituitary; *b*) hypophysectomized rats; *c*) immobilization of hypophysectomized rats; dot: $p<0.05$ in comparison with the intact control taken as 100%.

Fig. 2. Plasma concentration of hormones and height of thyroid epithelium in animals subjected to: immobilization, hypophysectomy , hypophysectomy in combination with immobilization. One dot: $p<0.05$; two dots: $p<0.01$, three dots: $p<0.001$. Indexes of the intact control are taken as 100%.

tact rats subjected to severe immobilization stress for 20 min; group 4 was made up of hypophysectomized rats subjected to the same influence. The pituitary was removed under ether anesthesia, the transsphenoidal approach being used. Immobilization stress was carried out for 20 min with rigid fixation of the head and extremities in a supine position. The rats were decapitated immediately after the immobilization. Blood was collected at the moment of decapitation for radioimmunological determination of hormones in the plasma. The brain and thyroid were fixed in a mixture of picric acid and formalin (3:5) for 7 days at 37°C. The VP- and OT-ergic elements were demonstrated immunohistochemically with the PAP-method in paraffin sections. Several criteria were used to monitor the changes in the rate of synthesis of nonapeptide neurohormones: first, we controlled the nucleolar volume and percentage of "polynucleolar" cells, i.e., cells containing nucleoluslike bodies in the nucleus, their increase testifying to a drastic inhibition of ribonucleoprotein formation processes [6]. In addition, we monitored the changes in the content of immunopositive material in NSC and their fibers and calculated the number of pycnomorphic cells in SON and PVN, which reflects degenerative changes resulting from hypophysectomy. The plasma level of 11-HOCS was evaluated fluorimetrically. Activity of the thyroid gland was assessed morphometrically from the height of the thyroid epithelium. The radioimmunological method was used to determine the hormonal level with the use of commercial kits: 1) "Immuno Nuclear Co.", USA (for determination of arginine-vasopressin); 2) CEA, "Sorin", France (ACTH); 3) "Mallincrodt Diagnostic", Germany (TTH). The results were processed statistically using the nonparametric *U*-test of Wilcoxson-Mann-Whitney.

RESULTS

In nonoperated rats the immobilization stress resulted in a significant elevation of the content of OT-immunopositive substance in pericaryons of OT-ergic NSC in both SON and PVN. The cell fibers revealed were more abundant in comparison with the control. The nucleolar volume of OT-ergic NSC in both hypothalamic centers practically did not alter (see Fig. 1), while the percentage of "polynucleolar" cells was reduced in SON to 33% ($p<0.05$) and in PVN to 84% ($p<0.05$).

On the other hand, a marked reduction in the content of VP-immunopositive material was revealed in VP-ergic NSC of the dorsolateral portion of PVN and in SON in the nonoperated rats under the immobilization stress. The nucleolar volume of these cells was reduced reliably only in PVN (Fig. 1). The number of "polynucleolar" cells increased to 166% ($p<0.05$) also exclusively in PVN. In the control rats both in the fibers of the hypothalamo-hypophyseal tract and in the external zone of the eminentia mediana we registered a marked decrease of VP-immunopositive material. This observation testifies that clearance of the material from the terminal prevails over its synthesis [10].

A reduction in VP content in the hypothalamic nuclei and in the fibers of the hypothalamo-hypophyseal tract is evidence of activation of its transport along the fibers. At the same time, the elevated VP level in the blood registered in this study (see Fig. 2) points to an intensive release of VP into the blood flow.

Thus, the examination of OT-ergic NSC in SON and PVN in nonoperated rats did not reveal any substantial changes in the intensity of the formation and demonstrated inhibition of its transport along the fibers. At the same time, the immobilization stress in the controls led to a significant decrease of the functional activity of VP-ergic NSC in PVN of the hypothalamus and activation of VP transport and clearance from SON cells.

Seven days after hypophysectomy, degenerative changes associated with injury of NSC axons in the hypophyseal cord appeared in the supraoptic and paraventricular centers of the hypothalamus. The total NSC number decreased and numerous wrinkled hyperchromic cells with a dark nucleus of irregular shape appeared. Degenerating NSC were surrounded by glial cells; cavities remained in the place of the lysed cells. In some cases we observed mitosis of glial cells. The number of pycnomorphic NSC was higher in SON both among OT-ergic (58%, $p<0.01$) and VP-ergic (55%, $p<0.01$) NSC.

In PVN the share of pycnomorphic NSC increased to 50% of the total number among OT-ergic cells ($p<0.01$) and to 48% ($p<0.01$) among VP-ergic cells. The nucleolar volume of the preserved NSC in SON and PVN was reliably reduced, this drop being more pronounced in VP-ergic NSC of both centers (see Fig. 1). The stress response of NSC in SON and PVN of the hypothalamus in the hypophysectomized rats exhibited the same pattern. A reduction in the nucleolar volume was registered in both centers of VP-ergic NSC but it was reliable only in PNV in comparison with the hypophysectomized control animals. Determination of VP level in the blood demonstrated that in the nonoperated rats its concentration increased to 220% for immobilization in comparison with the initial level ($p<0.01$), while in the hypophysectomized rats no stress-induced fluctuations were noted. The blood concentration of ACTH sharply increased upon stress in the nonoperated animals (up to 440%, $p<0.01$), while in the hypophysectomized rats its concentration was below the sensitivity threshold of the method. The stress response of the adrenal cortex was pronounced in the nonoperated rats (the 11-HOCS blood concentration rose to 174%, $p<0.001$) but disappeared after hypophysectomy. We failed to register a stress response of the thyroid gland in either the nonoperated or hypophysectomized rats (Fig. 2). However, the reaction pattern of VP- and OT-ergic elements in the hypophysectomized rats did not change. These observations provide grounds for concluding that the response of the neurosecretory centers to a nonspecific short-term stress is not influenced by the anterior pituitary and is independent of changes in the hormonal status of the organism.

Thus, in rats with an intact pituitary acute short-term immobilization stress provoked a diminution of VP formation in NSC of the dorsolateral portion of PVN and intensification of VP clearance from NSC and fibers of SON. The same pattern of reaction to nonspecific stress was revealed in the hypophysectomized rats. The results indicate that there is no correlation between the stress response of NSC in SON and PVN and the level of the anterior hypophyseal hormones in the blood. It may be assumed that the reaction of the nonapeptidergic hypothalamic centers to nonspecific short-term stress is regulated by some other structures of the central nervous system.

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