A Morphofunctional Study of the Hypothalamic Postoptic Nucleus in Rats Following Hypophysectomy, Cooling, or Immobilization

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Mammals have been shown to be the first animals in which the hypothalamic nonapeptidergic postoptic nucleus (PON) emerged as a separate neurosecretory center in the course of evolution [7]. During phylogeny its appearance is thought to have been preceded by division of the supraoptic nucleus (SON) into a medial and a lateral part, which is apparent in reptilians [7]. During ontogeny, the PON develops from the same source as the SON (the matrix zone of the preoptic bay) but is separated from the latter by optic tract fibers growing into the brain [10]. The commonness seen in the development of these two structures in phylogeny and ontogeny and their close topographic proximity to each other have led most authors to consider the PON as the medial or retrochiasmatic (caudal) part of the SON rather than as a distinct neurosecretory center [8,15]. For this reason, the number of studies attempting to demonstrate functional specialization of the PON is relatively small, and most of them were conducted in the late 1970s. As a rule, they are concerned with reactions of nonapeptidergic neurosecretory (NS) cells to disturbances of water-salt metabolism. In rats with such disturbances, similar changes were found to occur in the PON and SON [12,13]. Similar reactions of these two nuclei to stimulation of the cervical sympathetic nerve and of afferent fibers of the vagus were also demonstrated in cats [1]. However, in rats capable of tolerating hypobaric hypoxia as a result of prolonged training, reactions of PON cells were similar to those of the paraventricular nucleus (PVN) and, moreover, changes in morphometric parameters of PON cells were found to correlate with those of the thyroid [3]. No correlation between the reactions of PON and SON cells has been demonstrated. On the basis of the available evidence it can be assumed that the reactions of the PON to various influences have features that distinguish this nucleus from both the SON and the PVN. In this study on rats, PON reactions to short-term stresses were examined.

MATERIALS AND METHODS

Three groups of male Wistar rats weighing 120-150 g were used (a total of 25 animals). In rats of group 1, the pituitary body was removed by the transsphenoidal route, and the animals were decapitated 7 days later; group 2 rats were kept in individual chambers at 4°C for 2 h, while those of group 3 were subjected to rigid immobilization on the back with stretched legs. Animals of the latter two groups were decapitated immediately after exposure to the stress. For each group, intact rats served as controls.

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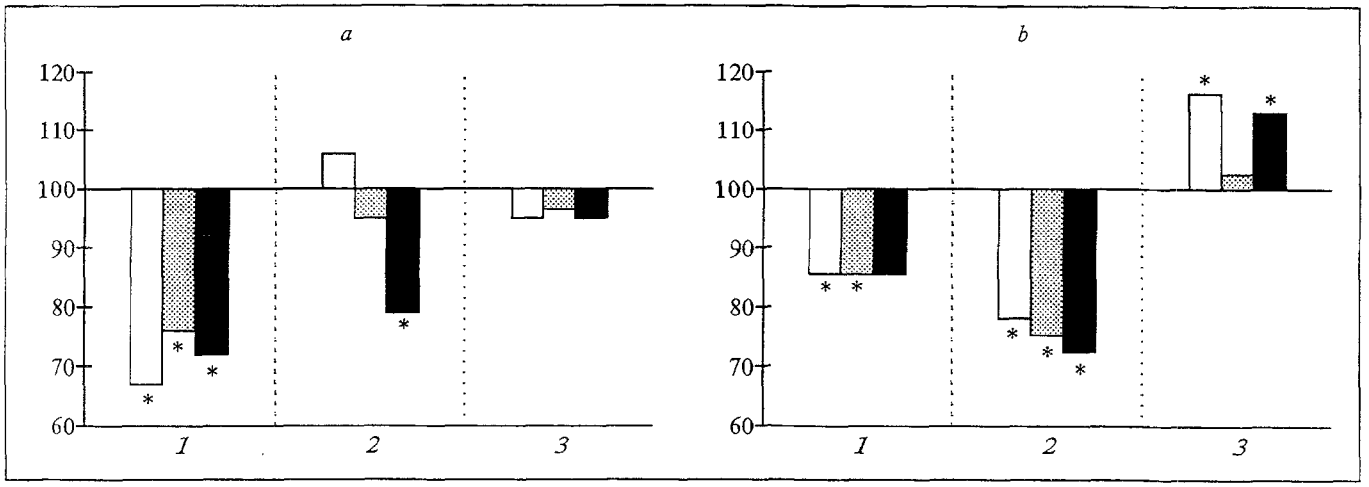

Fig. 1. Cross-sectional areas of nucleoli (white bars), nuclei (light grey bars), and perikarya (black bars) of oxytocinergic (*a*) and vasopressinergic (*b*) PON cells from rat hypothalamus after hypophysectomy (*1*), cooling (*2*), and immobilization (*3*), expressed as percentages of their values in the control taken as 100%. Asterisk: $p<0.05$; circle: $p<0.01$.

The brain of each rat was fixed in a mixture of picric acid and 40% formalin in a 3:5 ratio at 37°C for one week. In paraffin sections of the brain, oxytocinergic (OTE) and vasopressinergic (VPE) structures were demonstrated immunohistochemically by the PAP method. Some sections were stained with paraldehyde-fuchsin by Gomori-Gabe's method and counterstained with azan after Heidenhain. In the immunohistochemical preparations, diameters and cross-sectional areas of nucleoli ($S=¼\pi d^2$), nuclei, and perikarya ($S=¼\pi Dd$) of OTE and VPE cells were determined at a 15×90 magnification using an ocular micrometer, and the nucleolonuclear ratios were calculated. Measurements were performed in at least 30 OTE and VPE cells of the PON, SON, and PVN from each rat. The significance of differences was evaluated by Student's *t* test.

RESULTS

In the rat hypothalamus, the PON is located caudal to the SON, behind the optic chiasm. No NS cells between these two nuclei are detectable. In the PON, VPE cells predominate; the number of OTE cells is small.

At first sight, the morphology of NS cells from the PON of control rats did not seem to differ from that of these cells from the SON or PVN. However, comparison of morphometric parameters of OTE and VPE cells from the PON, SON, and PVN showed that the nucleolonuclear ratios were higher in PON cells than in SON and PVN cells (Table 1). This is because in intact rats nucleoli of NS cells from the PON are larger, which indicates that protein synthetic processes are more intensive in PON cells than in cells of the SON or PVN.

On day 7 after hypophysectomy, the nucleoli, nuclei, and perikarya of NS cells were significantly ($p<0.01$) decreased in size, especially in OTE cells (Fig. 1), and large numbers of polymorphous NS cells appeared in the PON. A similar observation has been made for the SON [14]. This suggests that an absolute majority of VPE and OTE cells of the PON, like those of the SON, send their axons to the posterior pituitary.

In rats exposed to cooling, VPE cells of the PON reacted more strongly than OTE cells: they had significantly decreased sizes of nucleoli (by 22% relative to the control; $p<0.01$), nuclei (by 24%; $p<0.01$), and perikarya (by 26%; $p<0.01$) (Fig. 1). In OTE cells, only perikarya had significantly decreased sizes (by 23%; $p<0.01$). These changes in the PON cells reflect their reduced functional activity.

It is of interest that under conditions similar to those of our experiment, no significant changes in morphometric parameters of SON cells were observed, and only the nucleoli of OTE cells tended to decrease in size [4].

In rats subjected to immobilization, no alterations in morphometric parameters were detectable in OTE cells of the PON, whereas its VPE cells

Table 1. Nucleolonuclear Ratios in Oxytocinergic (OTE) and Vasopressinergic (VPE) Neurosecretory Cells of Postoptic (PON), Supraoptic (SON), and Paraventricular (PVN) Nuclei from Intact Rats (Values are Means±SEM)

Cells	PON	SON	PVN
OTE	0.105±0.06 (4)	0.08±0.005 (6)	0.061±0.006 (6)*
VPE	0.101±0.048 (4)	0.08±0.017 (5)	0.06±0.003 (5)*

Note. Figures in parentheses denote the number of rats. The asterisk indicates the significance of differences for $p<0.05$ in comparison with the postoptic nucleus.

had significantly enlarged nucleoli (by 17% relative to the control; $p<0.05$) and perikarya (by 14%; $p<0.01$), indicating that hormone production was intensified in the latter cells. However, as shown earlier [2], NS cells of the SON did not respond to a 20-minute immobilization, while VPE cells of the PVN had nucleoli of diminished size.

These findings indicate that cooling and immobilization both led to greater changes in NS cells of the PON than of the SON or PVN. This is probably because the nucleolonuclear ratios in PON cells are considerably higher than in SON and PVN cells, reflecting more intensive processes of hormone production in PON cells. No polymorphous NS cells were detected in PON, SON, or PVN cells following immobilization or cooling.

The stressors used in our study (cold and immobilization) both cause rises in blood levels of adrenocorticotropic hormone and corticosteroids, i.e., they activate the adrenohypophyseal-adrenocorticoid system. In contrast, these stressors exert opposite effects on the adrenohypophyseal-thyroid system: cooling stimulates it, whereas immobilization brings about reductions in thyrotropic hormone levels and thyroid activity [5]. The changes in VPE cells of the PON after cooling and immobilization in our experiment were opposite to the responses of the thyroid in another, similar experiment [6]. Such a relationship between the functional state of the PON and that of the hypothalamic-adrenohypophyseal-thyroid system suggests that the PON experiences inhibitory influences as a result of enhanced activity of this system during cooling. The stimulation of VPE cells in the PON of rats subjected to immobilization may be due to the influence of somatostatin (an antagonist of thyroid releasing factor in the action on adenohypophyseal thyrotropes), whose blood level rises substantially under stress [9]. The reduced PON activity was most likely due to a fall in the blood level of vasopressin upon cooling [11], given that most NS cells of the PON, as shown by the results obtained after hypophysectomy, project their axons to this region.

It should be noted that NS cells of the PON responded to cooling and immobilization in the same manner as did those of the SON and PVN. This finding, and also the topographic separateness of the PON and its distinctive morphometric characteristics, have led us to conclude that this nucleus is indeed an independent neurosecretory center whose function appears to be associated with regulation of the thyroid gland.

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