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Accessory Groups of Nonapeptidergic Neurosecretory Cells of the Hypothalamus and Adjacent Regions of the Brain in Rats under Conditions of Dehydration

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Study of the hypothalamus and adjacent areas of the brain in adult rats revealed that, along with the osmosensitivity of neurosecretory cells, the transport of vasopressin and oxytocin along their axons and their release from the terminals into the bloodstream are impeded. This is due to the absence of axonal bonds between the accessory groups of neurosecretory cells and the posterior lobe of the pituitary.

Key Words: *hypothalamus; accessory groups; vasopressin; oxytocin; dehydration*

The hypothalamus and adjacent areas of the brain in rats contain a number of accessory groups (AG) in addition to the chief nonapeptidergic neurosecretory centers (supraoptic, postoptic, and paraventricular nuclei) [14]. At present, the neuroanatomy of these formations has been described in detail [5], but the functional specialization of AG is not quite clear. Our previous experiments (immobilization and cooling) permitted us to hypothesize that some AG are involved in the regulation of the functions of the peripheral endocrine glands: thyroid and adrenal [4].

The task of this study was to investigate the histophysiology of AG under conditions of dehydration, which is a specific exposure for the entire nonapeptidergic hypothalamopituitary neurosecretory system. The status of the nonapeptidergic neurosecretory cells (NSC) - the principal nonapeptidergic formations in dehydration - has been studied frequently [7,9,10,12]. In contrast, the contribution of AG to the regulation of water-salt metabolism is still unclear.

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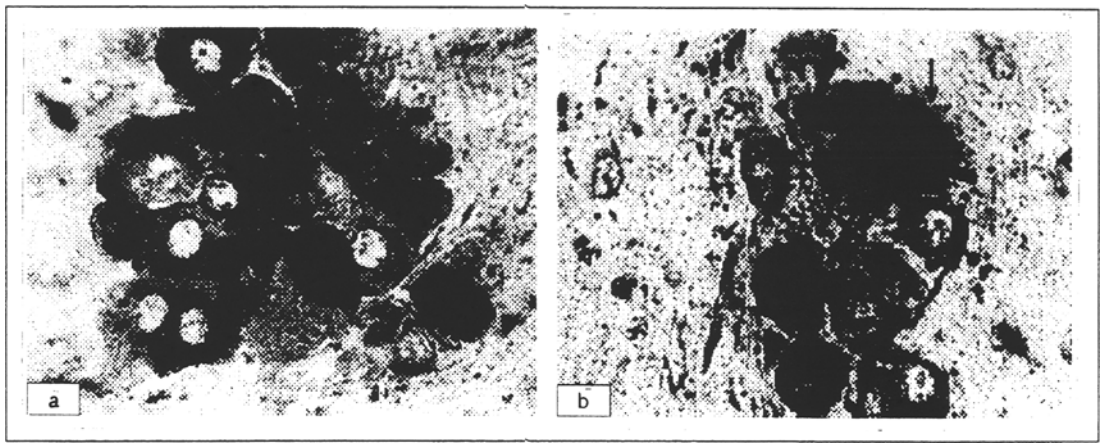

Fig. 1. The fornical accessory group in an intact rat (*a*) and a rat subjected to dehydration (day 8, *b*). Arrow shows a conglomerate of dilated fibers filled with vasopressin-immunoreactive material. Antibodies against vasopressin+Ehrlich's hematoxylin. ×200.

MATERIALS AND METHODS

Experiments were carried out in December-January on 20 adult male Wistar rats weighing 200 to 250 g. The rats were fed dry fodder and given 2.5% NaCl solution to drink. The animals were decapitated on days 2 and 8 of the experiment. Fragments of the brain and pituitary were fixed in a mixture of picric acid and 40% formalin for a week at 37°C, after which they were embedded in paraffin. Serial frontal slices were stained with paraldehydefuchsin (PAF) after Gomori and with azan after Heidenhain. Part of the material was treated immunohistochemically by the PAP method using unlabeled antibodies against vasopressin and oxytocin. The criterion of the reaction of the hypothalamopituitary neurosecretory system was the amount of PAF-positive material in the posterior lobe of the pituitary and the degree of its hyperemia (capillary status).

The size of sections of the nucleoli of Gomori-positive NSC from the AG ($S=\pi/4\times d^2$) was assessed with an ocular micrometer at 1350 magnification in intact and experimental animals. The nucleolus in NSC plays an exceptional role in the synthesis of ribonucleoproteins, and karyometry is still widely used for assessing the function of NSC [8]. The reliability of differences was assessed using Student's *t* test.

RESULTS

The following AG of the rat brain were studied: circular, fornical, dorsolateral, ventrolateral, and extrahypothalamic [2].

On day 2 of the experiment signs of activation of NSC of all AG appear, presenting as a reliable increase of the areas of the sections of their nucleoli (Table 1) and an increase in the number of type 1a NSC according to previously published data [7]. Phenotypically, type 1a NSC at the light-optic level are characterized by large nucleoli, nuclei, and perikarya. The cytoplasm of type 1a NSC contains little neurosecretory material. The dorsolateral group NSC, in which no reliable increase in the size of the nucleoli or alteration of their morphology are observed, are an exception. In parallel with this, the number of fibers filled with PAF-positive and vasopressin-immunoreactive contents increases in the activated AG. The number of type 1a cells increases in the main centers, particularly in the supraoptic and postoptic nuclei, but the amount of immunoreactive substance in NSC fibers appreciably decreases.

The amount of PAF-positive material in the posterior lobe of the pituitary drops markedly, and the number of dilated capillaries containing formed elements of the blood increases.

On day 8 of the experiment the size of the nucleoli in the NSC of all AG, including the dorsolateral, is reliably greater than in control animals. However, along with the increase in the size of the nucleoli, the amount of vasopressin-immunoreactive material increases in some NSC of AG (type 1b NSC appear), and numerous fibers filled with PAF-positive and vasopressin-immunoreactive neurosecretion appear in the AG (Fig. 1). The same kind of picture is observed in the paraventricular, but not in the supra- and postoptic nuclei.

On day 8 of dehydration very little PAF-positive substance is detected in the posterior lobe of the pituitary, particularly in its central part, which mainly contains the terminals of vasopressinergic NSC axons [6]. It is noteworthy that the general decrease in the amount of PAF-positive material in the terminal parts of NSC axons goes along with a reduction in the density of PAF-positive neurosecretion. In addition, numerous dilated capillaries filled with formed elements of the blood are found in the posterior lobe of the pituitary.

A statistically reliable increase in the size of the nucleoli measured in the PAF- and azan-stained preparations from day 2 to day 8 of the experiment is observed in the fornical, ventrolateral, and dorsolateral

TABLE 1. Changes in the Size of Sections of the Nucleoli (μ^2) of Gomori-Positive NSC in AG of Rats Subjected to Dehydration ($M \pm m$)

AG	Control	Day 2	Day 8
Circular	3.88±0.08	4.53±0.1**	4.56±0.11
Fornical	4.66±0.09	5.16±0.13**	7.01±0.23+
Dorsolateral	5.16±0.15	5.47±0.19	7.83±0.21+
Ventrolateral	4.89±0.09	5.46±0.16*	7.47±0.14+
Extrahypothalamic NSC	4.67±0.13	5.25±0.17*	6.11±0.42

Note. *$p<0.01$, **$p<0.001$ *vs.* the control; +$p<0.001$ *vs.* day 2 of the experiment.

AG, but not in the circular AG or NSC and groups thereof situated outside the hypothalamus. This may be due to a different ratio of the oxytocin- and vasopressin-immunoreactive NSC in the studied AG [1], because the size of the nucleoli was assessed in the NSC irrespective of their ergy. This may also explain the absence of signs of activation of NSC in the dorsolateral AG on day 2 of the experiment, because the dorsolateral group contains mainly oxytocin-immunoreactive cells (78%), whose reaction may be delayed.

Hence, the results indicate osmosensitivity of NSC of both AG and the main centers. Activation of the protein-synthesizing processes in the NSC of the supraoptic and paraventricular nuclei has been demonstrated in similar experiments by other scientists using *in situ* hybridization and immune cytochemistry [11,13].

Besides signs of NSC activation, accumulation of neurosecretion in the processes and perikarya of these cells is observed in AG (it being particularly well expressed on day 8), which reflects inhibition of the elimination of nonapeptide neurohormones (mainly vasopressin) from the perikarya and impeded transport of these neuropeptides along the fibers and from axon terminals into the bloodstream. Similar events were observed in the paraventricular nucleus on day 8.

The amount of neurosecretion is notably reduced in the posterior lobe of the pituitary as early as on day 2, and by day 8 very little of it is seen. This is paralleled by augmenting hyperemia of this neurohemal organ. These data attest to active release of nonapeptide neurohormones into the bloodstream [7]. Evidently, the accumulation of vasopressin and oxytocin is typical for perikarya and particularly NSC processes under conditions of dehydration, indicating impaired transport of these neurohormones. The reason may lie in the absence of axonal bonds between the absolute majority of NSC of AG and the posterior lobe of the pituitary. Therefore, the possibility of there being projections of these cells to the median eminence and other neurohemal organs, and to extrahypothalamic areas of the brain cannot be ruled out.

This hypothesis is based on our data obtained in experiments with hypophysectomized rats [3]. That study demonstrated that no pyknomorphous elements had appeared in AG on day 7 after removal of the pituitary, whereas in the supraoptic and postoptic nuclei they were abundant. As for the paraventricular nucleus, whose NSC axons terminate mainly in the median eminence, it was far less injured by hypophysectomy than the supra- or postoptic nuclei. It is interesting that salt loading also revealed a similar picture of neurosecretion accumulation in NSC fibers in the paraventricular nucleus and in AG, which fact, similarly as in hypophysectomy, indicates the similarity of the reactions of the paraventricular nucleus and AG.

We may conclude that under conditions of dehydration the NSC in AG are involved in the regulation of water-salt metabolism, but their reaction differs from that in the supra- and postoptic nuclei and is similar to the changes observed in the paraventricular nucleus, possibly due to specific features in the afferent-efferent bonds of the formations in question.

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