5 h later gives way to constriction, which affects the resistance by 6.2%. The increase of resistance mainly due to the rise in viscosity is reflected by parameter q.

If we considering the resistance as an index of a normal state and its change as a disorder, the aim of correction could be to stabilize the resistance. However, in the present case (Fig. 1) the left MCB index that is not affected by hemoconcentration is the viscosity of the venous blood, while on the right such an index is the bed area. The changes of all other indexes are geared towards preserving the conditions for maintaining a stable state. So, in this case correction for resistance remains disputable.

Returning to the problem of viscosity as a hematocrit function, it may be noted that the viscosity increased only by 8% when the hemoconcentration rose 8.5% (the right MCB). When the hemoconcentration increased by 50% there was no respective increase of the viscosity (it was only 16.5%). It is believed [7] that this result may be due to an interaction between the rheological and vascular mechanisms of homeostasis regulation, and this is indeed confirmed by the present analysis.

Thus, the findings prove that there are differences between the disorders in the paired MCB under hemoconcentration and support our previous results.

REFERENCES

1. Yu. Ya. Kislyakov, Yu. M. Levkovich, and T. E. Shumilina, *Fiziol. Zh. SSSR*, 72, № 9, 1199-1203 (1986).
2. L. A. Michailichenko and M. I. Reutov, *Byull. Eksp. Biol. Med.*, **108**, № 8, 162-163 (1989).
3. S. A. Seleznev, G. I. Nazarenko, and V. S. Zaitsev, *Clinical Aspects of Microhemocirculation* [in Russian], Leningrad (1985), p. 207.
4. H. Schmid-Schombein, *Kardiologia*, № 3, 82-85 (1982).
5. F. C. Fan, R. Y. Z. Chen, G. B. Schuessler, and S. Chien, *Amer. J. Physiol.*, **238**, H545-H551 (1980).
6. B. Klitzman, *Microvascular Res.*, **29**, 231 (1985).
7. H. H. Lipowsky and J. C. Firrel, *Amer. J. Physiol.*,. **250**, H908-H922 (1986).

Reaction of Nonapeptidergic Neurosecretory Cells of the Hypothalamic Accessory Groups to Cold and Immobilization Stress in Rats

V. V. Grinevich, I. A. Krasnovskaya, and A. L. Polenov

UDC 616.831.41-018.1-02:613.863]-092.9-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 8, pp. 201-203, August, 1993
Original article submitted March 29, 1993

Key Words: *hypothalamus; oxytocinergic cells; vasopressinergic cells; cooling; immobilization*

Rat hypothalamus nonapeptidergic neurosecretory cells (NSC) are located not only in the supraoptic, postoptic, and paraventricular nuclei, but also in small clusters called accessory groups. The localization and cellular composition of accessory groups have been previously described [13] but there are no reports about their functional role in the organism.

According to A. L. Polenov' concept, nonapeptide neurohormones synthesized in large neuro-

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Medical Sciences, St. Petersburg; Medical Institute, Kursk . (Presented by V. L. Sviderskii, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0008-1010$12.50

secretory centers contribute under specific conditions to trans- and paraadenohypophyseal regulation of peripheral endocrine gland function [4,6]. The role of the accessory groups in these processes was previously not paid proper attention. The effect of oxytocin and vasopressin on the peripheral endocrine glands is thought to manifest itself under stress [12]. We therefore decided to investigate accessory groups cells under two qualitatively different stress factors: cold and immobilization. The thyroid and the entire hypothalamo-adenohypophyseo-thyroid system are known to be stimulated during cold stress [8]. But cooling is known to activate the hypothalamo-adenohypophyseo-adrenocortical system as well, which may result in changes in accessory group NSC status. Immobilization stress is not associated with noticeable shifts in the TRF-thyrotropin-thyroid axis [3], and the pattern of CRF-ACTH-corticosteroid system reactions is the same as in cooling. Hence, we may suppose that a comparison of the changes occurring under both stress factors will help detect a functional correlation between NSC status in some accessory groups and changes in thyroid or adrenocortical activity.

The object of the present study was to explore the reaction of the hypothalamic NSC to cold and immobilization stress in rats.

MATERIALS AND METHODS

Two groups of male Wistar rats weighing 120 to 150 g (n=16) were used in the experiments. Group 1 animals were kept at 4°C for 2 h. Group 2 rats were rigidly immobilized (lying supine with the limbs stretched apart) for 20 min. The animals were decapitated immediately after the manipulations. Intact rats were controls. The brain was fixed in a 3:5 mixture of picric acid and 40% formalin at 37°C for one week. Paraffin sections were immunohistochemically treated by the PAP method, using unlabeled antibodies to oxytocin and vasopressin, and counterstained with hematoxylin after Ehrlich. Some sections were stained with paraldehyde-fuchsin after Gomori-Gabe and then with azan after Heidenhain. With a screw ocular micrometer the diameters of the nucleoli, nuclei, and perikaryons of all oxytocinergic and vasopressinergic NSC in every accessory group were measured at magnification 15×90, and the square of the nucleolar ($S=\frac{1}{4}\pi d^2$), nuclear, and perikaryon ($S=\frac{1}{4}\pi Dd$) sections was calculated. Since changes in nucleolar size reflect fluctuations in intensity of secretion production in NSC [10], we took this parameter as the major criterion of these cells' functional activity.

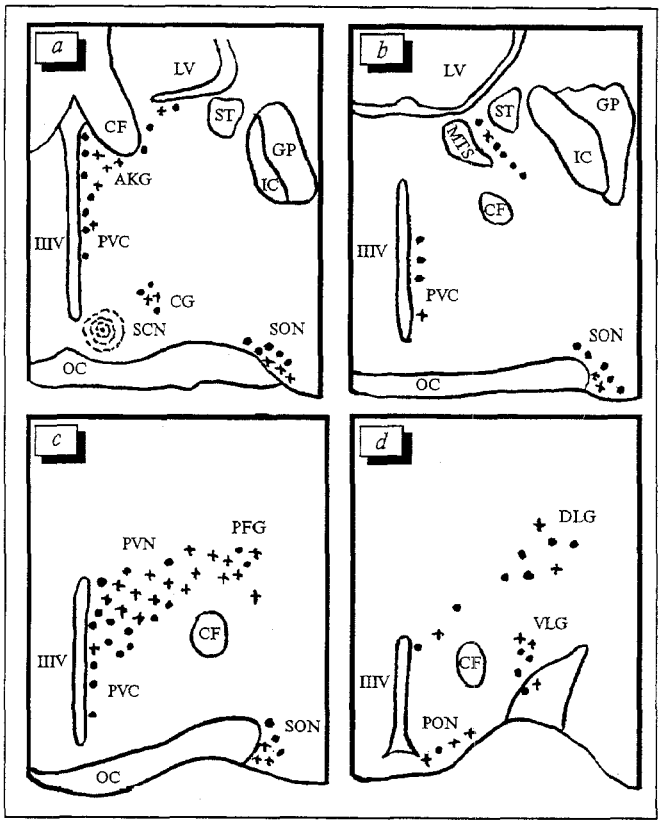

Fig. 1. Topography of rat hypothalamic nonapeptidergic cell nuclei and accessory groups on frontal sections ($a-d$). Circles show oxytocinergic cells, crosses vasopressinergic cells. IIIV: third ventricle; LV: lateral ventricle; GP: globus pallidus; IC: internal capsule; VLG: ventrolateral group; DLG: dorsolateral group; ST: stria terminalis; CF: columna fornicis; MTS: medullary thalamic stria; OT: optic tract; OC: optic chiasm; PVN: paraventricular nucleus; ACG: anterior commissural group; PON: postoptic nucleus; PFG: periforniceal group; SON: supraoptic nucleus; SCN: suprachiasmic nucleus; CG: circular group; EHG: extrahypothalamic group.

The reliability of differences was assessed using the Student t test. The results are presented as $M\pm$Set.

RESULTS

The following accessory groups were examined in the rat hypothalamus: circular, periforniceal, dorsolateral, and ventrolateral; periventricular cells, and also the extrahypothalamic accessory group (Fig. 1).

After exposure of rats to cold, signs of NSC activation are seen in some accessory groups (circular and extrahypothalamic groups, periventricular cells), this being manifested in the appearance of a large number of light, large cells with some neurosecretory material located perinuclearly (type 1a according to Polenov [5]).

Morphometric analysis showed increased nucleoli, nuclei, and perikaryons in the NSC of these accessory groups after cooling (Fig. 2). In the extrahypothalamic group the nucleoli were en-

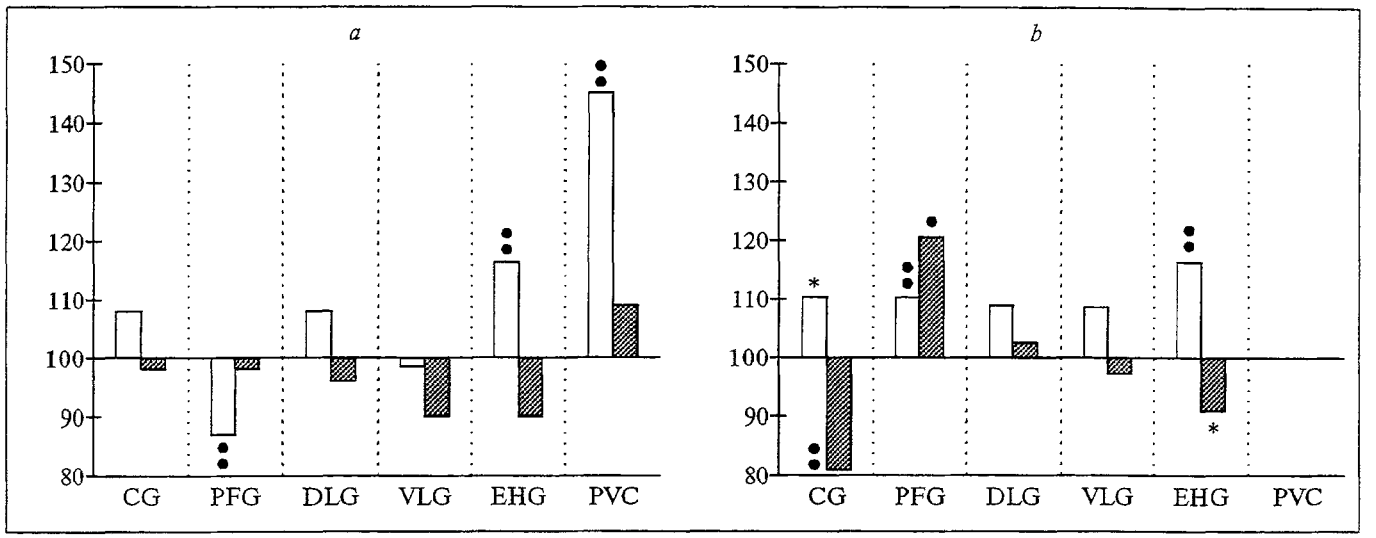

Fig. 2. Changes in size of nucleolar sections (*a*) in oxytocinergic and vasopressinergic cells (*b*) of accessory groups in the rat hypothalamus and adjacent brain areas during exposure to cold (light bars) and immobilization (black bars) in comparison with 100% in intact controls. Accessory group names as in Fig. 1. Asterisk: $p<0.05$; one dot – $p<0.01$, two dots $p<0.001$.

larged in both the oxytocinergic and vasopressinergic cells (from 4.51±0.11 to 5.18±0.15 and from 4.77±0.13 to 5.52±0.12 μ^2, $p<0.001$, respectively), whereas in the circular accessory groups only the vasopressinergic cell nucleoli were enlarged (from 4.93±0.1 to 5.4±0.17 μ^2, $p<0.001$). In the dorsolateral accessory group cells only the nuclei of both cell types were reliably enlarged. The changes were the most marked in the periventricular oxytocinergic cells (just a few vasopressinergic cells occur in this area): their nucleolar size was 144% that of the control (from 3.29±0.05 to 4.74±0.09 μ^2, $p<0.001$), this being associated with a reliable increase of the size of the nuclei and perikaryons. Of interest is the peculiar reaction of the periforniceal accessory group NSC: the cells from a sort of a sleeve round the arteriole. Oxytocinergic cells of this group undergo a marked decrease of nucleolar (from 5.85±0.1 to 5±0.1 μ^2, $p<0.001$) and perikaryon size. On the other hand, the vasopressinergic cell nucleoli were noticeably enlarged (from 6.11±0.1 to 6.75±0.12 μ^2, $p<0.001$).

Immobilization stress was not associated with any marked shifts in the oxytocinergic cells of almost all accessory groups. The only exception were the dorsolateral group oxytocinergic cells, in which only the size of the nuclear and perikaryon section area was decreased. The response of the vasopressinergic cells was different. In the circular group NSC the nucleoli were reliably reduced (from 4.94±0.25 to 3.97±0.21 μ^2, $p<0.001$), as well as the perikaryons. On the other hand, in the periforniceal group vasopressinergic cells the nucleoli were reliably ($p<0.01$) enlarged (from 6.85±0.41 to 8.18±0.36 μ^2). The absolute majority of periforniceal group cells belong to type 1a, this indicating their high functional activity.

Comparison of accessory group NSC morphometric parameters under stress and immobilization showed a clear-cut relationship between the circular group vasopressinergic cells and the type of stress: cooling stimulated these cells (the nucleoli, nuclei, and perikaryons increased in size), while immobilization reduced their activity. The extrahypothalamic group oxytocinergic cells were activated by cooling, like the vasopressinergic cells, although their less intensive functioning under immobilization stress could not be reliably recorded; still, it may be assumed that the responses of both cell types in the extrahypothalamic group were similar. The pattern of the hypothalamo-hypophyseo-thyroid system response was the same during these influences, which may reflect the involvement of the circular and extrahypothalamic accessory groups (just like the periventricular group) in thyroid regulation.

The activation of the NSC of these groups under conditions of cooling may be due to both TRF-ergic afferentation and the humoral effect of TRF on these cells [14]. It is possible that NSC activation in the circular and extrahypothalamic accessory groups and periventricular oxytocinergic cell activation are realized via the feedback principle and depend on changes in blood thyroid hormone levels. In addition, monoaminergic brain structures may be involved in the response to cooling [7]. Of course, the effects of cold stress on accessory group NSC are not limited to these factors, but the mechanisms of even these factors are still unknown.

Only the periforniceal group vasopressinergic cells undergo unidirectional changes under both stress factors. The nucleoli of these cells increased in size whatever the stress type. This gives us grounds for assuming that the periforniceal group response is connected with a nonspecific component of the stressor reaction, most likely with activation of the CRF-ACTH-corticosteroid system. Our hypothesis was confirmed by detection of CRF immunoreactive cells in the periforniceal group, whereas they were not detected in the other accessory groups.

Hence, the different pattern of NSC changes in accessory groups of various localization under every stress factor investigated appears to reflect the functional heterogeneity of these groups. This phenomenon may be explained by differences in accessory group afferentation and the specific reaction of the receptor apparatus of the cells to various hormonal signals.

The ventrolateral and dorsolateral accessory groups are of particular interest in this respect, for their vasopressin- and oxytocinergic cells undergo no appreciable changes in nucleolar size whatever the stress factor; this may be due to functional specificities the NSC of these groups [9,11].

The detected accessory groups NSC reaction differs considerably from the NSC reaction in the supraoptic and paraventricular nuclei in similar experiments [1,2]. We therefore believe that accessory groups are separate centers in the rat hypothalamus with a functional significance of their own in the organism. We think it possible that the circular, extrahypothalamic, and periventricular accessory groups contribute to thyroid regulation and the periforniceal group to adrenocortical regulation, but the routes and mechanisms of regulation of NSC function in these accessory groups are still unknown.

The authors are grateful to Dr. O. A. Danilova, who kindly provided hypothalamic preparations treated with antiserum to CRF, and to Dr. V. V. Kuzik for assistance in the immunohistochemical staining.

REFERENCES

1. L. S. Voropanova and I. A. Krasnovskaya, in: Neurobiological Aspects of Modern Neuroendocrinology [in Russian], Moscow (1991), p. 16.
2. I. A. Krasnovskaya, Probl. Endokrinol., **30**, № 2, 52 (1984).
3. I. A. Krasnovskaya and T. V. Sheibak, *Byull. Eksp. Biol. Med.*, **109**, № 1, 30 (1990).
4. A. L. Polenov, Arkh. Anat. Gistol. Embriol., **43**, № 9, 3 (1962).
5. A. L. Polenov, Hypothalamic Neurosecretion [in Russian], Nauka, Leningrad (1968).
6. A. L. Polenov, Usp. Fiziol. Nauk, **10**, № 1, 28 (1979).
7. E. M. Stabrodskii, *Byull. Eksp. Biol. Med.*, **87**, № 2, 414 (1979).
8. S. Arancibia, J. Epelbaum, R. Bayer, et al., Neurosci. Lett., **50**, № 1-3, 97 (1984).
9. R. Arevalo, F. Sanchez, J. R. Alonso, et al., Brain Res. Bull., **28**, 599 (1992).
10. J. C. Beauvillain, G. Tramu, and P. Poulain, Cell Tissue Res., **224**, № 1, 1 (1982).
11. R. Besson and R. Seite, Cell Tissue Res., **240**, 393 (1985).
12. A. L. Polenov, The Hypothalamic Neurohormonal Mechanisms of Adaptation, XXVIII Int. Congr. Physiol. Sci., Vol. 14 (1980), p. 36.