ROLE OF ADRENERGIC INNERVATION IN REGULATION OF SECRETORY ACTIVITY OF CELLS OF THE RAT SUPRAOPTIC NUCLEUS

M. S. Konstantinova

UDC 612.826:612.452.018

To study the role of the noradrenergic innervation in the production of peptide neurohormones by cells of the supraoptic nucleus (SON) the substance 6-hydroxydopamine (6-HDA), which causes selective destruction of noradrenergic terminals, was used. The intensity of fluorescence of these terminals in the region of SON 7 days after intraventricular injection of 6-HDA in a dose of 250 μg was sharply reduced and morphological pictures reflecting activation of hormone production by SON cells were observed. According to the results the noradrenergic innervation evidently inhibits synthesis of peptide neurohormones by SON cells and regulates the transport and secretion of peptide neurohormones from the posterior lobe of the pituitary into the general circulation.

KEY WORDS: hypothalamus; supraoptic nucleus; neurosecretory cells; noradrenergic innervation.

The neurosecretory cells of the supraoptic nucleus (SON) of the hypothalamus in rats have a rich noradrenergic innervation [1, 2, 9]. Noradrenergic receptors are found on the cells of SON [5]. There is as yet no general agreement whether noradrenalin inhibits or activates the secretion of peptide neurohormones by the cells of SON. The solution to this problem is extremely important for neuroendocrinology. The compound 6-hydroxydopamine (6-HDA) was used for this purpose. If injected into the cerebral ventricles, 6-HDA is incorporated selectively, destroys the terminals of noradrenergic neurons, and reduces the noradrenalin concentration in the hypothalamus sharply and for a long time [4, 6-8, 10, 12].

The object of this investigation was to study the functional activity of the neurosecretory cells of SON in rats after blocking of the noradrenergic innervation by means of 6-HDA.

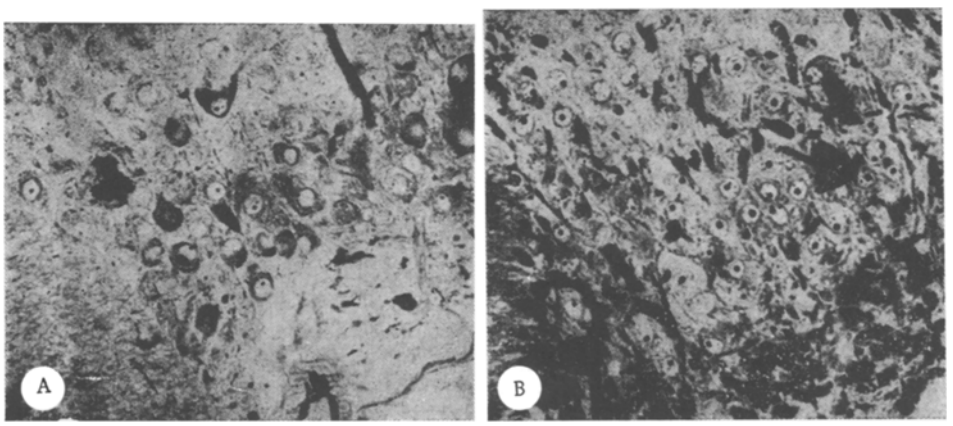

Fig. 1. Cells of rat SON after injection of 6-HDA. Compared with control (A), dimensions of nuclei of SON cells in experimental animal (B) are appreciably increased and processes of SON cells are packed with neurosecretory material. Stained with paraldehyde-fuchsin, ocular 3.2, objective 25.

Laboratory of Neuroendocrinology, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 11, pp. 518-520, November, 1979. Original article submitted June 28, 1978.

0007-4888/79/8811-1239$07.50 © 1980 Plenum Publishing Corporation

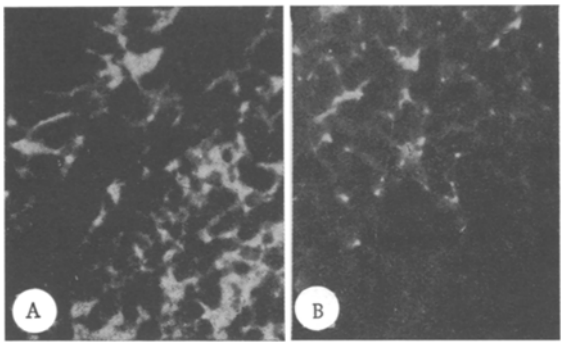

Fig. 2. Fluorescence of noradrenergic fibers in region of rat SON after injection of 6-HSA. Compared with bright fluorescence of noradrenergic terminals in control (A), intensity of fluorescence of these terminals around dark nonfluorescent bodies of SON cells in experimental section (B) was considerably reduced. Falk–Hillarp fluorescence method. Ocular homal 3, objective 40.

METHODS

Experiments were carried out in winter on sexually mature male Wistar albino rats weighing about 180-250 g. The substance 6-hydroxydopamine-HBr (from Sigma, USA) was dissolved before use in the cold in physiological saline with the addition of 0.1% ascorbic acid [12]. By means of a special device [13], 250 µg of 6-HDA in 0.02 ml of the above-mentioned solution was injected through the needle of a syringe into the lateral ventricle of an experimental rat under superficial ether anesthesia. The same volume of solvent was injected into a control rat. Respiration of the experimental rats quickened to a marked degree and their excitability was increased. The rats were decapitated 7 days after the injection. The hypothalamus and pituitary gland (four experimental and four control rats) were fixed in Bouin's fluid. Paraffin sections were stained with paraldehyde-fuchsin by the Gomori–Gabe method and counterstained with Heidenhain's azan. "Functional" types of neurosecretory cells [3] were counted in SON, their nucleoli were measured, and the content of neurosecretory material on the bodies and axons of the cells of SON and in the posterior lobe of the pituitary was determined visually by means of a five-point system with an accuracy of 0.5 point [11]. By means of the Falk–Hillarp histochemical fluorescence method noradrenergic fibers were revealed in the region of SON and in the posterior lobe of the pituitary (five experimental and five control rats). The antidiuretic activity of pituitary suspensions of the experimental and control rats was compared (suspensions from three experimental and three control rats, tested on 12 rats).

RESULTS

After injection of 6-HDA into rats in the region of SON changes were observed compared with the control (Fig. 1). In the experimental series about 90% of the cells were palely stained, with a very low concentration of neurosecretory material, whereas in the control series such cells numbered about 70%. The dimensions of the nucleoli in the cells of SON were increased statistically significantly ($P < 0.001$; Fig. 1B). In the ventral part of SON there were numerous large expansions of processes of neurosecretory cells up to 18 µ in diameter, filled with neurosecretory material (Fig. 1B). Large expansions of fibers, devoid of neurosecretory material, were less frequently found. The numerous capillaries were dilated and packed with blood cells. Along the course of the supraoptico-hypophyseal tract, in the median eminence, the content of neurosecretory material was 1 point higher than in the control. In the posterior lobe, where fibers of SON cells terminate, the content of neurosecretion also was greater in the experimental series (4.8 ± 0.42) than in the control (4.1 ± 0.28). The presence of numerous large expansions of fibers distended with neurosecretory material and of considerably dilated capillaries, packed with blood cells, was characteristic. Testing for biogenic amines in the experimental series revealed a sharp decrease in the intensity of fluorescence of noradrenergic terminals in the region of SON cells (Fig. 2B) and the brightly

fluorescent varicose thickenings of noradrenergic terminals, charcteristic of the normal state, disappeared (Fig. 2A). In the posterior lobe of the pituitary, close to the pars intermedia, large and brightly fluorescent masses (up to 10 μ) were found; they may have been caused by accumulation of noradrenalin in the preterminal portions of the destroyed noradrenergic terminals.

To compare the antidiuretic activity of pituitary suspensions of the rats of the experimental and control groups experiments were carried out to study the effect of these pituitary suspensions on water diuresis in intact rats [1]. The value of α reflects the level of excretion of urine and the level of the diuretic response at the 90th minute after injection of the suspension against the background of water loading [1]. The value of α for intact rats was about 70%, but after injection of pituitary suspensions from control rats, α was about 30%, and after injection of pituitary suspensions from the experimental rats, α was 19.4%. After injection of 6-HDA a very small increase was thus observed in the antidiuretic activity in the posterior lobe of the pituitary.

The mechanism of action of 6-HDA is not yet clear. It has been suggested that 6-HDA damages the mechanisms of uptake and accumulation of noradrenalin and also acts on the enzymes of its biosynthesis, without affecting cholinergic terminals [6, 10, 12]. The increase in size of the nucleoli in the cells of SON in the presence of numerous palely stained cells with a very low content of neurosecretory material and the dilated capillaries are signs of increased secretory activity of the cells of SON after administration of 6-HDA. Increased production of neurosecretion in the bodies of the SON cells was accompanied by its outflow into the processes. After injection of 6-HDA a very marked decrease in the intensity of fluorescence of the noradrenergic terminals in the region of SON, activation of synthesis of neurosecretory material in the perikarya of the SON cells, and its outflow into the axons were thus observed, in association with some degree of inhibition of transport of peptide neurohormones along the axons and their secretion from the posterior lobe of the pituitary into the general circulation.

The noradrenergic innervation evidently has an inhibitory effect on the synthesis of peptide neurohormones by the cells of SON and participates in the regulation of transport of these hormones and their secretion from the posterior lobe of the pituitary into the general circulation.

LITERATURE CITED

1. M. Konstantinova, Z. Zellforsch., 83, 549 (1967).
2. A. L. Polenov and Yu. I. Senchik, Nature, 211, 1423 (1966).
3. A. L. Polenov, Hypothalamic Neurosecretion [in Russian], Leningrad (1968).
4. É. Kh. Priimak, Probl. Éndokrinol., No. 4, 50 (1974).
5. J. L. Barker, J. W. Crayton, and R. A. Nicoll, J. Physiol. (London), 218, 19 (1971).
6. G. Bartolini, J. G. Richards, and A. Pletscher, Experientia, 26, 142 (1970).
7. F. E. Bloom, S. Algeri, A. Gropetti, et al., Science, 166, 1284 (1969).
8. A. C. Cuello, W. J. Shoemaker, and W. F. Ganong, Brain Res., 78, 57 (1974).
9. K. Fuxe and T. Hökfelt, in: Aspects of Neuroendocrinology, Berlin (1967), p. 165.
10. G. Jonsson, K. Fuxe, and T. Hökfelt, Brain Res., 40, 271 (1972).
11. A. Jasinski and A. Skraba, Endokrinologie, 64, 339 (1975).
12. N. J. Uretsky and L. L. Iversen, J. Neurochem., 17, 269 (1970).
13. J. Vaňaček, V. Krebs, E. Scheer, et al., J. Am. Pharm. Ass. Sci. Ed., 49, 178 (1960).