EFFECT OF INJURY TO HYPOTHALAMIC NUCLEI ON MORPHOLOGICAL AND FUNCTIONAL STATE OF THE HYPOTHALAMIC–PITUITARY SYSTEM AND THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

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Injury to individual nuclei of the anterior and middle parts of the hypothalamus in rabbits by electrocoagulation (anode, 5 mA, 20-25 sec) through previously implanted electrodes was followed by changes in the morphological and functional state of the hypothalamic–pituitary neurosecretory system (HPNS). The closer the focus of injury to the region of the supraoptic nuclei, the higher the functional activity of HPNS. If injury to individual hypothalamic nuclei was combined with administration of an atherogenic diet, marked morphological and functional changes were detected both in HPNS and in the adrenals, but in animals with experimental atherosclerosis, this disease assumed a more severe form under these conditions than in animals simply receiving the atherogenic diet. The results of these experiments point to a close connection between the genesis of atherosclerosis and neurogenic factors.

KEY WORDS: *electrocoagulation; hypothalamic neurosecretory system; experimental atherosclerosis.*

Recent publications contain data to show that after mechanical injury to the hypothalamic nuclei the intensity of secretory activity of the neurosecretory nuclei changes [3].

Clinical and experimental investigations also have shown that traumatic lesions of the hypothalamic region of the brain have an activating effect on the manifestation of atherosclerosis [6, 10, 13]. However, no attempt has been made to investigate the neurosecretory system of the hypothalamus under these conditions. Yet the writers showed previously that the development of experimental atherosclerosis is closely bound with the level of functional activity of the hypothalamic neurosecretory system [4, 5].

With these considerations in mind, the object of the present investigation was to study the morphological and functional state of the hypothalamic–pituitary neurosecretory system (HPNS) and the character of development of experimental atherosclerosis in rabbits after localized injury to the hypothalamic nuclei.

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EXPERIMENTAL METHOD

Electrocoagulation of the hypothalamic nuclei was carried out with a steady current (anode, 5 mA, 20-25 sec) through electrodes implanted previously by a stereotaxic technique into the supraoptic (SO), paraventricular (PV), or ventromedial (VM) nuclei so that it was possible to study HPNS both after injury to nuclei forming part of that system and also after local injury to regions of the brain anatomically more distant from it.

The experimental animals began to receive an atherogenic diet in accordance with a modified N.N. Anichkov's method [12] two weeks after electrocoagulation of the hypothalamic nuclei. The control group included animals with electrocoagulation of the hypothalamic nuclei only and also animals receiving the atherogenic diet only, and intact animals. The atherogenic diet was given for 30 days.

The location of the foci of injury in the hypothalamus was determined histologically with reference to coordinates of the atlas of Sawyer et al. The morphological and functional state of HPNS was assessed in serial sections through the hypothalamus and pituitary stained by the Gomori—Maiorova method [7], by determining the ratio between "palely stained" (high activity) and "darkly stained" (resting state) cells in 200 cells counted in the SO nuclei [9], and by karyometry of their nuclei [14]. In addition, the presence of neurosecretory substances was studied in the hypothalamic—pituitary tract and neurohypophysis. Histological investigations of the thyroid and adrenal glands were carried out and their gravimetric indices determined. The severity of the experimental atherosclerosis was judged from the serum cholesterol level and by a study of structural changes in the main and intramural blood vessels by the methods of Goldman and Steedman. The atherosclerotic index of the severity of the lesion (AI) was determined in the aorta [1].

EXPERIMENTAL RESULTS

Foci of electrocoagulation damage were located bilaterally in the region of the SO nuclei at the level of sections A-1, A-2, and A-3, in the region of the PV nuclei at the level of sections A-1, and in the region of the VM nuclei at the level of sections AP-0 and P-1. Small cavities or foci of necrotic brain tissue, separated from unchanged areas of brain by collections of glial elements and large macrophages (granular spheres), were arranged at sites corresponding to the location of the electrode tip 45 days after electrocoagulation. However, in sections through the hypothalamus cut orally or caudally to the center of the focus of brain tissue damage, the neurosecretory cells of the SO and PV nuclei preserved their characteristic structure; this was evidence that electrocoagulation with the parameters specified above induced only partial injury and did not completely block the hypothalamic nuclei under investigation.

As the results of morphometric investigation of HPNS showed, no neurosecretion could be detected in animals on an atherogenic diet in the hypothalamic—pituitary tract and in the neurohypophysis. The SO nuclei contained both "palely stained" and "darkly stained" cells, but counting showed a decrease of 8% below normal in the number of "pale" cells (Fig. 1a, b). However, the absence of neurosecretory substances in the other components of HPNS showed that the function of this system as a whole was in a state of moderate activity. Under these conditions the weight of the thyroid glands was within normal limits but the weight of the adrenals and the blood cholesterol level were raised by 1.8 and 3 times, respectively, compared with normal and AI for the aorta was 2.6% (Table 1).

Different results were obtained in animals receiving the atherogenic diet combined with injury to the hypothalamic nuclei (experimental group). For instance, after administration of the diet coupled with injury to SO, PV, and also VM nuclei, predominantly "pale" cells in the active phase of the secretory cycle were found in the residual parts of the SO nuclei, and their number was 11, 8, and 6% respectively

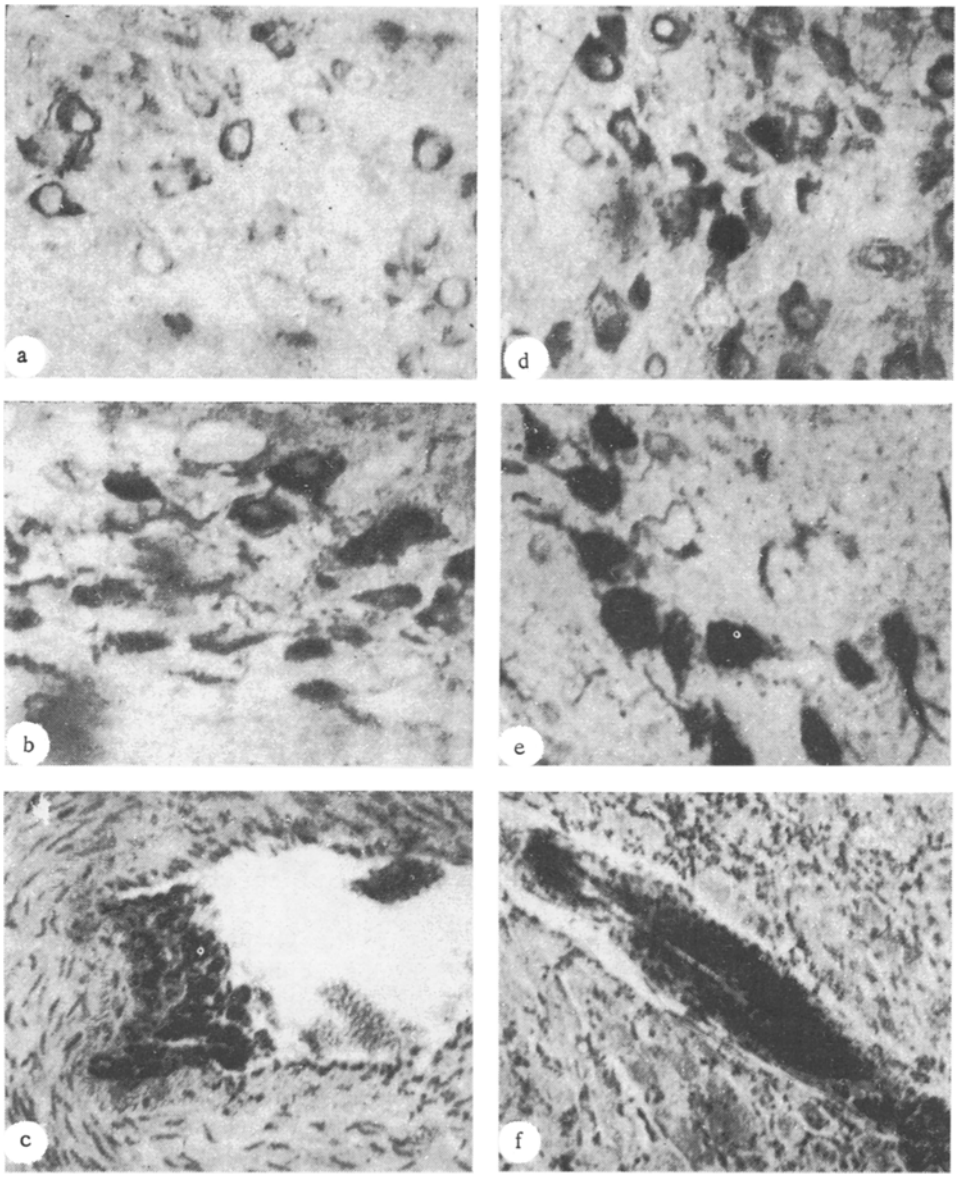

Fig. 1. Morphological changes in neurosecretory nuclei of the hypothalamus and blood vessels: a) supraoptic nucleus of hypothalamus of normal rabbit. Stained by Gomori—Maiorova method, 400 ×; b) supraoptic nucleus of hypothalamus of rabbit after receiving atherogenic diet for 30 days. Number of "dark" cells increased. Stained by Gomori—Maiorova method, 400 ×; c) atherosclerotic plaque in large artery of rabbit lung after administration of atherogenic diet + injury to VM nuclei of hypothalamus. Stained by Goldman's method, 140 ×; d) supraoptic nucleus of rabbit hypothalamus after administration of atherogenic diet + injury to SO nuclei. "Pale" cells with large nucleus predominate. Stained by Gomori—Maiorova method, 400 ×; e) paraventricular nucleus of rabbit hypothalamus after administration of atherogenic diet + injury to cerebral cortex. Deposition of neurosecretory substance in cells and their appendages. Stained by Gomori—Maiorova method, 400 ×; f) atherosclerotic changes in artery of rabbit myocardium after administration of atherogenic diet + injury to SO nuclei. Stained by Goldman's method, 140 ×.

higher than normal and 19, 16, and 14% higher than in animals receiving the atherogenic diet only. The most marked morphological and functional changes were found in the group "coagulation of SO nuclei + atherogenic diet." The volume of the nuclei of

TABLE 1. Changes in SO Nuclei, Endocrine Glands, and Aorta after Injury to Hypothalamic Nuclei + Administration of Atherogenic Diet

Character of experiment	Number of animals	Ratio of "dark" to "pale" cells in SO nucleus, %	Volume of cell nuclei in SO nucleus (in μ)	Weight (in mg) of		Blood cholesterol level (in mg %)	Atherosclerotic index of severity of aortic lesion
				thyroid gland	adrenal glands		
Normal	5	34—66	31,5±0,85	122,3±21,7	292,9±26,9	46,5±7,0	0
Coagulation of SO and PV	4	25—75	36,3±0,92	148,0±27,4	283,7±55,0	22,0±7,2	0
Coagulation of nuclei in middle part of hypothalamus	3	32—68	27,9±0,58	141,0±22,0	336,0±108,0	40,0±7,7	0
Atherogenic diet	5	42—58	30,0±0,68	85,6±5,5	545,0±165,0	143,0±83,0	2,6
Coagulation of SO nuclei + atherogenic diet	6	23—77	34,7±0,67	125,0±10,0	628,0±25,0	323,0±39,0	5,5
Coagulation of PV nuclei + atherogenic diet	3	26—74	20,0±0,62	98,6±5,4	560,0±59,0	182,0±40,0	0,5
Coagulation of VM nuclei + atherogenic diet	3	28—72	—	113±19,0	658,0±35,0	638,3±105,5	6,4
Coagulation of cortical brain structures + atherogenic diet	2	42—58	19,5±0,63	135,0	702,0±225,0	18,5±12,0	0

the neurosecretory cells was greater than normal and the perikarya were enlarged and contained only solitary granules of neurosecretion (Fig. 1d). No neurosecretion could be detected in the other components of HPNS, except in animals with injury to VM nuclei, in which the neurohypophysis contained single small Herring's bodies. The morphometric data as a whole indicated higher activation of HPNS function in the experimental animals than in the animals receiving the atherogenic diet alone. Under these conditions the weight of the thyroid gland did not differ significantly from normal, but the weight of the adrenals was increased, especially in rabbits receiving the diet together with injury of the SO and VM nuclei. In these cases the lipid content in the cells of the cortical layer was increased, morphological evidence of their reduced function. In the animals receiving the atherogenic diet and injury to both SO and VM nuclei the blood cholesterol level was increased by 2.2 and 4.4 times respectively, whereas AI for the aorta was increased by 1.9 and 2.4 times compared with animals on the atherogenic diet alone (Table 1). At the same time widespread atherosclerosis was found in the intramural blood vessels, including the vessels of the lungs and heart, which are known to be affected at later stages of the experiment when atherosclerosis is produced in rabbits in the usual way (Fig. 1c, f). Administration of the atherogenic diet together with injury to the PV nuclei led to a smaller increase in the blood cholesterol level and AI for the aorta (Table 1).

In two cases foci of electrocoagulation were found entirely within the cortical structures of the brain at the level of sections A-1; for that reason the animals re-

ceived the atherogenic diet in conjunction with brain injuries as indicated. In these animals all components of HPNS contained an increased amount of neurosecretion; large "dark" cells in a state of deposition of neurosecretion predominated not only in the SO, but also in the PV nuclei under these circumstances (Fig. 1e). The volume of the nucleus in these cells was increased by 1.6 times compared with normal and this, in conjunction with the other evidence, points to reduced function of HPNS. Although the weight of the endocrine glands was similar to their weight in the experimental animals, the cholesterol level in the blood and vessels was unchanged.

In control animals undergoing coagulation of the SO and PV nuclei, just as in the experimental animals (coagulation of the nuclei + atherogenic diet), the morphometric indices of HPNS indicated high functional activity of the system as a whole (Table 1). Under these conditions the weight of the endocrine glands varied, but the blood cholesterol level was within normal limits and the vessels showed no visible changes. This result was evidently due to compensatory, adaptive mechanisms developing in the animal in response to trauma.

Analysis of the experimental results showed that focal injury to the hypothalamus and other brain structures modify the morphofunctional state of HPNS. The nearer the foci of injury were located to the region of the SO nuclei, the higher the activation of HPNS function. However, after injury to the hypothalamic nuclei alone, the blood cholesterol level remained within normal limits throughout the experiment.

With a combination of injury to the hypothalamic nuclei and administration of the atherogenic diet morphological and functional changes developed both in HPNS and in the adrenals. Under these conditions it can be postulated that increased amounts of neurohormonal substances, including vasopressin [9], circulated in the body. Disturbance of the hormonal equilibrium led to the more rapid development of experimental atherosclerosis. The possibility likewise cannot be ruled out that injury to the hypothalamic nuclei, especially to VM nuclei, disturbed the central regulation of lipid-cholesterol metabolism [13, 15].

The results thus point to a close connection between the genesis of atherosclerosis and neurogenic factors.

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