EFFECT OF DIOSPONIN ON THE HYPOTHALAMIC NEUROSECRETORY SYSTEM AND THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

L. N. Lebedeva, L. P. Kobozeva, and L. N. Sokolova*

UDC 615.22:547.918].015.45:612.826,4+ 616.13-004.6-092.9

In rabbits simultaneously receiving an atherogenic diet and diosponin the functional activity of the hypothalamic-pituitary neurosecretory system is weakened, and this is accompanied by a decrease in the level of hypercholesteremia and in the severity of the vascular lesions by comparison with animals receiving exogenous cholesterol without diosponin. Restoration of the normal neuro-hormonal relations evidently inhibits the manifestation of atherosclerosis.

Recently diosponin has been used successfully for the treatment of organic atherosclerosis [8, 15]. It has a marked hypolipidemic action, it increases diuresis, lowers the arterial pressure, slows the heart, and restores normal sleep [8, 12, 15, 17, 19]; i.e., it acts on the autonomic functions whose regulation is closely linked with the hypothalamus and, in particular, with the structures of the magnocellular nuclei forming part of the hypothalamic-pituitary neurosecretory system (HPNS) [4, 6, 18, 23, 27, 28]. A connection between the character of the morphological and functional state of the HPNS and the severity of experimental atherosclerosis has been demonstrated previously [9-11].

In this connection it was decided to investigate the functional morphology of the HPNS during the treatment of experimental atherosclerosis with diosponin.

EXPERIMENTAL METHOD

Twenty male rabbits weighing 3 kg were used. Cholesterol was given to the animals of group 1 by Anichkov's method [3]. In group 2 the administration of exogenous cholesterol was combined with simultaneous administration of diosponin (10 mg/kg by mouth in aqueous solution). The animals of group 3 received diosponin alone in the same dose as in group 2. Group 4 consisted of intact rabbits. Observations continued for 30 days.

The neurosecretory nuclei of the hypothalamus (supraoptic – SO, paraventricular – PV) and the neurohypophysis were investigated in serial brain sections stained by Gomori's method in Maiorova's modification [14] and by Nissl's method. The functional state of the HPNS was evaluated by Polenov's scheme, based on determination of the quantity of Gomori-positive material in the neurosecretory cells, the hypothalamic-pituitary tract, and the neurohypophysis. The percentage of "pale" cells actively producing and secreting neurosecretion, and of "dark" cells, in a state of rest or beginning to synthesize secretion [16], was determined in the SO nuclei. The nuclei of the neurosecretory cells were measured [25]. The weight of the thyroid and adrenal glands of the animals was determined and the character of their struc-

*Deceased.

Laboratory of Experimental Pathomorphology, Institute of Normal and Pathological Physiology, Academy of Medical Sciences of the USSR. Laboratory of Pharmacology and Folk Medicine, All-Union Institute of Medicinal and Aromatic Plants. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 5, pp. 65-68, May, 1974. Original article submitted June 20, 1973.

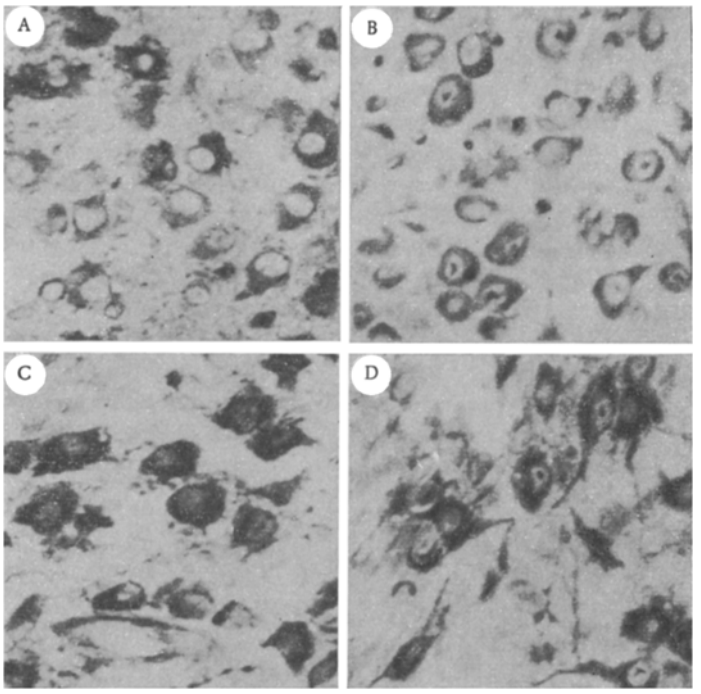

Fig. 1. Structure of neurosecretory cells of the supraoptic (SO) nucleus of the rabbit hypothalamus under normal conditions and after various treatments: A) cells of SO nucleus, normal; B) cells of SO nucleus on 30th day after administration of exogenous cholesterol; C) cells of SO nucleus on 30th day after combined administration of cholesterol and diosponin: accumulation of granules of neurosecretion in perikarya and processes; D) cells of SO nucleus on 30th day after administration of diosponin: accumulation of neurosecretory granules in cells and processes. Gomori–Maiorova method, 400×.

tural changes studied by the methods of Mallory and Goldman. Fatty infiltration and changes in the mucopolysaccharides in the ground substance were studied in the walls of the aorta and the blood vessels of the organs by the methods of Goldman and Steedman. The severity of atherosclerosis of the aorta was estimated [1] and the blood cholesterol level determined [22].

EXPERIMENTAL RESULTS

On the 30th day after administration of exogenous cholesterol, the neurosecretory cells in the PV and SO nuclei as well as in the intact rabbits still retained their oval shape and the perinuclear arrangement of the small granules of neurosecretion in the cytoplasm (Fig. 1A, B). However, the percentage of "pale" cells in these nuclei was a little higher than normally (Fig. 2). The volume of the nuclei of the neurosecretory cells was reduced from $46.5 \pm 1.7\ \mu^3$ in the intact rabbits to $31.3 \pm 0.3\ \mu^3$ ($P < 0.05$). No neurosecretion was present in the hypothalamic-pituitary tract or in the neurohypophysis. On the whole, therefore, the function of the HPNS could be described as increased [2, 5, 13, 16]. The weight of the adrenal and thyroid glands in the animals of this group was almost indistinguishable from normal, but the thyroid glands contained large follicles, filled with dense colloid, more often than normally. The blood cholesterol level was raised and the severity of the atherosclerotic lesions in the aorta was assessed as 21.3% . Microscopically, established atherosclerotic plaques were found in the aorta and in some cases lipidosis of the small intramural arteries was present.

After the combined administration of cholesterol and diosponin to the rabbits, the neurosecretory cells of both PV and SO nuclei were enlarged and their perikarya were completely filled with tiny granules of Gomori-positive material, which penetrated also into the processes arising from the cells (Fig. 1C). The number of "pale" cells was 27% below normal, and 32% below that found in the previous group (Fig. 2). The volume of the nuclei in the neurosecretory cells was reduced to $24.8 \pm 0.6\ \mu^3$ ($P < 0.05$). The hypothalamic-pituitary tract was filled with tiny granules of neurosecretion and, in addition, large Herring's bodies were found in the neurohypophysis.

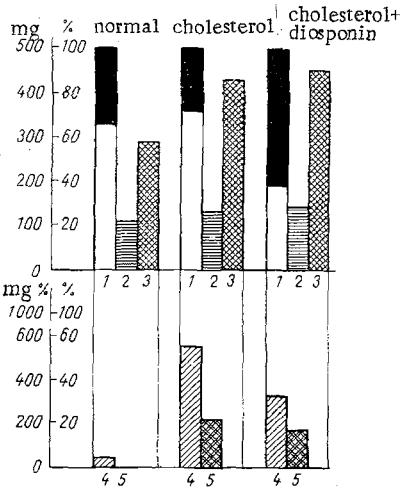

Fig. 2. Parameters of cholesterol metabolism and state of the endocrine system in the rabbits: 1) ratio of "pale" to "dark" cells in the SO nucleus of the hypothalamus (in %); 2) weight of thyroid glands (in mg); 3) weight of adrenals (in mg); 4) blood cholesterol (in mg %); 5) severity of atherosclerotic lesions in aorta (in %).

According to some investigators, increased accumulation of neurosecretion in the main components of the HPNS in conjunction with a decrease in size of the nuclei of the neurosecretory cells is a morphological criterion of reduced functional activity of that system [2, 5, 16]. The thyroid glands were indistinguishable from normal both structurally and in weight, whereas considerable accumulation of lipids occurred in the adrenals in the cells of the zona fasciculata. The blood cholesterol concentration was halved and the severity of the atherosclerotic lesions in the aorta was assessed at 1.3 times less than in animals receiving cholesterol alone (Fig. 2). The histological picture of the aorta was dominated by lipidosis, and established atherosclerotic plaques were found only in isolated cases.

Administration of diosponin was accompanied by considerable accumulation of Gomori-postive neurosecretory material in the hypothalamic-pituitary tract and neurohypophysis, and in the cells of the SO nuclei it filled the perikaryon and penetrated into the processes arising from the cells (Fig. 1D). The number of "pale" cells in the SO nuclei was reduced, while the number of "dark" cells was increased by 31% over normal. The volume of the nuclei of the neurosecretory cells was smaller than in the preceding groups, namely $22.1 \pm 0.6\ \mu^3$ ($P < 0.05$). The thyroid and adrenal glands in this group were indistinguishable both in weight and structurally from those of the intact animals. The blood cholesterol also remained within normal limits.

Administration of cholesterol to rabbits for 30 days thus led to an increase in the functional activity of the HPNS. Under these conditions an increased supply of biologically active substances, in particular vasopressin [2,7, 24, 26], into the general circulation could have occurred, with a resulting disturbance of neuro-hormonal relations [20, 21], and conditions favoring the development of hypercholesteremia and atherosclerosis of the blood vessels were created. Administration of diosponin, however, weakened the functional activity of the HPNS and this evidently contributed to the antiatherosclerotic effect of the preparation.

LITERATURE CITED

1. G. G. Avtandilov, Arkh. Pat., No. 11, 21 (1963).
2. B. V. Aleshin, Histophysiology of the Hypothalamic-Pituitary System [in Russian], Moscow (1971).
3. N. N. Anichkov, Trudy Obshch. Russkikh Vrachei SPb., 1, 80 (1912).
4. I. E. Ganelina et al., Lipid Metabolism and Atherosclerosis [in Russian], Leningrad (1965).
5. A. N. Golubitsa and L. I. Korochkin, Dokl. Akad. Nauk SSSR, 195, No. 6, 1439 (1970).
6. N. I. Grashchenkov, The Hypothalamus and Its Role in Physiology and Pathology [in Russian], Moscow (1964).
7. S. V. Zhukova, Arkh. Anat., No. 4, 26 (1969).
8. B. V. Il'inskii, Klin. Med., No. 1, 7 (1973).
9. L. N. Lebedeva et al., in: The Physiology and Pathology of the Circulation [in Russian], Moscow (1969), p. 14.
10. L. N. Lebedeva et al., in: General Neurophysiology and Experimental Pathology of the Nervous System [in Russian], Moscow (1970), p. 51.
11. L. N. Lebedeva and L. M. Chuvil'skaya, in: Proceedings of the 5th All-Union Congress of Pathological Anatomists [in Russian], Erevan (1971), p. 51.
12. V. Ya. Legchaev, Farmakol. i Toksikol., No. 5, 424 (1959).
13. L. A. Lushnikova, in: Atherosclerosis. Problems in Pathogenesis and Clinical Aspects [in Russian], Kazan' (1970), p. 12.
14. V. F. Maiorova, Arkh. Anat., No. 8, 101 (1960).
15. L. A. Myasnikov et al., Kardiologiya, No. 8, 26 (1971).

16. A. L. Polenov, Hypothalamic Neurosecretion [in Russian], Leningrad (1968).
17. L. N. Sokolova, Farmakol. i Toksikol., No. 1, 42 (1959).
18. A. V. Tonkikh, The Hypothalamic-Pituitary Region and Regulation of the Physiological Functions of the Body [in Russian], Moscow – Leningrad (1965).
19. A. D. Turova, Medicinal Plants of the USSR and Their Use [in Russian], Moscow (1967), p. 356.
20. A. M. Chernukh, Pat. Fiziol., No. 2, 3 (1972).
21. A. S. Shvarts, The Functional Pathology of Atherosclerosis [in Russian], Saratov (1969).
22. V. A. Éngel'gardt and L. G. Smirnova, Textbook of Clinical and Laboratory Investigations [in Russian], Moscow (1960).
23. W. Bargman, Verh. Anat. Ges. (Jena), 100, 30 (1954).
24. R. Barer et al., Proc. Roy. Soc. B., 15, 388 (1963).
25. J. Fischer and W. Inke, Acta Morphol. Acad. Sci. Hung., 7, 141 (1956).
26. F. La Bella and M. Sanwal, J. Cell. Biol., 25, 179 (1965).
27. H. Olivecrona, Acta Physiol. Scand., 40, Suppl. 136, 179 (1957).
28. E. Scharrer and B. Scharrer, Recent Prog. Hormone Res., 10, 183 (1954).