7. A. Berthelot, C. Desplan, F. Pernot, et al., Horm. Metab. Res. (Stuttgart), 10, 3 (1978).
8. J. M. Davidson and S. Levine, Annu. Rev. Physiol., 34, 375 (1972).
9. A. G. Gilman, Proc. Natl. Acad. Sci. USA, 67, 305 (1970).
10. M. D. Goldberg, in: Role of Cyclic AMP in Cell Function, New York (1970), p. 67.
11. N. Lavin, G. Rachelefsky, and S. A. Kaplan, Horm. Metab. (Stuttgart), 7, 253 (1975).
12. T. P. Lee and C. E. Reed, Biochem. Biophys. Res. Commun., 78, 998 (1977).
13. B. S. McEwen, J. M. Weis, and L. S. Schwartz, Nature, 220, 911 (1968).
14. J. Schmidtke, T. Wienker, M. Flügel, et al., Nature, 262, 593 (1976).

REGIONAL DIFFERENCES IN THE NORADRENERGIC AND CHOLINERGIC INNERVATION OF THE PIAL ARTERIES OF THE BRAIN

A. V. Sakharova

UDC 611.133.33:611.839

Considerable regional differences in the noradrenergic innervation of the pial arteries of rats were found histochemically (by the condensation reaction with glyoxal). The distal portions of the arteries (arterial bordering zones) were not innervated or were much less richly innervated then the proximal portions (the zones of supply of the individual vessels). Fibers detected by the reaction for acetylcholinesterase were more uniformly distributed. It is suggested that insufficiency of the noradrenergic innervation may be one factor responsible for the lower resistance of vessels of the arterial bordering zones to an acute rise of arterial pressure.

KEY WORDS: pial arteries; arterial bordering zones; noradrenergic innervation.

Experiments on animals showed that during an acute rise of arterial pressure compensatory constriction of the pial arteries does not arise everywhere: Some arteries are passively dilated and show signs of failure of the response of autoregulation of the cerebral blood flow. According to data obtained by Gannushkina and Shafranova [9], failure of the response of autoregulation of the cerebral blood flow followed by damage to the blood–brain barrier (BBB) and the brain substance, takes place chiefly and preferentially in zones of anastomosis between distal branches of the main arteries of the brain, which are known as arterial bordering zones [2]. The hypothetical sites for these pathological reactions are vessels running a straight course, vessels branching off at an acute angle, and end-to-end anastomoses [8]. To explain this uneven response the hypothesis of the role of the "geometry" of the arteries, which may determine the hemodynamics and so affect the intravascular pressure [1], has been suggested. Other conditions being the same, this may probably be the leading factor. But are other conditions the same? In particular, is there no difference in the innervation of those portions of the arterial system which differ in their responses? Publications in which regional differences in the adrenergic innervation of the pial and intracerebral vessels are described [4, 7, 12] do not give the answer to this question.

In this investigation the innervation of arteries was compared in those parts of the vascular system in which a normal autoregulatory response predominates and in those parts where the response of autoregulation of the cerebral blood flow is disturbed, i.e., in arterial bordering zones. Systems of nerve fibers revealed by staining for catecholamines and for acetylcholinesterase (AChE) were studied.

EXPERIMENTAL METHOD

The innervation of the pial arteries was studied in two-dimensional preparations of the pia mater taken from 20 noninbred albino rats. Biogenic amines were detected by the condensation reaction with glyoxal [13] in the writer's modification for membranous tissue. The rat brain was perfused through the left ventricle with

Laboratory of Experimental Pathology of the Nervous System, Research Institute of Neurology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR E. V. Shmidt.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 141-143, February, 1980. Original article submitted April 19, 1979.

0007-4888/80/8902-0105$07.50

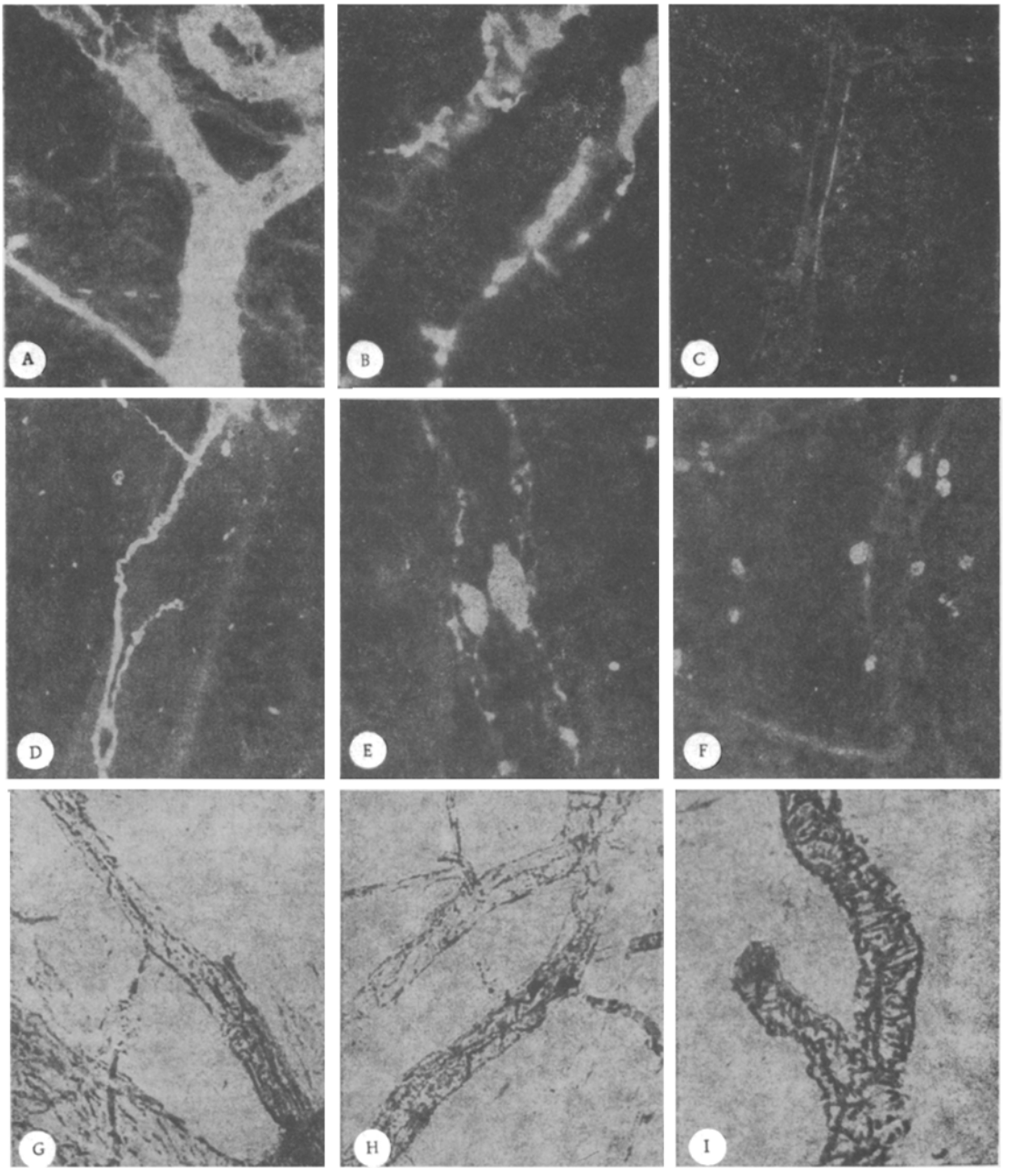

Fig. 1. Innervation of pial vessels of a rat. A-F) reaction for catecholamines and serotonin; G-I) reaction for AChE. A) proximal branches of middle cerebral artery close to circle of Willis. Diameter about 15, 40, and 50 μ. Objective 10, ocular homal 3; B) two second-order branches about 20 and 25 μ, leaving circle of Willis. Objective 40, ocular 3; C) artery about 20-30 μ in diameter from bordering zone supplied by anterior and middle cerebral arteries. Objective 10, ocular homal 3; D) distal part of vessel from territory of middle cerebral artery, solitary noradrenergic fibers. Objective 40, ocular homal 3; E) serotonin-containing mast cells in wall of vessel from arterial bordering zone (arrow). Objective 40, ocular homal 3; F) two arterial branches about 25 μ in diameter with anastomosis 20 μ in diameter in arterial bordering zone. Objective 40, ocular homal 3; G) proximal portions of middle cerebral artery. Objective 10, ocular 10; H) veins with nerve fibers and intramural nerve cells (arrow). Objective 20, ocular 10; I) vessel about 20 μ in diameter from arterial bordering zone. Objective 40, ocular 10.

the incubation solution for the histochemical reaction in order to prevent displacement of the biogenic amines, and to produce fixation of the brain tissue, thereby facilitating removal of the pial membrane. After removal of the brain, areas of the pia were separated from the territory of supply of individual arteries and from arterial bordering zones, immersed in incubation solution for 2-3 min, straightened out on slides, dried in a jet of warm air for 15-20 min, heated in a thermostat at 80°C for 10-15 min, mounted in mineral oil, and again heated for 15 min. The preparations were studied in the luminescence microscope with a standard set of filters. AChE was detected by the reaction of Karnovsky and Roots [10].

EXPERIMENTAL RESULTS AND DISCUSSION

Noradrenergic nerve fibers formed dense plexuses on the vessels at the base of the brain and their main branches supplying the brain (Fig. 1A, B). With increasing distance from the circle of Willis the intensity of innervation gradually diminished, and in the most distal parts only one or two fibers could be seen in each, and in arterial bordering zones nerve fibers of this chemical type were totally absent (Fig. 1C, E, F). Nerve fibers or plexuses accompanied vessels lying both on the brain surface and in its parenchyma. Comparison of blood vessels of different diameters lying in arterial bordering zones and in the territories supplied by the main arteries of the brain revealed sharp differences in their noradrenergic innervation: intensive innervation of blood vessels in the territory supplied by the main arteries (Fig. 1A, C, D) and no innervation in the arterial bordering zone (Fig. 1C, E, F). Veins in the proximal part were sparsely innervated, and in arterial bordering zones they were completely uninnervated, although serotonin-containing mast cells were most frequently present on them (Fig. 1E).

Fibers giving a reaction for AChE, like noradrenergic nerve fibers, formed plexuses consisting of six bundles on the main arteries of the brain. As the vessels branched the bundles broke up and some fibers from the thicker bundles could be traced along vessels of subsequent orders. This pattern was repeated many times, and arteries most distant from the sources contained one or two fibers. On vessels located in arterial bordering zones innervation also was present, for single fibers or thin plexuses of fibers were found along both arterial and venous branches, and also on anastomoses connecting branches of arteries of different territories, and on arterial and venous branches of the smallest caliber (Fig. 1G, H, I).

This investigation revealed considerable differences in the intensity of the noradrenergic innervation of the proximal and distal portions of the pial arteries. The intensity of innervation was found to depend not so much on the diameter of the vessel, as was hitherto considered, as on its remoteness from the circle of Willis. The arterial neighboring zones – the most distal portions of each of the main arteries of the brain – had the scantiest noradrenergic innervation. The question posed at the beginning of this paper, namely whether other conditions are the same, can thus be answered by saying that using the noradrenergic innervation as a sign, the conditions are not the same in the scale of the arterial system of the whole surface of the brain, whereas different areas within arterial bordering zones are under relatively identical conditions.

Fibers giving a reaction for AChE were found on vessels in arterial bordering zones and on the proximal portion of vessels.

The noradrenergic and cholinergic innervation is the source of opposite (constrictor and dilator) influences on the tone of the muscular wall of the brain arteries [6, 11]. Constrictor responses to an acute rise in arterial pressure are aimed at stabilizing the cerebral blood flow and maintaining the integrity of the vessel wall. The fact that failure of the response of autoregulation and damage to the BBB takes place primarily in arterial bordering zones in response to an acute rise of arterial pressure can be explained by the absence or deficiency of the local source of vasoconstrictor substance (noradrenalin), which is present on arteries of similar diameter in the proximal portions. The deficiency of the noradrenergic innervation of the arteries in arterial bordering zones can thus be regarded as an additional factor, besides the hemodynamic factor, responsible for the lower resistance of the vessel wall to an increase in intravascular pressure.

The presence of a cholinergic innervation in the arterial bordering zone evidently does not ensure more favorable conditions when the arterial pressure falls, for in response to a fall in arterial pressure failure of the response of autoregulation and damage to the BBB also arise in the arterial bordering zone [1]. In this case the pathological reaction can be explained on the ground that acetylcholine acts as a vasodilator only in the presence of adequate tone of the smooth muscles of the vessels [14], which is not the case when the pressure falls.

The noradrenergic and cholinergic innervation is only part of the chemically specific innervation system participating in the formation of vascular tone known at the present time [3], and a further study of regional differences in the innervation of the cerebral vessels must include their histochemical identification.

LITERATURE CITED

1. I. V. Gannushkina, V. P. Shafranova, and T. V. Ryasina, Functional Angioarchitectonics of the Brain [in Russian], Moscow (1977).
2. B. N. Klosovskii, The Circulation of the Blood in the Brain [in Russian], Moscow (1951).
3. J. Cervos-Navarro and F. Matakas, Neurology, 24, 282 (1974).
4. L. Edvinsson, Acta Physiol. Scand., Suppl. 427, 35 (1975).
5. I. V. Gannushkina et al., Acta Neurol. Scand., 56, Suppl. 64, 392 (1977).
6. I. V. Gannushkina and V. P. Shafranova, in: Cerebral Vascular Diseases, Stuttgart (1975), p. 61.
7. M. Karnovsky and L. Roots, J. Histochem. Cytochem., 12, 219 (1964).
8. R. E. Purdy and J. A. Bevan, Stroke, 8, 82 (1977).
9. J. C. De la Torre and J. W. Surgeon, Histochemistry, 49, 81 (1976).

ULTRASTRUCTURAL AND FUNCTIONAL CHANGES IN RAT BRAIN SYNAPTOSOMES DURING ELECTRICAL STIMULATION IN VITRO

V. I. Rodina, L. K. Salata, V. V. Rozhanets, O. M. Pozdnyakov, and R. N. Glebov

UDC 612.823.5.014.424

Electrical stimulation of a suspension of rat brain synaptosomes leads to significant Ca^{++}-dependent liberation of endogenous noradrenalin and to a Ca^{++}-dependent increase in its concentration in the synaptosomes themselves. Cyclic nucleotide phosphodiesterase activity is lowered significantly under these same conditions. No disturbance of synaptosomal ultrastructure is found during stimulation. An increase in the number of electron-dense synaptosomes is observed.

KEY WORDS: ultrastructure of synaptosomes; electrical stimulation; secretion of noradrenalin; phosphodiesterase.

Synaptosomes (isolated nerve endings), isolated from different parts of the CNS of animals, are convenient objects for the neurochemical study of synaptic processes. During depolarizing procedures in vitro – electrical stimulation (ES), an increase in the K^+ concentration, addition of veratrine and ouabain – biochemical changes associated with the Ca^{++}-dependent liberation of neurotransmitters contained in the synaptosomes take place [1, 6].

The object of this investigation was to study the effect of ES of a suspension of synaptosomes on their ultrastructure and also on functional indices such as the rate of Ca^{++}-dependent liberation of noradrenalin (NA) and cyclic nucleotide phosphodiesterase (PDE) activity.

EXPERIMENTAL METHOD

Synaptosomes were isolated from rat brain without the cerebellum by the method described previously [3]. After sedimentation in modified Krebs–Ringer solution (104 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 1.3 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 10 mM glucose, 20 mM Tris-HCl buffer, pH 7.6, at 37°C) the synaptosomes were suspended in a fresh portion of the same solution (1-3 mg protein/ml and preincubated (15 min, 37°C) with agitation by means of a magnetic stirrer. Part of the suspension was left under the same conditions during ES (control), the rest was subjected to ES by means of platinum ring electrodes at 37°C and with constant

Laboratory of General Pathology of the Nervous System, Laboratory of Experimental Pathomorphology and Ultrastructural Characteristics of Pathological Processes, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 143-145, February, 1980. Original article submitted October 3, 1978.

0007-4888/80/8902-0108$07.50