DISTRIBUTION AND ACTIVITY OF CHOLINESTERASE IN NERVE STRUCTURES OF THE PIAL ARTERIES: HISTOCHEMICAL INVESTIGATION

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UDC 612.824.3.015.14.014.1

It was shown by the Koelle–Gomori histochemical method that arteries of the pia mater in rabbits, cats, and rats are innervated by nerve fibers containing specific cholinesterase. The number of bundles of nerve fibers containing the enzyme was counted in the periarterial plexuses of pial vessels of different diameters, and the pattern of their distribution is described.

The pial arteries are the principal vascular mechanism for maintaining an adequate blood supply to the cerebral cortex. When the metabolic requirements of the cortex are increased (for example, during a marked increase in its activity or after interruption of its blood supply for a short time) the pial arteries regularly dilate, whereas the smaller arteries and arterioles in the cortex actually constrict slightly [5, 6]. There is physiological evidence of the existence of a nervous mechanism of functional dilatation of the pial arteries [4], but no humoral mechanism has yet been demonstrated [8]. From this point of view it was important to study the cholinergic nerve structures in the walls of the pial arteries which could posible participate in the mechanism of their functional vasodilatation.

The object of the present investigation was to examine the nerve plexuses in the walls of the pial arteries for cholinesterase and to investigate these nerve structures in pial arteries quantitatively in relation to location and caliber.

EXPERIMENTAL METHOD

The main investigations were carried out on 15 adult rabbits. For comparison the pial arteries were studied in cats (5 animals) and the mesenteric and pial arteries in albino rats (5 animals). True (specific) and nonspecific cholinesterase were demonstrated in the nerve structures of the arterial walls (of the basilar and middle and posterior cerebral systems), mainly in total preparations of the pia. The Koelle histochemical method as modified by Gomori was used. Control experiments were performed with butyrylthiocholine iodide and DEP [14]. To demonstrate the global pattern of innervation of the pia and to compare it with the histochemical data, the same vascular regions were stained with methylene blue by Shabadash's method [15].

The number of nerve structures of the pial arteries in the histochemical preparations of the rabbit pia was counted every 0.5 mm along the arteries. In each preparation all branches of the pial arteries were investigated except when the vessels were wrinkled. The number of longitudinal and transverse bundles of nerve fibers was counted in the periarterial plexuses. Bundles of fibers were regarded as longitudinal if they intersected the cross section of the pial arteries at an angle of between 0 and 45° to their axis, and transverse if they did so (often as segments not less than 20 μ in length) at an angle of between 45 and 90° to the axis. For statistical analysis the investigated arteries were divided into groups depending on their diameter: 400-700, 250-400, 150-250, 100-150, 60-100, and 30-60 μ (the diameter was measured by an ocular micrometer). This method was used to investigate more than 300 segments of pial arteries of different caliber.

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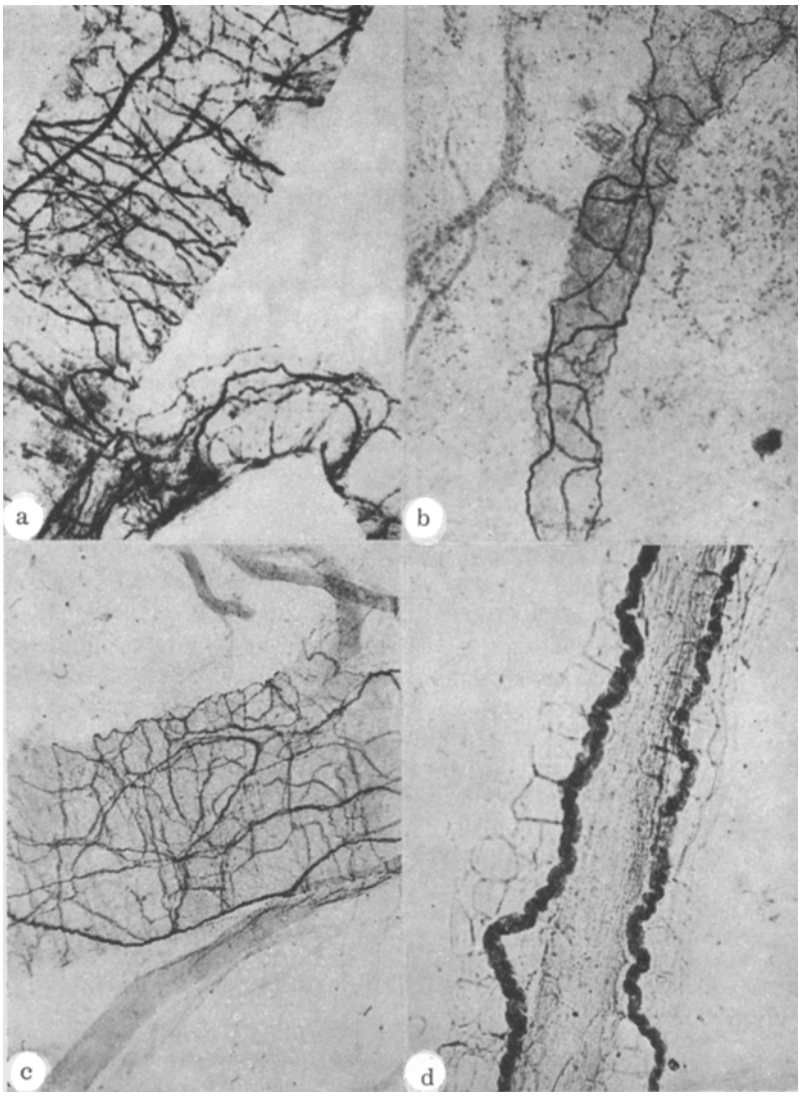

Fig. 1. Nervous plexus containing active acetylcholinesterase in the walls of the pial arteries. a) Posterior cerebral artery of a rabbit 600 μ in diameter (100 ×); b) middle cerebral artery of a rabbit 200 μ in diameter (100×); c) posterior cerebral artery of a rat 400 μ in diameter (70 ×); d) mesenteric artery of a rat 200 μ in diameter from the same experiment as (c): the periarterial plexus does not contain active acetylcholinesterase, which is found only in branches of the mesenteric nerves running at the periphery of the vessel (70×).

EXPERIMENTAL RESULTS

The pial arteries of rabbits, cats, and rats are innervated by nerve fibers possessing a large amount of active specific cholinesterase. No nonspecific cholinesterase could be detected in these nerve fibers.

Along the course of the main trunks of the basilar and posterior and middle cerebral arteries two plexuses were detected: superficial and deep. The main trunks of nonmedullated nerve fibers containing active enzyme run in the superficial layers of the adventitia along the vessel wall. Among them solitary medullated sensory fibers not possessing active enzyme can be seen. The main trunks give off thinner bundles of nerve fibers which form a periarterial plexus. At the point of division of the basilar artery

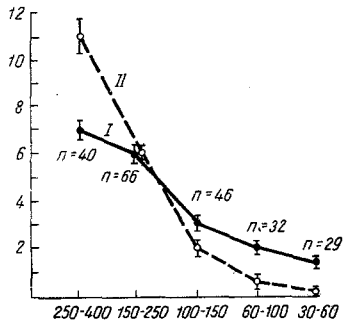

Fig. 2. Relative numbers of longitudinal (I) and transverse (II) bundles of nerve fibers containing acetylcholinesterase in the walls of the pial arteries of different caliber (branches of the middle and posterior cerebral arteries) of the rabbit (arithmetical mean values and their mean errors are given). Abscissa, caliber of arteries (in μ); ordinate, number of bundles.

into the cerebral arteries, collections of neurons evidently of parasympathetic nature, rich in active enzyme, are found in the pia. The axons of these neurons participate in the formation of vascular plexuses.

Bundles of nerve fibers of the superficial adventitial plexus break up into thinner trunks and separate fibers, which penetrate into the depth of the adventitia and form a deep plexus at the border with the muscular coat of the artery. The participation of these nerve structures in the innervation of the muscular coat of the vessels could not be demonstrated histochemically. However, in preparations stained with methylene blue the spread of very thin nerve fibrils of the terminal plexus among the muscle cells of the superficial layer of the media could be seen. In the adventitia of the arteries of large caliber (400-700 μ) the network of nerve fibers was especially dense (Fig. 1, a). With a decrease in caliber of the pial vessels (400-200 μ or less) the number of nerve elements in the periarterial plexus gradually decreases (Fig. 1, b).

Counts of the number of nerve fibers exhibiting active acetylcholinesterase in different segments of the pial arteries of rabbits showed that they are more numerous in the periarterial nerve plexus of the basilar artery than in the corresponding plexus of the middle and posterior cerebral arteries. The later plexuses have about equal numbers of nerve structures innervating them. Analysis of the preparations showed that arteries of large diameter (over 100 μ) contain both longitudinal and transverse bundles of nerve fibers. As the caliber of the vessel decreases, the number of transverse fibers falls faster than the number of longitudinal, and as a result the very small pial arteries (less than 100 μ) contain mainly longitudinal bundles of nerve fibers (Fig. 2). This ratio between the number of nerve structures of the periarterial plexus and the diameter of the vessel is easily explained by the fact that with a decrease in the diameter of the arteries the number of muscle cells in their wall decreases correspondingly.

Nerve fibers in the region of the walls of the pial arteries have been found by many histologists [1, 2, 16]. More recently it has been shown that numerous nerve plexuses containing noradrenalin, i.e., adrenergic in character, are present in this region [9], while the present investigation demonstrated the presence of nerve fibers containing active acetylcholinesterase (evidently cholinergic in character). From the standpoint of the functional significance of these adrenergic and cholinergic nerve structures their numerical ratio is important. Considerably fewer adrenergic fibers are contained in the walls of the pial arteries than in the arteries of other parts of the body, for example of the mesentery [10, 11]. This corresponds to physiological data: sympathetic control over the pial arteries is comparatively weak [1, 13], while over the mesenteric arteries it is well marked [12]. So far as nerve fibers containing acetylcholinesterase are concerned, under the same experimental conditions (on the same animals, with identical times of incubation, in the same substrate), highly active acetylcholinesterase was found in the perivascular plexus of the pial vessels (Fig. 1, c) but not in the nerve structures in the walls of the mesenteric arteries (Fig. 1, d). All these facts correspond to the principles governing the functional behavior of the pial arteries: vasodilatation is their typical reaction under various physiological and pathological conditions and constriction is observed more rarely [7].

The relative abundance of cholinergic nerve fibers in the walls of the pial arteries is a new fact giving evidence of a nervous mechanism of the functional vasodilation of these vessels, as some earlier experiments have indicated [3, 4].

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