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A histochemical study on the innervation of the cerebral blood vessels in the carp

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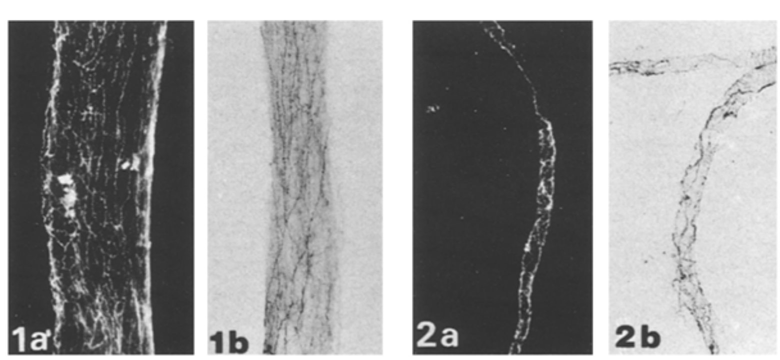
Summary. Adrenergic and cholinergic innervation of the cerebral blood vessels were studied in the carp using fluorescence and cholinesterase histochemistry. Most of the major cerebral and pial arteries were densely innervated with both nerves. Moreover, both nerves, being probably of central origin, were observed frequently on the walls of parenchymal arterioles and capillaries in the sections of the inferior lobe but were only rarely found in the other brain sections.

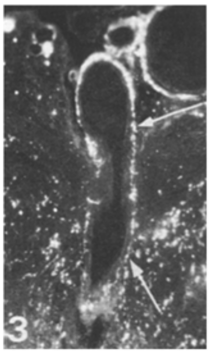
In the visceral and cardiovascular systems of vertebrates, the adrenergic fibres are a relatively late step in the vertebrate evolution[1]. However, recent comparative histochemical studies on innervation in the cerebral blood vessels support the assumption that the evolutionary trend for the autonomic nerves in the cerebral blood vessels is a unique one[2-7].

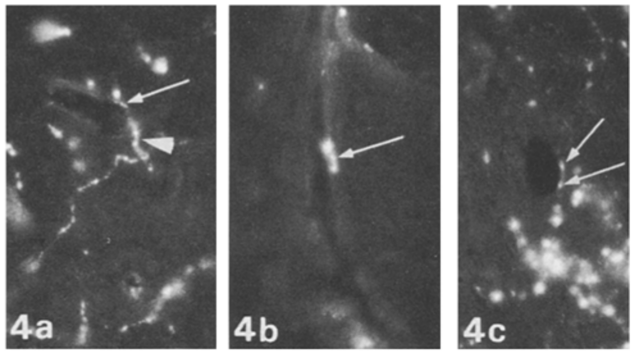
Material and methods. 35 carps, *Cyprinus carpio*, were used in this study. For whole mount preparation, the cerebral arteries were stretched on glass slides for fluorescence histochemistry, or were fixed with 4% formaldehyde buffered to pH 7.4 for 20 min for cholinesterase histochemistry. Small blocks of brain, frozen in dry-ice isopentane, were treated according to the freeze-drying or cryostat technique in order to demonstrate the innervation in the parenchymal vessels. For demonstration of adrenergic nerves, air-dried or freeze-dried materials were treated in formaldehyde vapor from paraformaldehyde for 1 h at 80 °C.[8] As for the cholinergic innervation, formaldehyde-fixed whole mount preparations or cryo-cut sections (20 µm) were maintained in substrate (acetylthiocholine-iodide)-free Karnovsky's medium[9] for 30 min at 4 °C, and then incubated in the complete medium, containing 2×10^{-4} M iso-OMPA as an inhibitor of nonspecific cholinesterases, for 1 h at 20 °C.

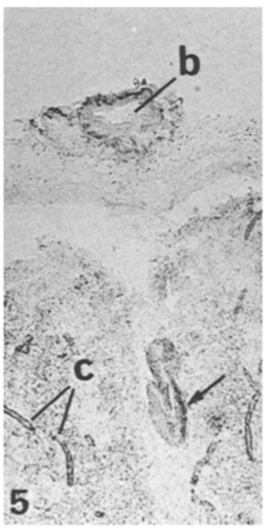
Results. Although the nerve fibres were rather fine as compared with those found in the other animals, most of major cerebral and pial arteries were innervated with dense adrenergic nerve plexuses (figure 1, a). Even the small pial arteries, especially those on the optic lobe, were also densely innervated with adrenergic fibres (figure 2, a). Although the AChE-activity of the nerve fibres was weak in comparison with that in mammals, the cholinergic nerve plexuses in those arteries showed almost the same density as those of adrenergic (figures 1, b and 2, b). The parenchymal arteries just penetrating into the brain parenchyma were accompanied by the adrenergic nerve plexuses (arrows in figure 3) continuing with those of the pial arteries. Moreover, the adrenergic nerve fibres and varicosities could be seen on the walls of arterioles and capillaries (arrows in figures 4, a–c), and a direct connection by the adrenergic nerves between the brain parenchyma and the walls of those small parenchymal vessels was sometimes observed (arrowhead in figure 4, a). This feature of the adrenergic innervation in the small parenchymal vessels was found frequently in the cross sections of the inferior lobe, but only rarely in the other brain sections. Some of the penetrating arteries within the brain parenchyma were also accompanied by the cholinergic nerves (arrow in figure 5). The cholinergic innervation in the parenchymal arteries, however, was much scarcer than that in the pial arteries of the same diameter and than the adrenergic innervation in the parenchymal arteries. The AChE-positive nerves contacting with the arterioles and the capillaires



Figs. 1 and 2. The adrenergic (a) and cholinergic (b) innervation in the posterior ramus of cerebral carotid artery (1) and the small pial artery on the optic lobe (2). a×67; b×64.







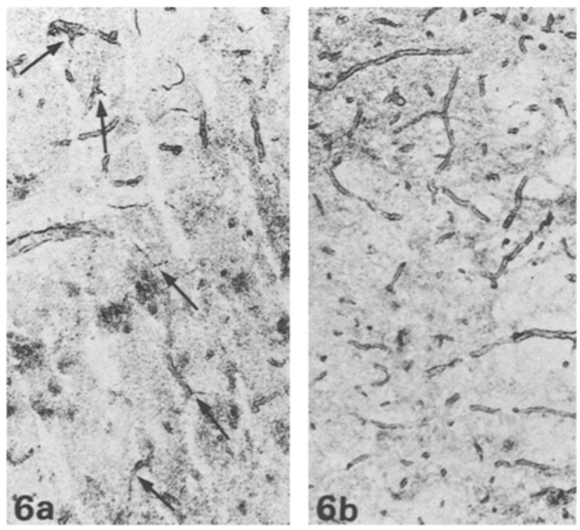


Fig. 3. Photograph showing that the parenchymal artery just penetrating into the brain parenchyma is accompanied by the adrenergic nerve plexuses (arrows) continuing with those of the pial artery. × 67.

Fig. 4. Photographs showing the central adrenergic innervation in the arterioles (arrows in a, b) and in the capillary (arrow in c). Note direct connection by the adrenergic fibre between the brain parenchyma and the arteriole wall (arrowhead). × 396.

Fig. 5. Cross section of the basilar artery (b) and the medulla oblongata. Arrow indicates the cholinergic nerve fibre in the parenchymal artery. The walls of the parenchymal small vessels, especially those of capillaries (c), exhibit diffusely the AChE-activity. × 100.

Fig. 6. Cross sections of the inferior lobe (a) and the medulla oblongata (b). Note that the AChE-positive nerve fibres contacting with capillaries (arrows) are observed frequently in the cross section of the inferior lobe but rarely in the other section. a × 96; b × 75.

were also observed frequently in the cross sections of the inferior lobe (figure 6, a) but rarely in the other sections (figure 6, b), as in the case of the adrenergic innervation. The walls of almost all parenchymal small vessels exhibited diffusely AChE-activity, and it was the greatest in the walls of capillaries (figures 5 and 6).

Discussion. Although it has for a long time been a controversial problem whether the cerebrovascular bed is under neural influence or not, a rich innervation in the cerebral arteries of carp, the walls of which are rather thick, indicates that the neural elements in the cerebral arteries may have some important effects on the cerebral blood flow (CBF). It is well-known that the cerebral blood vessels of several species receive a dual adrenergic and cholinergic innervation. The plexuses of perivascular cholinergic nerves are as well-developed as those of adrenergic in some mammalian species[10]. The cholinergic innervation, however, declines in density and also in AChE-activity as the species become less evolutionary advanced. The cholinergic nerve plexuses are not so rich as the adrenergic ones in the snake[4]. Those in the turtle, the lowest species in reptiles, are considerably less than the adrenergic nerve plexuses[3], and a similar disproportional development of both nerve plexuses is also observed in the cerebral arterial system of the domestic fowl[5]. Finally, the cerebral blood vessels of bullfrog are innervated solely with the adrenergic nerve plexuses[6], and also solely with the serotonergic nerves in the lamprey[7]. In the present study on the carp, however, the cholinergic nerve plexuses showed approximately the same density as the adrenergic ones, though the AChE-activity of nerves were not so great as in mammals. This suggests that the carp is a rather well-evolved species as regards the cerebral vascular innervation. It seems that the connections of the adrenergic and the AChE-positive fibres with the walls of parenchymal arterioles and capillaries represent the existence of a dual central innervation on the parenchymal small vessels. In fact, a dual central innervation on the parenchymal small vessels of the rat was demonstrated electron microscopically[11,12]; and the existence of central adrenergic[13,14] and cholinergic[15] control of the CBF was suggested as well.

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