Innervation of Brain Intraparenchymal Vessels in Subhuman Primates: Ultrastructural Observations

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SUMMARY  Sympathetic innervation of intraparenchymal blood vessels in the basal ganglia was demonstrated by transmission electron microscopy in arteries, arterioles, and capillaries of the subhuman primate brain. Small arteries (40–120 μm) and some arterioles (12–40 μm) are innervated only at branching sites. However, arterioles occasionally may be innervated at points distal to their origin. Capillary innervation was very infrequently observed.

IT IS CURRENTLY WELL accepted that there are, in several animal species, adrenergic and cholinergic fibers in the tunica adventitia of the subarachnoid (or pial) vessels of the brain. The extent and type of this innervation of the intraparenchymal vessels is less clear. Nerve fibers and nerve endings in the adventitia of intraparenchymal brain vessels have been demonstrated by transmission electron microscopy in the cat, and the rat, but not in subhuman primates. Establishing the existence and nature of vascular innervation to the brain vessels in the subhuman primate may be of considerable interest because (a) electrical stimulation of sympathetic fibers under normal conditions produces a significant decrease in cerebral blood flow in the cynomolgous monkey while in other laboratory animals, the response to sympathetic stimulation is less pronounced, and (b) a comparable response to that of the monkey’s has also been reported in humans following stimulation of the cervical sympathetic chain.

The two main objectives of this study were: (1) To determine the type and extent of nerve fibers in intraparenchymal brain blood vessels of the subhuman primate, and (2) to determine if there are any anatomical differences between the nerve fibers and nerve terminals described in other species (ie: cat, rat, rabbit) and those observed in the subhuman primate.

Materials and Methods

Two male hypertensive and two male normotensive cynomologus monkeys (Macaca fascicularis) with an average body weight of 5.5 kg, were used in this study. All four animals are part of a larger study designed to evaluate the effects of chronic hypertension on the large cerebral arteries. Detailed data on hypertension are not included in this study. This information will be reported later in a separate publication. Because of the small number of animals studied, no attempt was made to detect possible changes in the nerve fibers attributable to the effects of hypertension. In vivo fixation of monkey brains was completed under general anesthesi (nembutal 30 mg/kg) via vascular perfusion through the thoracic aorta at a pressure equivalent to the animals’ mean arterial blood pressure. A rinsing solution of heparinized physiologic saline was circulated before fixation. The fixative solution is composed of a combination of 1% glutaraldehyde and 2% paraformaldehyde in a 0.15 M phosphate buffer at a pH of 7.4. All cerebral hemispheres were serially cut along the horizontal plane; slabs of tissue from both cerebral hemispheres were placed on an Oxford model G vibratome and 1200 μm thick sections from the basal ganglia were generated. The specimens obtained in this manner were then processed and embedded in epon/araldite; blocks were cut at one micron on an LKB ultramicrotome and sections were stained with toluidine blue. After locating at least two small intraparenchymal arteries in each brain, serial sections (50 nm in thickness) were cut in the direction of the distal branches of the lenticulo striate arteries. Every tenth section was evaluated so as to examine a length of approximately 1 mm in each vessel. Examination of vascular innervation was completed with a Philips 400 electron microscope. A total of 32 branching sites containing nerve terminals were examined.

Observations

No significant differences were noted between the two groups of monkeys, i.e.: normotensives and hypertensives; however, two animals in each group may not generate a sufficiently large number of samples to warrant valid comparisons.

Nerve fibers are not found in the tunica media of intraparenchymal vessels. Unmyelinated adrenergic nerve fibers accompany small penetrating arteries and travel within the arterial tunica adventitia for long distances (approximately 80 μm) before reaching a branching site (fig. 1a). The nerve fibers are embedded in Schwann cell cytoplasm and each nerve fiber contains 10–40 axons varying in diameter from 180 to 500 nm (figs. 1b & 1c). The axons are associated with basement membrane presumably derived from the Schwann cell; some axons contain a large central mitochondrion and all contain abundant neurofilaments and microtubules (fig. 1c). Dense core vesicles (30–50 nm) typical of those which are known to contain catecholamines by fluorescence histochemistry are found only at branching sites (fig. 1d). As the nerve fiber approaches a branch it begins to enlarge until it reaches a diameter equal to approximately 5 times its original...
diameter (fig. 2a). The nerve terminals are located in a cleft between the parent vessel and its branch and are surrounded by Schwann cell processes and fibroblasts; the latter form a bridge between the two vessels (fig. 2b). The nerve/muscle separation ranges between 1100 and 2300 nm. In addition to the adrenergic nerve terminals, two nerve terminals which resemble the afferent terminal described in blood vessels from human myocardium were observed at arterial branching sites (fig. 2c). Afferent nerve endings have not been described previously in association with intraparenchymal vessels, but such endings were observed in pial vessels by Burnstock.

Intraparenchymal arterioles are usually innervated at their origin but they may occasionally have nerve terminals on a segment of the vessel located at variable distances from the point of bifurcation. These nerve terminals are confined to the tunica adventitia and approach the vascular smooth muscle as close as 80 nm (Fig. 2d). The nerve fibers are separated from smooth muscle tissue of the tunica media by the basement membrane of the smooth muscle cells. These arteriolar nerve terminals are identical in structure to the adrenergic terminals found at branching sites.

Almost all arteries were surrounded by nerve fibers; a minority of arterioles had scanty adventitial nerve fibers. The vessels whose pattern of innervation is reported here were selected for the study because of their nerve fibers. Only arterioles in the basal ganglia were studied in detail (i.e., through serial sections) in this portion of the study; patterns of innervation similar to those existing in the basal ganglia have been casually noted in arteries and arterioles from other locations, e.g.: the cerebral cortex.

Capillary innervation was observed in only two of the many capillaries examined. Both innervated vessels had a diameter of 8 μm and contained a single layer of endothelial cells surrounded by a pericyte. Dense core vesicles apparently free of limiting membranes were embedded in the basement membrane of the capillaries and approached the pericyte cytoplasm as close as 15 nm (fig. 3a & 3b).

Comments

Evidence for the autonomic innervation of extraparenchymal and intraparenchymal vessels of the brain in humans and some other mammals has been derived from a number of physiologic investigations. The role of neurogenic vasomotor mechanisms in the regulation of cerebral blood flow (CBF) is still unclear. The caliber of the adrenergic nerve fibers and their nerve terminals as described in this
INNERVATION OF INTRAPARENCHYMAL BLOOD VESSELS/Briggs et al

FIGURE 2. a. Arterial branching site from the putamen of an adult monkey. The nerve fiber contains many enlarged ovoid-shaped nerve endings. (12,500 ×) b. A vascular branching site from the putamen of an adult monkey. The nerve terminals (arrow) are located between a small artery (VI) and an arteriole (V2). (4600 ×) c. Afferent nerve terminal at an arterial branching site in an adult monkey. The afferent nerve endings are enclosed in a capsule-like structure; the fibers contain large dense inclusion bodies (*), dense core vesicles (DV) of variable sizes, and a large number of pleomorphic mitochondria (M). The nerve terminal also contains adrenergic nerve endings (arrows). (16,500 ×) d. An arteriole from the putamen of an adult monkey. Dense core vesicles are seen at the base of the nerve terminals (N). The distance between the nerve terminals and the smooth muscle (SM) is 80 nm; nerve terminals are separated from one another only by smooth muscle basement membrane (BM). (46,000 ×)

study of subhuman primates is not substantially different from that observed in the adventitia of intraparenchymal or extraparenchymal vessels of other animal species. The new finding in this study is the observation that nerve endings containing adrenergic vesicles occur primarily at branching sites. Using a fluorescence histochemical technique Peerless, et al20 found a high concentration of nerve fiber enlargements (which they called "varicosities") located at the origin of small arteries and arterioles on the intracranial, extraparenchymal vessels of the rabbit; these authors suggested that such structures were appropriately placed to effect some variation in the distribution of regional blood, and perhaps in the total blood flow as well. In contrast, Purdy et al21 also using a fluorescence histochemical technique on the same animal species failed to find structures at vascular branching sites that might account for the regional inhomogeneity of the normal CBF, eg: selective increase of regional CBF in the precentral gyrus upon exercising the contralateral limbs.

Some authors have suggested that species variation and/or regional variations may explain some of the conflicting reports regarding CBF and vasomotor responses to sympathetic stimulation.10, 11, 22 Since resistance to blood flow is regulated through changes in the diameter of peripheral branches rather than at trunk arteries, it is likely that vascular innervation at branching sites may assist in producing minimal changes in cerebral blood flow. Local variations in cerebral blood flow (both physiologic and pathologic) have been confirmed by studies of brains in a number of species including cats, rats, rabbits, monkeys.5, 12, 18, 28, 29 These local changes in CBF may be so subtle that they may be detected only by techniques designed to measure local CBF. Nerve terminals at branching sites may also serve other important functions, such as the protection of the blood brain barrier from disruption during acute hypertension1, 8, 9, 16, 25 or they may serve as modulators that temper the effects of other vasoactive stimuli.18

Electrical stimulation of the cervical sympathetic ganglia has produced primarily two types of circulatory responses in the brain. CBF in dogs and cats has generally remained unchanged after cervical ganglionic stimulation while sensitive responses have been recorded in monkeys and baboons.11, 22 Heistad, et al11 demonstrated a 26% reduction in total CBF following
Figure 3. a. A capillary from the putamen of an adult monkey. Dense core vesicles (arrows) are seen within the basement membrane (BM). The capillary is ensheathed by an astrocytic process (AS). (12,500 x) b. A higher magnification of the vesicles within the capillary in figure 3a. Dense core vesicles are 15 nm from a fragment of pericyte cytoplasm (P). The dense core vesicles are apparently not surrounded by limiting membranes. (35,500 x)

electrical stimulation of the sympathetic ganglion in the monkey. Meyer17 found a 37% reduction in internal carotid artery flow after stimulation of the cervical chain also in the monkey. James et al (1979)13 observed a marked reduction in the CBF/PCO2 response curve during stimulation of the cervical sympathetic chain in the baboon. These responses to electrical stimulation were confirmed in the baboon by Harper et al. Krog (1964)15 observed reduction of carotid artery flow in man following sympathetic stimulation. The observations in the present study provide a possible anatomical explanation for the blood flow responses in the primate. However, we lack evidence indicating whether branch site innervation is species specific (monkey) or is a feature which is common to other species (ie: dog, cat, rat).

Three important facts should be noted: (1) Neurogenic stimuli can alter cerebral blood flow; (2) Structures (nerve endings) that may mediate these responses to neurogenic stimuli have been demonstrated in the intraparenchymal vessels; and (3) These nerve terminals are located primarily at branching sites where control of blood distribution may very well occur. This ultrastructural study of nerve fibers in the penetrating or intraparenchymal vessels of the basal ganglia has demonstrated that vascular innervation in the subhuman primate is comparable to that observed in the penetrating arteries and arterioles in other species, and that nerve terminals are confined primarily to branching sites. The latter finding is particularly relevant because it exists in an animal species in which intracranial vessels respond appropriately to sympathetic stimulation, ie: they constrict.

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References

Effect of Recirculation and Regional Counting Rate on Reliability of Noninvasive Bicompartamental CBF Measurements

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SUMMARY Based on data from routine intravenous Xe133-rCBF studies in 50 patients, using Obrist's algorithm the effect of counting rate statistics and amount of recirculating activity on reproducibility of results was investigated at five simulated counting rate levels. Dependence of the standard deviation of compartmental and noncompartmental flow parameters on recirculation and counting rate was determined by multiple linear regression analysis. Those regression equations permit determination of the optimum accuracy that may be expected from individual flow measurements. Mainly due to a delay of the start-of-fit time an exponential increase in standard deviation of flow measurements was observed as recirculation increased. At constant start-of-fit, however, a linear increase in standard deviation of flow measurements was observed as recirculation increased. By multiple linear regression analysis the effect of arterial PCO2 on perfusion following global ischemia. Stroke 11: 534–541, 1980


FOR MANY YEARS, regional cerebral blood flow (rCBF) has been measured successfully on a routine basis using various radioactive inert gas methods, although some of the underlying assumptions of the model (e.g., complete and instantaneous equilibration of the indicator gas between capillary blood and tissue, homogeneous perfusion of each compartment) may not always be fully valid, especially in pathologic tissue, and the distribution coefficient cannot be determined by external detection of radiation in two-dimensional projections. Despite those limitations injection of radioactive inert gases into the internal carotid artery allows rCBF measurements with reasonable accuracy. High spatial resolution can be achieved either by means of a gamma-camera or by a multicrystal system.\(^2\)

\(^3\)In order to avoid arterial puncture noninvasive pro-
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