Topographical Distribution of Barrier Function In Cervico-Cephalic Arteries of Dog

Major Cerebral Arteries Possess Definite Barrier Function?

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SUMMARY Topographic distribution of barrier function in normal canine cervico-cephalic arteries was studied using horseradish peroxidase (HRP) and Evans blue as tracers. The carotid sinus of the internal carotid artery (ICA) was conspicuously permeable to HRP when compared to other areas of major cerebral arteries. The cavernous portion of the ICA also showed prominent permeation of HRP, especially through the outer surface, which is covered with venous endothelial cells. On the luminal side of the cavernous portion of the ICA, barrier deficiency was noted at angulated segments such as the carotid siphon. Intracranial segments of both ICA and vertebral arteries demonstrated incomplete barrier function of the first 1 to 4 mm from the origin of the intradural segments. These areas were considered to be transitional zones in barrier function between extra- and intracranial arteries. Focal, but definite, barrier disruption was also noted at the distal ends of the ICA and other arterial branching sites of major intracranial arteries. While opening of the interendothelial junctions was considered to be one of the mechanisms causing increased permeability in the cavernous ICA, the mechanisms for the permeation of HRP into the major cerebral arteries could not be confirmed ultrastructurally.

ENDOTHELIAL CELLS in the intracranial major arteries are distinctive structures with unique morphologic properties which contribute significantly to barrier function as in the blood-brain barrier of the cerebral microvessels. However, there have been no precise topographical observations on barrier function in the cervico-cephalic arteries of normal animals. In the intracranial arteries, it is also unknown whether the barrier occurs abruptly beyond the dura mater, or if there are transitional zones of barrier between extra- and intracranial arteries. The purpose of the present study is to investigate the topographical distribution of barrier function in the major cervical and cerebral arteries of normal dogs. In order to evaluate changes in the barriers of major arteries under various conditions, particularly in subarachnoid hemorrhage and systemic hypertension, it will be necessary to recognize the topographical differences in barrier function.

Materials and Methods

Five adult female mongrel dogs, weighing between 5.1 and 6.6 kg, were anesthetized with intravenous sodium pentobarbital (Nembutal, 30 mg/kg body weight). Endotracheal intubation was performed and anesthesia was supplemented with a mixture of 60% N2O and 40% O2 administered during ventilation. The femoral artery and vein were catheterized to monitor blood pressure and to administer drugs. A capnometer was used to monitor expiratory CO2 concentration, which was held between 35 and 40 mmHg. During the experiment, arterial blood pressure, PaCO2, and pH of each animal were maintained within normal range. Evans blue (2% solution, 2.5 ml/kg body weight) was injected intravenously 30 minutes prior to fixation. Diphenhydramine hydrochloride (5 mg/kg body weight) was used in order to prevent hypotension and the histamine release reaction induced by horseradish peroxidase (HRP) administration. Ten minutes before fixation, HRP (200 mg/kg body weight, Sigma Type II) was injected intravenously and each animal was then sacrificed by perfusion fixation using 2.5% paraformaldehyde — 2% glutaraldehyde — 0.1 M phosphate buffered fixative (pH 7.3) under a pressure of 100—120 mmHg for 15 minutes. The brain and cervical arteries were removed, including cavernous and cervical portions of the internal carotid arteries (ICA), external carotids and common carotid arteries, and the specimens were kept in 0.1 M cacodylate buffered fixative (pH 7.3) for five hours. After washing overnight, the specimens were incubated for determination of peroxidatic activity. The incubated sections were examined with an operating microscope and were prepared for electron microscopy. The sections were post-fixed in osmium tetroxide, stained en bloc with uranyl acetate, dehydrated through graded acetone, and embedded in epoxy resin. One micron sections were cut and appropriate areas were selected for ultrathin sectioning. The sections were examined with Hitachi (H-600) electron microscope.

Results

The results in the present study are illustrated in figure 1.

Macroscopic Findings

While HRP-reaction products in cervico-cephalic arteries were relatively easily detected as brown-colored areas with operating microscopy, it was essential-
ly impossible to detect Evans blue staining of the arterial segments, except for the carotid sinus and cavernous portions of the internal carotid artery (ICA).

**Cervical Arteries and Cavernous Internal Carotid Arteries**

The common carotid, external carotid, and internal carotid arteries were diffusely but weakly stained with HRP-reaction products. Characteristic staining was found at the carotid sinus and the stained area was consistent with the anatomical localization of the carotid sinus (fig. 2A). The carotid bodies were also densely stained with HRP-reaction products (fig. 2A), and the ostia of the branches from the external carotid arteries were focally stained. The cavernous portion of the internal carotid artery was conspicuously stained with HRP-reaction products, especially at the outer surface which was covered with the endothelial cells of the cavernous sinus. The arterial lumen was also densely stained, but the densely stained areas were focally located at the angulated segments such as the carotid siphon. The dura mater around the internal carotid artery was also densely and diffusely stained (fig. 2B).

**Intracranial Arteries**

The proximal portion of the intradural internal carotid artery was stained with HRP-reaction products in continuity of the stained area of the cavernous portion of the ICA (fig. 2B, 2C). The vertebral arteries were also stained at the proximal portion, 1 to 4 mm from the point of dural penetration (fig. 2D). Small areas of focal staining were found at arterial bifurcations such as the apex and lateral wall of the distal ends of the internal carotid artery, the origin of the azygous trunk of the anterior cerebral artery, the bifurcation of the anterior spinal arteries and basilar arteries, and other branching sites of the major cerebral arteries (fig. 2B, 2C & 2D). Brain parenchyma was not stained with HRP-reaction products except at the hypophyseal infundibulum, median eminence and choroid plexus.

**Electron Microscopic Findings**

**Carotid Sinus**

HRP-reaction products were present in the subendothelial space, but the density of staining was relatively low in contrast with that of the macroscopic and light microscopic staining. HRP-labeled vesicles were noted on the abluminal membranes of the endothelium (fig. 3A). We did not detect continuous staining with HRP-reaction products throughout the entire thickness of the interendothelial junctions from the lumen to the subendothelial space.

The subendothelial space of the adventitial surface of the cavernous portion of the internal carotid artery contained HRP-reaction products. A small number of HRP-labeled micropinocytic vesicles were found at the abluminal membrane of the endothelial cells, and reaction products were present at the interendothelial junctions. The subendothelial space of the arterial lumen contained varying densities of HRP-reaction products, the highest being just beneath the interendothelial junctions (fig. 3B). Some intercellular junctions were stained throughout the entire thickness of the vessel wall from the arterial lumen to the subendothelial space (Figure 3C). A large number of the HRP-labeled vesicles were noted at the junctional area and the abluminal membrane of the endothelial cells (fig. 3B, 3C). There were very few HRP-labeled vesicles on the luminal surface or within the cytoplasm of the endothelium.

**Intracranial Major Arteries**

In the intracranial arteries, except in the focally stained areas, HRP-reaction products were not found in the subendothelial space. There were no HRP-labeled micropinocytic vesicles attached to the abluminal membrane nor were there any HRP-labeled vesicles on the luminal surface of the endothelial cells (fig. 3D). HRP-reaction products were detected in the subendothelial spaces of the focally stained areas. The staining was usually so low in density, however, that even under electron microscopy reaction products were detectable only as HRP-labeled vesicles attached to the relatively dense abluminal membrane of the en-
endothelial cells (fig. 3E). Among the stained areas in the intracranial arteries, the distal portion of the internal carotid artery showed HRP-reaction products in the endothelial spaces. In these areas, abluminal HRP-labeled vesicles were relatively increased in number (fig. 3F). However, luminal HRP-labeled vesicles were not detected. Despite the presence of HRP-reaction products in the subendothelial spaces, opening of the interendothelial junctions was not found in the intracranial arteries, probably due to the small amount of HRP permeation into the macroscopically stained area.

**Discussion**

Though it is generally assumed that major cerebral arteries have barrier function similar to the parenchym-
mal microvessels of the brain and that systemic arteries do not possess definite barrier function. The present study has revealed that there were topographic differences in barrier function between the various parts of the cervico-cephalic arterial system.

Among the cervical arteries, the carotid sinus showed a well demarcated stained area, corresponding to its anatomical localization. It may be speculated that the specific absence of barrier function at the carotid sinus is related to the presence of baroreceptors or...
vasoregulatory function at this area, and perhaps also to the anatomical characteristics of the vascular wall components. The cavernous portion of the internal carotid artery was characterized by diffuse staining with HRP-reaction products through the adventitial side. The endothelial cells covering the outer surface do not possess definite barrier function. In contrast, the endothelial cells covering the arterial lumen showed focal barrier deficiency, especially at the angulated segments. It is likely that the deficiency of barrier function at the carotid sinus might be physiological, since the carotid sinus showed clear demarcation of the stained area with homogeneous staining which was overlapped by relatively dense staining at the ostia of the branches. On the other hand, in arterial segments showing barrier disruption on the luminal side of the cavernous and distal portion of the internal carotid artery and vertebo-basilar systems staining was definitively localized at the angulated area and arterial bifurcation. The barrier disruption might be attributed to hemodynamic stresses or other pathologic processes related to atherogenesis and aneurysm formation.

Proximal segments 1 to 4 mm from the origin of the intradural segments of both internal carotid artery and vertebral arteries showed increased permeability. These areas are probably transitional zones of endothelial barrier function between extra- and intracerebral arteries. Bevan8 investigated precise topographic differences in pharmacological vascular properties between major cerebral and systemic arteries of rabbits. He reported that the transition site in the carotid artery was just proximal to the carotid canal while the transition site in the vertebral artery occurred just rostral to the emergence of that artery from the foramen of the lateral process of the atlas. There was no evidence for a gradual transition of characteristics but rather there appeared to be an abrupt change. It is interesting that at the site of transition there are differences between the pharmacological responses of medial smooth muscle and barrier function in the endothelium.

There are a few reports concerning barrier function or increased permeability in the major cerebral arteries. Sadoshima et al. reported that deposition of fibrinogen-fibrin as a result of increased permeability was seen within intimal pads at the bifurcation of human major cerebral arteries even in neonates, and the amount of deposition increased with age. Topographic distribution of the arterial segments permeable to HRP in the dog were compared with those showing atherosclerotic changes in human.

Increased permeation of the plasma components is one of the most important causative factors in atherogenesis.11 Conspicuous, and characteristic staining with HRP at the carotid sinus indicates that the endothelium at the carotid sinus is extraordinarily permeable to plasma proteins. Since atherosclerotic changes are generally most frequently seen in the region of the carotid sinus among the cervico-cephalic arteries, increased permeation seen in the present study indicates that barrier deficiency could be closely related to atherogenesis. Focal barrier-disrupted areas in the major cerebral arteries corresponded to those sites which were most directly influenced by hemodynamic stress. These focal increases in the permeability at the branching sites may be related to the development of cerebral saccular aneurysms.

In the present study, we could not delineate all of the mechanisms for the HRP permeation through the endothelial cells, because the amount of permeated HRP was usually too small to detect ultrastructurally despite the macroscopic presence of the stained areas. We could not find continuous staining of interendothelial junctions at the carotid sinus, despite macroscopic and light microscopic dense staining. It is possible that HRP in the intercellular opening was completely washed out during perfusion fixation. Except for the carotid sinus and cavernous portion of the internal carotid artery, the staining with Evans blue in the cervico- cephalic arteries was too low in density to detect under an operating microscope, so Evans blue dye may be inappropriate as a tracer for investigating barrier function in the cervico-cephalic arterial system of normal animals.

Recognition of the physiological absence of barrier function in the major cerebral arteries is essential for evaluation of barrier changes or disruption in these vessels under various experimental conditions. The physiological barrier deficiency in the cerebral arteries may influence not only the arterial response to some vasoactive substances, but also the vascular reaction following subarachnoid hemorrhage, systemic hypertension, and atherosclerosis.

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