Normal perfusion pressure breakthrough: the role of capillaries

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Excision of human cerebral arteriovenous malformations (AVMs) can be complicated by postoperative edema and hemorrhage in adjacent brain tissue, despite the complete excision of the malformation. Various theories have purported to explain the hemodynamic basis for this predisposition, including disordered autoregulation causing “normal perfusion pressure breakthrough” and obstruction of venous drainage leading to “occlusive hyperemia.” This study did not evaluate the arterial or venous circulations in this scenario, but rather examined the capillaries in adjacent brain parenchyma for any structural deficiencies that would predispose the brain to the postoperative formation of edema and hemorrhage.

Arteriovenous fistulas (AVFs) were created surgically in the necks of 10 male Sprague–Dawley rats, which caused chronic cerebral hypoperfusion with a reduction in cerebral blood flow of between 25% and 50%. Ten age-matched animals were used as controls. Twenty-six weeks after AVF formation the animals were killed and perfusion fixed. Their brain tissue was prepared for light microscopic studies by staining for glial fibrillary acidic protein or for transmission electron microscopy. In the CA1 pyramidal cell region of the hippocampus, it was found that in the animals with AVFs there was increased capillary density and absent astrocytic foot processes in some of these vessels. It was concluded that these vessels had developed as a result of neovascularization in response to chronic cerebral ischemia and that their anatomical configuration made them prone to mechanical weakness and instability following the increase in perfusion pressure that occurs in adjacent brain parenchyma after AVM excision.

The authors believe that this study pinpoints a structural accompaniment to the hemodynamic changes that occur in brain tissue in the vicinity of cerebral AVMs that predispose these areas to the formation of edema and hemorrhage after AVM excision.

KEY WORDS • normal perfusion pressure breakthrough • arteriovenous malformation • capillary • blood-brain barrier • rat

In 1978 Spetzler, et al.5 described several cases in which total excision of an intracranial arteriovenous malformation (AVM) was followed by massive brain swelling and bleeding, and this has subsequently been described by many others.3,5,6,8,9,17,18,20,27,28,30,32,35–38,40,48,49,51,52 Spetzler, et al., suggested that as a result of the hypoperfusion of surrounding brain regions, normal autoregulation of cerebral blood flow (CBF) was impaired and that flow would increase beyond that seen in the absence of disease once the arteriovenous fistula (AVF) was occluded. Capillary breakthrough would occur because of these changes, resulting in cerebral edema and, if severe enough, hemorrhage. They called this phenomenon “normal perfusion pressure breakthrough.”

Recently, Al-Rodhan, et al.,1 suggested that “occlusive hyperemia” may be the actual pathophysiological process explaining postoperative hemorrhage and brain edema that might otherwise be described as normal perfusion pressure breakthrough in AVM patients. The basis of occlusive hyperemia is obstruction of the venous outflow system of adjacent brain parenchyma, with subsequent passive hyperemia and engorgement. Stagnation of arterial flow in former AVM feeding vessels and their parenchymal branches also occurs, with subsequent worsening of the existing hypoperfusion, ischemia, and hemorrhage or edema in these areas. Regardless of whether arterial autoregulatory override (that is, normal perfusion pressure breakthrough) or venous occlusion is responsible for the pathological process, both are manifested by capillary rupture and breakdown of the blood-brain barrier.

What has not been addressed is whether there is any structural abnormality at the capillary level that would predispose the brain to edema and hemorrhage. It is the purpose of this study to use an established model of an AVF in the rat to examine the capillary bed structure.

Materials and Methods

All experiments were approved by our institutional animal care and ethics committee. The construction of the AVF has been described in detail elsewhere.24–26,41,42 Briefly, 10 Sprague–Dawley rats, approximately 8 to 10 weeks old and weighing between 250 and 350 g, received a general anesthetic consisting of 1.6% halothane. Under direct magnification, a carotid–jugular fistula was created between the right internal carotid artery (ICA) and external
jugular vein. This was achieved by ligating the external carotid artery at its origin and the external jugular vein and common carotid artery caudally in the neck. The common carotid artery and the external jugular vein were each divided above their ligatures and subsequently anastomosed in an end-to-end fashion with interrupted 10-0 nylon. This formed a functional AVF between the anterior intracranial circulation and extracranial venous circulation and resulted in chronic cerebral hypoperfusion without an acute ischemic insult (Fig. 1). Ten age-matched rats were used as controls.

The CBF in all regions of both hemispheres of the brain, as measured using 14C-autoradiography, has been previously shown in this model to be reduced by 25% and 50% in the absence of cerebral infarction.

The results of this study were based on findings in nine rats with a carotid–jugular AVF and 10 healthy control animals. Twenty-seven weeks after AVF formation, five animals in the control and four in the AVF group were again anesthetized with halothane and perfusion fixed by cannulation of the ascending aorta and administration of a primary fixative of chilled 6.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4. This was preceded by a 30-second rinse with 0.9% saline at room temperature and 100 IU of sodium heparin. All solutions were infused via gravity feeding from a height of 2 m.

Light Microscopy Examination

The brains taken from animals in the control and AVF groups showed no evidence of poor perfusion fixation encompassing hydroptic cell change, dark neurons, or changes in perivascular or perineurial spaces. On gross examination of all regions of the brains stained with cresyl violet in animals in the AVF group, no evidence of infarction was apparent. An increase in general vascularity became evident on survey of all regions of the hippocampus. Numerous vessels were visible in all regions, with capillaries in the CA1 and CA3 subfields. The large CA1 is clearly visible. No increase in overall astrocytic density in the AVF group, particularly in the CA1 subfield. There was a greater than 100% increase in the number of capillaries when compared with controls, particularly in the CA1 subregion (Fig. 2). In all the animals in the AVF group, a small number of capillaries were notable for the absence of pericapillary GFAP staining, indicating a deficient surrounding astrocytic foot process layer. All control animal specimens had GFAP-stained periluminal rims, indicating intact astrocytic foot process layers. There was no increase in overall astrocytic density in the AVF group, and right and left hippocampi were equally affected in terms of these changes in vascularity.

Electron Microscopy Examination

The CA1 region of the hippocampus was the primary

Fig. 1. Depictions of an AVF model used to create chronic hypoperfusion in the rat. Upper: Schematic representation of the right carotid–jugular fistula anastomosis demonstrating some of the major connections. CCA = common carotid artery; ECA = external carotid artery; Ext. jug. v = external jugular vein; ICA = internal carotid artery; Int. jug. v = internal jugular vein. Lower Left and Right: Angiograms obtained in rats in the control and AVF groups, respectively. The closed arrow indicates the right ICA and the open arrow indicates the transverse sinus. The large AVF is clearly visible.

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brains were embedded in paraffin and adjacent 10-μm sections were cut and stained for glial fibrillary acidic protein (GFAP) to identify pericapillary astrocytic foot processes. All subsequent assessments were made in a blinded fashion by three independent observers, concentrating on the CA1 region of the hippocampus. Interpretation of results was qualitative. The sections stained with toluidine blue allowed precise localization of regions of interest. A systematic survey of all neurons, glial cells, and capillaries was made at low power before commencing a high-power survey of each structure.

Thirty-two weeks after AVF formation, the remaining 10 animals (five each in the control and AVF groups) were anesthetized with halothane and perfusion fixed by cannulation of the ascending aorta and administration of a primary fixative of chilled 6.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4. This was preceded by a 30-second rinse with 0.9% saline at room temperature and 100 IU of sodium heparin. All solutions were infused via gravity feeding from a height of 2 m. The brains were left in situ for 4 hours, then removed and placed in storage in cold fixative solution. After 24 hours the hippocampi were dissected and sliced at 1-mm intervals perpendicular to the long axis. Further dissection yielded wedges centered on the CA1 band of the hippocampus, which were placed in cacodylate buffer at 4°C for 24 hours. The specimens were then postfixed in 1% osmium tetroxide in cacodylate buffer for 1 hour and 0.5% uranyl acetate for 45 minutes. After dehydration in a graded ethanol and acetone series, the specimens were infiltrated and embedded in Spurr’s resin. Semithin (0.5-μm) and ultrathin (90–150-nm) sections were then prepared on an ultramicrotome.

The former sections were stained with toluidine blue and examined using light microscopy for precise anatomical localization. The latter sections were placed on formvar-coated grids and stained with lead citrate and saturated uranyl acetate prior to examination using an electron microscope. Emphasis was placed on assessment of capillaries in the CA1 subfield. Again, all assessments were made by three independent observers in a randomized, blinded fashion. Three to six sections were surveyed for each animal, and only features that could be found consistently in all specimens were reported. At least five to 10 capillaries were reviewed per section prior to assessing the next section. In total, at least 30 to 50 astrocytes and approximately 30 capillaries were assessed per animal.

Results

The brains taken from animals in the control and AVF groups showed no evidence of poor perfusion fixation encompassing hydroptic cell change, dark neurons, or changes in perivascular or perineurial spaces. On gross examination of all regions of the brains stained with cresyl violet in animals in the AVF group, no evidence of infarction was apparent. An increase in general vascularity became evident on survey of all regions of the hippocampus. Numerous vessels were visible in all regions, with capillaries particularly prominent. There was a greater than 100% increase in the number of capillaries when compared with controls, particularly in the CA1 subregion (Fig. 2). In all the animals in the AVF group, a small number of capillaries were notable for the absence of pericapillary GFAP staining, indicating a deficient surrounding astrocytic foot process layer. All control animal specimens had GFAP-stained periluminal rims, indicating intact astrocytic foot process layers. There was no increase in overall astrocytic density in the AVF group, and right and left hippocampi were equally affected in terms of these changes in vascularity.

Electron Microscopy Examination

The CA1 region of the hippocampus was the primary
area of interest and was the only region investigated in detail. Capillaries were only rarely seen in the specimens from the control group. Those vessels that were identified had clearly recognizable endothelial cells that rested on a basal lamina of uniform thickness and density (Fig. 3A). The capillary lumina were devoid of red blood cells, as expected after perfusion fixation, and beneath the basal lamina the clear cytoplasm of the astrocytic foot process layer was clearly identifiable. At higher magnification, all structures were easily identified, with the astrocytic foot processes appearing rich in glial fibrils and mitochondria (Fig. 4A). In specimens from the AVF group, the density of capillaries was greatly increased and many capillaries (> 40/animal) were examined. Most capillaries had an intact astrocytic foot process layer, but the smaller vessels were notable for an absence of astrocytic end-feet (Figs. 3B and 4B). The basal lamina was identified and did not appear to be altered in thickness, density, or uniformity; the endothelial cells did not appear to be shrunken or swollen and were typically electron dense. These findings were confirmed on closer inspection at high power, with a definite finding of an absent astrocytic foot process layer in many capillaries from animals that had undergone chronic cerebral hypoperfusion.

Discussion

These results show that chronic cerebral hypoperfusion in an AVF model in the rat induces capillary proliferation in the hippocampus, probably as a result of neovascularization, with some of the resultant vessels lacking an astrocytic foot process layer. This finding is important in the context of chronic cerebral hypoperfusion, in that this pathological finding has not been previously described, and it may provide a structural accompaniment to the proposed hemodynamic changes that occur after AVM excision and that are associated with the formation of edema and hemorrhage. Further studies will need to incorporate full morphometric analyses.

In a healthy brain, capillary density is known to correlate with the density of synapses and is thought to be related to the degree of oxidative metabolism in a region. However, it seems unlikely that either of these correlates would explain our findings. Purves showed that prolonged hypoxia could lead to increased capillary density in the brain, yet previous studies have not shown a similar result with chronic cerebral hypoperfusion. In the rat, maximum capillary density is achieved 20 days after birth. One of the factors thought to control capillary growth is adequacy of oxygenation of the tissue, and this

![Fig. 2. Light micrographs of the CA1 subregion of the hippocampus of rats in the control (left) and AVF (right) groups showing the greatly increased vascularity of the region notable in the AVF group. Some of the capillaries in the animal in the AVF group appear to lack a clear GFAP-stained rim, indicating an absent astrocytic foot process. GFAP, original magnification × 240.](image)

![Fig. 3. Low-power electron micrographs of capillaries in the stratum radiatum of the hippocampus from animals in (A) the control and (B) the AVF group. Tissue from the control rats shows a well-defined basal lamina (bl) and the clear cytosol of astrocytic foot processes (f), which are notably absent around the capillary from the animal in the AVF group. An adjacent CA1 cell (c) is also shown that in the rat in the AVF group contains a large lipofuscin body (l). A nearby dendrite (d) is also seen. Bars = 1 μm.](image)
may be relevant in this model of chronic cerebral hypoperfusion.

Embryologically, capillaries develop from capillary sprouts that are composed of solid cords of cells (angioblasts), and immature intracerebral vessels develop by sprouting from preexisting ones. It appears that astrocytic foot processes emerge at the time of opening of the capillary lumen, with narrowing of the perivascular space and formation of a basal lamina. In the rat cerebral cortex, the capillaries are surrounded by perivascular glia at the end of the 1st postnatal week. Bär and Wolff have suggested that the final differentiation of the cerebral capillaries begins at the end of the 2nd postnatal week. Astrocytic foot processes form a single layer without overlap, and this layer has an approximately constant thickness. Oligodendrocytes and microglial cells can be found next to capillaries, with the notable absence of any specializations comparable to those of astrocytic foot processes. It has been qualitatively estimated that the astrocytic foot process “sheath” is approximately 85% complete. From these studies, it becomes apparent that in the postnatal animal, all capillaries in the central nervous system should have astrocytic foot processes (as was the case in the control group). Therefore, at least in part, the increased capillary density seen in the animals with chronic cerebral hypoperfusion is due to neovascularization, because the absence of astrocytic foot processes cannot be explained by mere recruitment of a normal mature capillary. It must be emphasized that all capillaries should thus have had astrocytic foot processes, as was seen in the control group. This did not occur in the AVF group, and this finding is clearly an aberration from the norm.

The onset of neurological dysfunction caused by cerebral hyperemia after the occlusion of a cerebral artery was suggested by Gowers in 1888. Prior to 1950, Elvidge, Oliveira and Rives, and Norlén all commented on increases in cerebral perfusion following excision of cerebral AVMs. In 1978, Spetzler, et al. described several cases in which total excision of intracranial AVMs was followed by massive brain swelling and bleeding. They suggested that, as a result of the arterial hypotension, the surrounding brain CBF is maintained by maximum vasodilation and that after a prolonged period the normal autoregulatory response is impaired. On excision of the AVM and restoration of pressure within the arteries, there is a failure in the appropriate arterial vasoconstrictor response, with high pressure levels transmitted to the smallest blood vessels. Morgan, et al. have subsequently suggested that this occurs in an experimental setting. Capillary breakthrough occurred because of these changes, resulting in cerebral edema and, if severe enough, hemorrhage. Spetzler and colleagues described this phenomenon as normal perfusion pressure breakthrough and suggested that a stepwise reperfusion of the ischemic cerebral tissue via staged AVM resection may allow normal autoregulation to be reestablished or that normalization of CBF by maintenance of an artificially low blood pressure after total excision may avoid this hyperemic complication. This theory has yet to be substantiated on a pathophysiological basis and remains controversial. Despite this, many authors have reported the onset of brain edema with or without hemorrhage in the normal brain parenchyma surrounding the bed of a totally excised AVM and restored pressure within the arteries, there is a failure in the appropriate arterial vasoconstrictor response, with high pressure levels transmitted to the smallest blood vessels. This was responsible for this recognized complication. However, Batjer and colleagues and Young, et al. have provided evidence that vasoreactivity is intact and may be enhanced in patients developing a normal perfusion pressure breakthrough syndrome.

Furthermore, Al-Rodhan, et al. suggested that the phenomenon of “occlusive hyperemia” may be the actual pathophysiological process underlying swelling and hemorrhage consequent on AVM removal. They deduced that in such cases the circulation time ranged from consistently average to very slow (with no case showing a fast circulation time, as defined by their own criteria) and impairment of the venous drainage system was present.

Edema and hemorrhage after excision of human cerebral AVM rarely occurs, yet it can have devastating results for the patient. Until now, various theories have been put forth to explain this predisposition on the basis of hemodynamic factors. It seems likely that a combination of normal perfusion pressure breakthrough and occlusive hyper-

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**Fig. 4.** High-power electron micrographs of the walls of capillaries from the stratum radiatum of the hippocampus from animals in (A) the control and (B) the AVF group. At this magnification, in the animal from the control group the bundles of glial fibrils are clearly identifiable (double arrows) lying in the clear cytosol of an astrocytic foot process (f) adjacent to several mitochondria (m) and an endothelial cell (e). In the animal from the AVF group, no astrocytic foot process is noted, although the basal lamina (bl) is intact. Bars = 1 \( \mu \)m (A) and 0.5 \( \mu \)m (B).
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emia are at play with other as yet unidentified hemodynamic processes. This study of a rat model shows that an experimentally created AVF is associated with an increase in capillary density, with some of these capillaries becoming structurally defective in their walls. By inference these capillaries are mechanically weaker and less able to withstand distending forces that occur when cerebral perfusion is reestablished after ablation of the arteriovenous communication. The regulatory function provided by astrocytes to this region may also be disturbed. Consequently, the capillaries in these animals signal a structural weakness that would accompany the hemodynamic perturbations and that may explain the predisposition of some patients to edema and hemorrhage after AVM excision. Whether these changes also occur in humans remains to be seen, and the changes in these vessels over time is a matter for future study.

Acknowledgments

We thank the Electron Microscope Unit, The University of Sydney, for the use of their electron microscope and the Department of Pathology, The University of Sydney, for preparation of specimens for light microscopy.

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Manuscript received March 11, 1996. Accepted in final form October 28, 1996.

This work was supported in part by the following: National Health and Medical Research Council; Clive and Vera Ramaciotti Research Foundation; Royal Australasian College of Surgeons; and the Australian Brain Foundation. This research is part of an ongoing project investigating the pathophysiology of chronic cerebral hypoperfusion and intracranial arteriovenous malformations.

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