Cerebral Autoregulation: An In Vitro Study

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SUMMARY Cerebral autoregulation can be duplicated in vitro using the large middle cerebral arteries from a calf. The limits of autoregulation were between 50 and 150 mm Hg. Excessively high pressures may lead to the appearance of the 'sausage- or bead-string' response followed by forced dilation. These results suggest the existence of an intrinsic myogenic mechanism responsive to intraluminal pressure changes.

CEREBRAL autoregulation is defined as the maintenance of a relatively constant blood flow in the face of moderate changes in perfusion pressure. In vivo it is seen as vasoconstriction after increased intraluminal pressure and vasodilation in response to pressure decreases.1,2 The normal range of autoregulation in cerebral vascular systems is between 45 and 170 mm Hg, but the range can be altered by pathological factors such as hypertension.3 Excessively high pressures may lead to the appearance of the 'sausage- or bead-string' phenomenon and then forced dilation, concurrent with the loss of autoregulation. In the absence of autoregulation, cerebral blood flow is passively dependent upon perfusion pressures.

Previous in vitro studies with cerebral arteries have described the effects of metabolic, ionic and pH changes that may mimic those occurring in vivo during normal and abnormal conditions.4,5 This report describes an in vitro method for the study of autoregulation in the cerebrovascular system. As such, it is the first quantitative study of autoregulation in vitro in a cerebral vessel. The vascular responses to pressure duplicated those found in vivo which suggests the existence of an intrinsic myogenic mechanism responsive to intraluminal pressure changes.

Methods

Within 15 minutes after death, middle cerebral arteries were collected from a calf and placed in modified Krebs solution. Modified Krebs solution had the following mM composition: NaCl 118.8, KCl 4.7, KH₂PO₄ 1.2, CaCl₂ 1.2, MgSO₄ 1.2, NaHCO₃ 14.9, dextrose 5.6. Osmolarity of the solution was 276 miliosmoles, and pH was 7.4. Sections of white matter were removed along with each vessel to preserve intact branching. The vessels were threaded onto a glass rod in Krebs solution maintained at 37°C and equilibrated with 12% oxygen and 5% carbon dioxide.

The vessel was then cannulated between 2 glass tubes in a whole mount apparatus similar to that used by Farrar.6 After cannulation, the artery was stretched longitudinally (about 15%) between the glass rods to its approximate in vivo length. The vessel was perfused, both extraluminally and intraluminally, with oxygenated Krebs solution (37°C) with a peristaltic perfusion pump (Harvard Apparatus). Extraluminal solution was replaced every 5 minutes while intraluminal solution was continually recirculated. Intraluminal pressure was monitored at both inflow and outflow sites with Statham pressure transducers. Pressure changes were made in the system via an inverted reservoir with an air pressure bulb. Isometric Grass transducers were attached along the center top of the artery at 4 evenly spaced locations with 10-0 sutures. Resting tension was maintained at 1.0 gram at each suture point. Suturing of the artery was accomplished with a Zeiss operating microscope equipped with a micrometer eyepiece which was also used to measure changes in vessel outside diameter (O.D.). Generated tension due to changes in O.D. was recorded on a Grass polygraph. The absence of leaks was determined by the ability of the vessel to maintain a constant pressure. Preliminary experiments with serotonin established the viability of the tissue handled in this manner. Serotonin hydrochloride (1.0 µg/ml) consistently contracted the artery until pressures of 150 mm Hg were reached, at which point the contractions decreased. After forced dilation the vessel no longer responded to serotonin at any pressure. To eliminate active smooth muscle participation, some vessels (passive arteries) were frozen in 0.9% isotonic saline for 24 hours. All vessels were equilibrated for one hour at a flow rate of 4.1 ml/min. At 5 minute intervals, arteries were subjected to incremental pressure changes of 25 mm Hg starting at zero pressure. Changes in O.D. were recorded within the first 30 seconds of the pressure change, and again 5 minutes later at the 2 sites where transducers were attached.

Results

When investigating blood flow in vivo, the ability to autoregulate is a recognized sign of a normal functioning vascular bed. In the present in vitro experiments, autoregulatory responses to pressure occurred in the range of 50 to 150 mm Hg (fig. 1). This response was noted even in the absence of flow. The vessel only dilated when pressure was increased from zero to 25 mm Hg. In the lower pressure range, maximum arterial dilation was reached before autoregulation was evident; a fact noted in vivo in cat pial arteries.7 The first indication of autoregulation occurred at 50 mm Hg, connoted by a decrease in vessel O.D. in response to increased pressure. Between 50 and 125 mm Hg the vessel's initial response to increased pressure was dilation followed within seconds by constriction. Maximum constriction occurred between 100 and 125 mm Hg. Above 150 mm Hg the vessel con-

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FIGURE 1. Middle cerebral arteries (MCA) response to increases in perfusion pressure. O . . . o passive vessels (non-functional smooth muscle); • • • • active vessels (functional smooth muscle). Changes in outside diameter (O.D.) of the artery were recorded immediately after pressure was increased (0 time) and 5 minutes later. The large increase in O.D. at 225 mm Hg indicates the point where forced dilation usually occurs. Each point is the mean ± se of 8 vessels. The O.D. for each artery was the mean of 2 different measurements.

sistent responded to increases in pressure by only dilating, reaching maximum dilation at 300 mm Hg. Alternating constriction ("sausage- or bead-string" phenomenon) has been described in cerebral arteries in vivo at high pressures. Though not consistently reproducible, the beadstring response was observed in this preparation (fig. 2a) between 175 and 250 mm Hg. Eventually, the artery became uniformly dilated (fig. 2b).

Passive arteries (non-functional smooth muscle)
only dilated when subjected to increases of pressure until 250–300 mm Hg was reached, at which point O.D. no longer increased (fig. 1). Active arteries (functional smooth muscle) expanded past their forced dilation point behaved like passive arteries when pressure was returned to the autoregulating range (50–150 mm Hg). Neither passive nor forced dilated active arteries contracted when stimulated with serotonin at any pressure level.

Discussion

Regulatory mechanisms controlling cerebral blood flow include metabolic and neurogenic factors, changes in chemical milieu, and autoregulation (for review see refs. 3 and 12). Autoregulation has been attributed to myogenic changes in the lumen size of large arteries of the brain and is known to occur in other vessels. Active contraction in response to stretch occurs in helical strips of the dog cerebral artery and isolated segments of small arteries develop basal tone during perfusion. The myogenic or Bayliss explanation for autoregulation hypothesizes that there is an intrinsic mechanism in the smooth muscle cells of an artery which responds to intravascular pressure changes or tension in the wall of the vessel. The fact that this in vitro model closely duplicates in vivo autoregulation and was dependent upon active smooth muscle participation suggests the existence of just such a mechanism.

Baroreceptors, such as those found in the middle cerebral artery, may act as sensors of vascular pressure changes and thus implement myogenic contractions. Baroreceptors are nerve endings that respond to deformation or strain in the vessel wall. Constant high pressure, as found in hypertensive animals, can cause baroreceptors to adapt to higher operating points. Similar autoregulatory adaptability to hypertension has been pointed out in vivo in cerebral arteries.

This study does not indicate a major role for the myogenic regulation of cerebral blood flow. However, it does demonstrate in vitro autoregulatory responses to intraluminal pressure changes in the resting physiologic range, and is strong evidence for the existence of an intrinsic myogenic mechanism operative in cerebral autoregulation.

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