Adrenergic Innervation of Large Cerebral Blood Vessels of the Rabbit Studied by Fluorescence Microscopy

Absence of Features That Might Contribute to Non-Uniform Change in Cerebral Blood Flow

RALPH E. PURDY, PH.D., and JOHN A. BEVAN, M.D.

SUMMARY The literature provides evidence for non-uniform regional changes in cerebral blood flow under a variety of circumstances. Possible causes of such changes were sought in the larger cerebral arteries of the rabbit prepared according to the glyoxylic acid fluorescence histochemical technique. The adrenergic innervation of the circle of Willis, the basilar artery and their main branches, although showing small differences, is essentially of uniform density. There is no evidence for collars of adrenergic nerves around the origin of small branches nor for cushions at their orifices. Innervation density appears to diminish as the pial vessels get smaller and all vessels seem to be innervated. Thus non-uniform alterations of cerebral blood flow cannot be accounted for by these factors.

ADRENERGIC INNERVATION of the cerebral arterial vasculature has been well documented in a variety of species. There is general agreement that the basilar artery and the arteries that make up the circle of Willis are richly innervated and that the innervation of their pial branches becomes less dense as the smaller the vessels and the greater their distance from their origin.

Histochemical demonstration of the presence of such adrenergic innervation has stimulated efforts to elucidate its physiological role. In vivo studies have failed to provide consistent answers, possibly because the experimental variables have not been adequately controlled. Recent studies have reported that cerebral blood flow can be altered by electrical stimulation of the superior cervical ganglion, by ganglionectomy and by injection of α-adrenergic receptor blocking agent, but these have not been confirmed by careful studies with labeled microspheres. Isolated segments of cerebral blood vessels respond to adrenergic agonists and also to activation of their intramural vasoconstrictor nerves. Although these studies demonstrate that cerebral blood vessels have the capacity to respond to adrenergic stimulation, they do not prove that such a capacity subserves an important physiological function nor what that function is.

Cerebral blood flow remains relatively constant and is independent of modest fluctuations in arterial pressure, presumably because of its capacity for autoregulation. After cervical sympathectomy, however, one group reported that cerebral blood flow rate is no longer constant but varies with arterial pressure. The obvious conclusion that the sympathetic innervation of cerebral blood vessels is essential to the autoregulation of cerebral blood flow, however, is not universally accepted.

Regional variation in blood flow may occur as a result of neuronal activity. Eklof and Siesjö observed that ischemia combined with a lowered systemic blood pressure produced differences in blood flow between cerebral hemispheres and between cerebral lobes within each hemisphere in the rat. Unilateral electrical stimulation of the cervical sympathetic nerve in the cat also resulted in a non-uniform change in arterial blood flow. Aubineau and coworkers observed that injections of epinephrine or isoproterenol produced regional differences in cerebral blood flow in the rabbit. These experiments raise the possibility that mechanisms exist in the cerebral vasculature of, at least, the rat, cat and rabbit for non-uniform alterations in blood flow as a result of neuronal activity.

The present study was undertaken to determine if a non-uniform variation in regional flow could be the result of differences in the density of the sympathetic innervation to the circle of Willis and its main pial branches, or could be accounted for by other unusual anatomical features, such as arterial cushions at the orifices of pial arterial branches or collars of sympathetic nerves around the origins of pial branch vessels. Either of these phenomena could convert small smooth muscle responses into large non-uniform regional circulatory effects.

Methods

New Zealand rabbits weighing 2.2 to 2.8 kg were stunned and exsanguinated, and the brains were removed rapidly and placed in Krebs' bicarbonate solution at 0°C. Cerebral arteries were isolated and prepared for histological study of their adrenergic innervation according to a modification of the glyoxylic acid fluorescence method of Lindvall and Bjorkland. Glyoxylic acid solution (2%) was prepared immediately before use. Glyoxylic acid was dissolved in Krebs' bicarbonate solution, adjusted to pH 7.5 to 8.5, and chilled to 0°C. Tissues were immersed in this solution for three to five minutes with occasional stirring, immediately after their isolation from the brain, and then were prepared for transverse section or whole-mount viewing as follows.

Transverse Section

After exposure to glyoxylic acid, vessel segments were blotted, frozen rapidly in isopentane chilled with liquid nitrogen, and freeze-dried for 24 hours. After freeze-drying they were heated in an oven at 100°C for six minutes, and vacuum-embedded in paraffin according to standard histological technique. The paraffin-embedded tissues were sectioned serially at 10 μ.

Department of Pharmacology, University of California School of Medicine, Center for Health Sciences, Los Angeles, California 90024. Present address: Medical Pharmacology and Therapeutics, California College of Medicine, University of California-Irvine, Irvine, California 92717. This work was supported by U.S. Public Health Service Grant HL 15809 and by the American Heart Association-Greater Los Angeles Affiliate Grant 408 I.G.
Whole Mounts

After exposure to glyoxylic acid, cerebral artery segments of varying length with branches intact were positioned on a microscope slide in a drop of glyoxylic acid solution. The drop was blotted, allowing the tissue to settle upon the slide surface, and the tissue was dried initially in a stream of warm air for 15 minutes. Then it was further dried in vacuo over fresh phosphorus pentoxide for 24 hours, placed in an oven at 100°C for six minutes, and coverslipped for observation. Tissues were observed using a large Zeiss fluorescence microscope with type II/FL vertical illuminator (Carl Zeiss Co.). Fluorescence was produced by an HBO 200 mercury vapor lamp, a 3-mm BG3 excitation filter, and a 500-mm barrier filter. Photographs were taken with a Zeiss-Ikon camera, using Kodak Tri-X film.

Results

Serial sections were cut transversely along the whole length of the basilar artery and each of the arteries of the circle of Willis of four rabbits. In agreement with previous studies in the rabbit \cite{7} and other species, \cite{16,8} the adrenergic innervation was limited to the adventitia in these vessels. Figure 1a shows the innervation was composed of an outer periadventitial and an inner adventitiomedial plexus. Serial sections did not reveal any increase in innervation at the origins of branches from the larger arteries. In fact, density of innervation often appeared diminished at these sites.

A total of two arterial cushions were observed in these transverse sections from the cerebral vasculature of the four rabbits. Using the same technique, serial sections of the distal parts of four rabbit ear arteries, 1 cm in length, revealed one to three arterial cushions in each.

While transverse sections demonstrate the position of the neural plexuses within the thickness of the vessel wall, whole mounts provide a topographical viewpoint useful in assessing nerve density. Whole-mount preparations were made from cerebral vessels of 14 rabbits. Figure 1b illustrates the anterior cerebral, figures 2a and b the basilar, and figure 3a the posterior cerebral artery. The basilar artery and the arteries of the circle of Willis are richly supplied with adrenergic nerves. Although density of innervation appeared to be similar in all of these vessels, that in the anterior cerebral artery (fig. 1b) was probably greater than in the others.

Nodal swellings usually characteristic of the adrenergic terminal plexus were not easy to distinguish in the larger vessels — the plexus did not show discrete areas of high fluorescence density. Fluorescence was often diffuse in the bands or branches of the plexus.

FIGURES 1a and b. Figure 1a is a transverse section across the posterior cerebral artery within the circle of Willis. The 50 µ scale applies to all photomicrographs. The solid arrows (figs. 1a and b) indicate characteristic fluorescence associated with adrenergic innervation. The broken arrows (fig. 1a) indicate origins of branches (Br) where fluorescence is absent. Figure 1b is a whole mount of the anterior cerebral artery.
Figures 2a and b. Whole-mount views of the basilar artery taken of the center (2a) and near the edge of the preparation (2b) where a medullary branch originates.
Innervation density declined steadily (1) passing away from the circle of Willis or basilar artery, and (2) with decreasing vessel diameter. Thus, figure 2b shows the basilar artery with a less densely innervated branch. Figures 3a and b show the posterior cerebral artery, within the circle of Willis and at some distance away, respectively. The latter vessel is less densely innervated. Finally, small pial branches taken from the surface of the temporal lobe exhibited the least density of innervation observed (figs. 4a and b). In the smaller vessels varicosities could be more readily distinguished. Because of the nature of the preparation and the form of the neural plexus, quantification of the innervation density in the larger vessels could not be made.

At bifurcations of pial vessels into branches of similar diameter, there was no specialization of adrenergic innervation (fig. 4a). In agreement with the studies of transverse sections, branches issuing from the larger vessels often exhibited a decrease in innervation density at their origins compared to that more distally along their length (fig. 4b). Adrenergic nerves were seen in all the vessels studied.

The specificity of the glyoxylic acid fluorescence method for visualization of adrenergic neurons has been reported by Lindvall and Bjorklund. Six days after bilateral superior cervical ganglionectomy, the neuronal fluorescence seen in innervated vessels completely disappeared.

Discussion

Sympathetic adrenergic innervation was approximately equally dense in the components of the circle of Willis and the basilar artery and the origins of their main branches. Innervation density, however, declined as these branches became smaller. Although Peerless and Yasargil have reported finding an accumulation of fluorescent nerves around the origin of pial branches in the rabbit, it was our observation that there was more commonly a decrease in innervation density at these sites. In whole-mount vessel preparations, when the artery wall is folded at the level of the origin of the branch, an appearance of increased fluorescence density around the branch sometimes can be observed. This is because the origin of the branch is viewed tangentially through the parent vessel wall. However, observation of vessels originating at other orientations belies this impression.

Arterial cushions at the orifices of branches which could presumably serve to cut off blood flow with only a small movement of the vessel wall were rare and inconsistently observed. These findings support the conclusion that in the rabbit there are no adrenergic neuronal or vessel wall specializations either in the large arteries of the circle of Willis or its main branches which could form a basis or substrate for substantial non-uniform changes in blood flow.
must be borne in mind, however, that when whole-mount preparations are viewed under the microscope, the observer is looking through four neuronal plexuses occurring in the two superimposed layers of the vessel wall. The outer peri-avitdinal plexus probably contains neurons passing distally. Since each bundle contains a number of neuron plexi, this probably accounts for the difficulty in seeing varicosities and the apparent diffuseness and uniformity of the fluorescence within the plexus. The inner plexus is probably masked by the outer. However, the transverse sections do reveal that no dramatic variation in density of the adven-titiomedial plexus occurs, although this is difficult to assess.

Basal or resting cerebral blood flow varies widely between the various regions of the brain. It has been suggested that this is determined by differences in blood vessel geometry and number.31 Gerard32 and Sokoloff33 demonstrated that illumination of the eyes resulted in an increase in blood flow in discrete areas of the visual cortex, superior colliculus, and lateral geniculate ganglion. These changes were attributed to vasodilation of blood vessels within the brain substance caused by the local accumulation of metabolic products. The demonstration that non-uniform changes in blood flow between cortical lobes and between hemispheres could be induced by ischemia23 and neurogenic stimulation24 probably implicates neurogenic factors. There is some evidence for heterogeneity of the function of the sympathetic neurons originating from the superior cervical ganglion.34

Since the cerebral regional bed is unusual in that its larger vessels appear to be densely innervated, and there are no precapillary sphincters in this organ, it would seem reasonable to look to the larger vessels for a possible basis for non-uniformity of response to excitation. However, within the limitations of this study no such basis exists. This study does not eliminate the possibility of any number of preadrenergic and postadrenergic synaptic factors which could be responsible. Recent observations that the α-adrenergic receptor sensitivity can be dramatically altered, for example, by low concentrations of histamine suggests at least one possible mechanism.20 Histamine-containing mast cells occur in relative but non-uniform abundance in the meninges, particularly in association with cerebral blood vessels.28

The finding that the richest supply of adrenergic nerves occurs in the large cerebral blood vessels is in agreement with others.2, 3 These earlier observations led to the speculation that the larger vessels are the site of nerve-induced resistance to blood flow in the cerebral vasculature. Such speculation may seem reasonable since in most peripheral vascular beds the most densely innervated vessels are also the sites of greatest resistance to blood flow.28 However, it must be remembered that here the increased nerve density and the decreased vessel diameter work in concert to maximize the magnitude of the neurogenic resistance.

It is of some interest to speculate how much more dense the innervation of a large blood vessel would have to be to effect the same proportionate change in resistance as a vessel, for example, one-quarter of its internal diameter. Sufficient data are not available to permit a precise solution to this problem. However, for the sake of argument, let it be assumed that intravascular pressure, wall thickness, muscle contractility, transmitter sensitivity, and so on, will be the same in both vessels. And furthermore, that the mean effective transmitter concentration is proportional to density of adrenergic varicosities — which is probably not correct.27 Then according to the La Place law the smooth muscle in the wall of the larger vessel would have to develop four times the active tension to overcome the corresponding tangential wall stress. Since, in fact, intravascular pressure and wall thickness would be less in the smaller vessels, this factor would be in excess of this. The matter becomes more complex since there is an approximately loglinear relationship between neurotransmitter concentration and contractile response. Thus an increase in transmitter concentration in excess of this factor would have to be achieved to bring about a proportionate change in blood flow in larger vessels compared with smaller vessels.

On the basis of these very approximate considerations it is reasonable to suggest that the apparent greater density of innervation found in the large cerebral vessels of the rabbit may only bring these vessels into parity with the less densely innervated pial branches with respect to their ability to produce changes in resistance to the flow of blood.

References

22. Keyes SS, Schmidt CF: The effect of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consump-

Failure of Prolonged Hypocapnia, Hypothermia, or Hypertension to Favorably Alter Acute Stroke in Primates

JOHN D. MICHENFELDER, M.D.,* AND JAMES H. MILDE†

SUMMARY The effects of induced hypocapnia, hypothermia, and hypertension were surveyed in a primate model of acute stroke during and following a 48-hour period of intensive care. The results were compared to a group of nine control animals previously studied. Hypocapnia (PaCO₂ = 25 torr) was examined in five animals and did not appear to alter the expected mortality, degree of neurological deficit, or frequency of infarction. There was, however, a suggestion that the size of infarction may be reduced. Hypothermia (29°C) in five animals had a detrimental effect in that no animals survived following the intensive care period and all had infarction with massive edema. We speculate that hypothermia caused a sufficient increase in blood viscosity as to compromise collateral flow, thereby accounting for this detrimental effect. Induced hypertension (to 20% above control levels) was abandoned after three animals because of severe systemic effects (cardiac failure and pulmonary edema) resulting in death during the period of intensive care.

Introduction

A VARIETY OF MEASURES has been proposed as possibly being therapeutic in the management of acute stroke. Recommendations have been variously based upon laboratory investigations, theoretic considerations, and/or clinical impression. Laboratory investigations which attempt to document the effect of such measures in acute stroke can often be criticized for any one of several reasons. Studies of acute stroke in non-primates are vulnerable to criticism since the observations are made in animals with a collateral circulation that differs markedly from that of man. The method of creating an ischemic lesion might in-validated any results if the surgical exposure requires brain retraction, possible brain trauma, significant disruption of the cranial vault, or insertion of a foreign body. Finally, studies which examine only the acute effects of a proposed therapeutic measure may be misleading, since any observed beneficial effects might be only transient or even ultimately detrimental if the test treatment were continued.

Recognizing the validity of these criticisms, we have studied Java monkeys after occlusion of a middle cerebral artery via a transorbital approach which requires no brain retraction and minimal disruption of the cranial vault. Thereafter, these animals have been maintained for 48 hours in a laboratory-created intensive care environment during which time the test therapeutic intervention is introduced and maintained and following which the effects are examined. Using this preparation, we previously studied the effects of 48 hours of barbiturate anesthesia and demonstrated a significant beneficial effect in terms of mortality, neurological deficit, and frequency and size of infarction. The present report details our observations in a survey of three other proposed therapeutic interventions: hypocapnia, hypothermia, and hypertension.

Methods

Thirteen Java monkeys of both sexes and weighing 1.0 to 1.9 kg were studied. The protocol for the study is summarized in table 1. The monkeys were studied in groups of two...
Adrenergic innervation of large cerebral blood vessels of the rabbit studied by fluorescence microscopy. Absence of features that might contribute to non-uniform change in cerebral blood flow.

R E Purdy and J A Bevan

Stroke. 1977;8:82-87
doi: 10.1161/01.STR.8.1.82

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/8/1/82

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/