Adrenergic Blockade of Hypocapnic Cerebral Arterial Constriction

BY RICHARD A. R. FRASER, M.D., BENNETT M. STEIN, M.D., AND J. LAWRENCE POOL, M.D.

Abstract: Adrenergic Blockade of Hypocapnic Cerebral Arterial Constriction

Hypocapnic constriction of the basilar and vertebral arteries was produced and pharmacologically modified (by intrathecal administration) in nine Rhesus monkeys. The arteries were then removed and studied with a catecholamine fluorescent technique. Alterations in Pco2 were associated with significant changes in the caliber of large arteries. Mere depletion of the periarterial norepinephrine stores did not prevent hypocapnic vasoconstriction. The latter was reversed, however, by alpha adrenergic blockade (phenoxybenzamine). The alpha adrenergic receptor appears to be a mediating site for hypocapnic constriction of the intracranial vessels. We have proposed that the alpha receptor may be H+ sensitive so that changes in pH alter the responsiveness of the adrenergic receptor to transmitter substances.

ADDITIONAL KEY WORDS cerebral blood flow phenoxybenzamine alpha receptor catecholamine fluorescence autoregulation

Concepts concerning the regulation of cerebral vascular resistance have undergone many revisions since Roy and Sherrington, almost 80 years ago, demonstrated the relative independence of cerebral blood flow from systemic arterial pressure. The role played by carbon dioxide (CO2) currently receives the most experimental attention in this regard. This stems from the repeatedly confirmed observation that an increase in arterial Pco2 and a decrease in cerebral blood flow occurs with hypocapnia. As CO2 is the ultimate end product of cerebral metabolism, it would appear that a suitably flexible homeostatic mechanism then exists, whereby an increase in cerebral metabolism causes an increase in CO2, which in turn causes arterial dilatation and an increase in the blood supply to the brain—in effect, autoregulation. Certain clinical and experimental observations, however, have provided paradoxical data that suggest that other factors beside CO2 levels influence cerebral blood flow. Three examples are: (1) Despite a low Pco2, cerebral blood flow is increased in diabetic metabolic acidosis; (2) In a period of chronic respiratory alkalosis cerebral blood flow is normal despite moderate to severe hypocapnia; and (3) In an experimental setting with Pco2 levels maintained at normal levels, cerebral blood flow does not change despite induced alterations in arterial pH in either direction. These apparently paradoxical events, however, are compatible with the thesis that extracellular pH is the main factor controlling cerebral blood flow. Whether either H+ or CO2 is the primary metabolic regulator of cerebral vascular resistance, the mechanism by which either of these agents exerts its effect is unknown. The evidence for a direct CO2 effect on vascular smooth muscle has been summarized by Shalit and co-workers. These same authors have suggested the existence of a brain stem center sensitive to changes in CO2, which remotely controls cerebral blood flow.
Considerable data have been developed by others in support of the existence of such a center, but contrary evidence has recently been provided by Skinhøj and Paulson.

The possibility of participation by the sympathetic periarterial fiber plexus in the regulation of cerebral blood has been raised. This concept was explored by earlier workers and has been recently reviewed by White and Lassen. Little support developed for neural regulation of cerebral vascular tone because of the failure to demonstrate significant changes in CBF after conventional sympathetic manipulation by either surgical or pharmacological means.

The recent fluorescent demonstration of a profuse noradrenergic innervation of cerebral vessels in the cat and in monkeys and humans in our laboratory has stimulated a revival of interest in the function of these nerves and has suggested the possibility that they may participate in the regulation of cerebral blood flow.

Prior studies in our laboratory have demonstrated the participation of this noradrenergic system in the mediation of experimentally induced cerebral vascular spasm. These studies indicate that: (1) a pathological constriction of the intracranial vessels occurs in some, but not all, animals following the introduction of blood into the subarachnoid space, (2) this constricted vascular state is reversed by the topical application of an alpha adrenergic blocking agent to the adventitial surface of the vessel, and (3) the application of such an agent produces vascular dilatation of both normal and spastic vessels.

These data would suggest that the (as yet unidentified) vasoconstrictor substance contained in blood exerts its effect at the alpha adrenergic receptor of the intracranial vascular smooth muscle. They would also suggest that the periarterial noradrenergic fiber plexus maintains a tonic vasoconstrictive state which can be reversed by noradrenergic blockade.

The present study, utilizing a transclival exposure to the arteries of the posterior circulation, was designed to explore further the functional significance of this vascular adrenergic plexus, and specifically to determine whether this plexus participated in CO2-induced changes in cerebrovascular tone and caliber.

**Methods**

Nine Rhesus monkeys (3 to 4 kilos of either sex) underwent transclival exposure of the vertebral and basilar arteries according to the method of Echlin.

Under barbiturate anesthesia, a femoro-aortic cannula was placed, allowing frequent measurement of arterial PCO2, PO2, and pH (Beckman model 160 gas analyzer), as well as providing a route to obtain blood aliquots for application to the basilar and vertebral arteries for the induction of spasm. Blood pressure, respiratory and EKG were constantly monitored. After a tracheostomy, microsurgical techniques were used to remove the clivus from the foramen magnum to the intercavernous sinus using small drills and bone rongeurs. The dura was opened and tented, thus exposing the ventral surface of the brain stem with the basilar and vertebral arteries lying in the subarachnoid space. After ensuring that meticulous hemostasis of the previously dissected tissues was obtained, the
ADRENERGIC BLOCKADE

Arachnoid was carefully removed from its attachments to the underlying vessels. A photograph was then taken through a reticule eyepiece in the operating microscope to record vessel size and establish a baseline diameter. Blood gases were monitored at this time in order to establish that physiological conditions of $P_{CO_2}$, $P_{O_2}$, and pH were present.

Arterial reactivity was first established by respiratory alterations of $P_{CO_2}$ levels. First, hyperventilation via a volume respirator was performed. Constant photographic documentation of alterations in vessel size was carried out concomitant with measurements of blood gases. After maximum vasoconstriction of the basilar and vertebral arteries was observed and photographed (fig. 1), the animal was allowed to return to a normocapnic state by passive ventilation at a decreased tidal volume until spontaneous respirations had returned. Changes in arterial size were again documented until near baseline diameter was observed and photographed (fig. 2).

After normocapnia had persisted for 10 to 15 minutes without change in arterial diameter, the animal was allowed to spontaneously respire a mixture of 4% CO$_2$ and 96% O$_2$. Blood gases were monitored during this hypercapnic period, and photographs were taken of alterations in arterial diameter through the microscope (fig. 3).

In five of these animals arterial reactivity was further demonstrated by the application of fresh arterial blood to the vessel wall, in three during a period of normocapnia, and in two during a hypercapnic period. This consistently resulted in marked vasoconstriction of the vessel, with a 30% to 50% reduction in external diameter and an even greater reduction in the intraluminal blood column, an index of lumen diameter, as previously described by us and other workers.$^{34,35}$

With arterial responsiveness established to subarachnoid blood and to alterations in arterial $P_{CO_2}$, physiological conditions of normal blood $pH$, $P_{CO_2}$, and $P_{O_2}$ were awaited. The animal was allowed to spontaneously respire until blood gases and arterial diameter were at baseline levels.

---

**FIGURE 2**

Normal caliber vessels during normocapnic conditions ($P_{CO_2}$ 38, pH 7.39). Rhesus 317. ×8

**FIGURE 3**

Same animal during hypercapnia. Note marked dilation of large and small caliber vessels ($P_{CO_2}$ 66, pH 7.27). ×8
Photographical documentation of vessel size was then obtained.

At this point hypocapnia was again induced by passive hyperventilation. Blood gases and vessel size were again monitored. When maximal vasoconstriction of the basilar and vertebral arteries was observed, photographs were taken and simultaneous blood gases were recorded.

Modification of Hypocapnic Vasoconstriction

Various pharmacological agents were utilized to modify this vasoconstriction response.

All concentrations of each agent used to modify or prevent the hypocapnic vasoconstrictive response were dissolved in isotonic Ringer's solution buffered to pH 7.35 and maintained at 37.5°C in a constant temperature water bath. Three cubic centimeter aliquots of each agent used were gently irrigated into the subarachnoid space surrounding the brain stem basilar-vertebral artery complex, and allowed to remain for three minutes. Each solution was then removed by suction and irrigated with isotonic normothermic Ringer's solution. The exposed vessels remained submerged in spinal fluid, supplemented as necessary by isotonic normothermic Ringer's solution throughout the experiment except when photographs were taken. While the initial concentration of each agent was known because of dilution by CSF surrounding the brain stem, the actual concentration of the agent in contact with the vessels was considerably reduced. Each drug was prepared in 1 x 10^(-2) molar concentration, and subsequently diluted so that successively more diluted concentrations from 1 x 10^(-2) molar to 1 x 10^(-6) molar were available.

At the conclusion of each experiment the basilar and vertebral arteries were removed and prepared for fluorescent studies—a small portion submitted as a stretch preparation on a glass slide while the remainder was treated by freeze drying. Both specimens were vacuum dessicated and subsequently incubated in paraformaldehyde vapor for an appropriate interval. Using appropriate excitor and barrier filters and the ultraviolet microscope, the presence or absence of catecholamine-containing periarterial nerves was demonstrated in both longitudinal and cross section.

Results

Hyperventilation-induced hypocapnia (PCO_2 10 to 15 mm Hg) and associated respiratory alkalosis (pH 7.57 to 7.68) are associated with a marked reduction in the caliber of the basilar and vertebral arteries when these vessels are directly observed (fig. 1). Maximum constriction is observed after six to eight minutes of hyperventilation. Though reticule measurements are subject to a certain error of magnitude, a comparison of photographs of the basilar and vertebral arteries during hyperventilation-induced hypocapnia (pH 7.63, PCO_2 11) and the same vessel under normocapnic (PCO_2 38, pH 7.39) conditions (fig. 2) reveals that the reduction in arterial caliber associated with hypocapnia is marked. Severe vasoconstriction is also noted in the long and short circumferential branches of the basilar and vertebral arteries. The average reduction in caliber of the basilar artery under these conditions was 30% to 40%. Frequently during hyperventilation, a mild reduction in blood pressure and pulse pressure occurred. Presumably this resulted in some degree of myogenic-induced vasodilation and to some degree reduced the net effect of hypocapnic-induced vasoconstriction.

Hypercapnia (secondary to active inhalation of 4% CO_2 and 96% O_2 mixture) results in marked enlargement of the basilar and vertebral arteries—a maximal increase in diameter was observed after six to ten minutes of ventilation. Blood gas studies reflect this time period with PCO_2 levels varying from 55 to 70 mm Hg and a pH shift from normal levels of 7.38 ± 0.03 to 7.26 ± 0.04 being achieved at the point of maximum vasodilation. Comparison of baseline vessel diameter during normal blood gas conditions (fig. 2) with the engorged dilated vessels during observed respiratory acidosis (fig. 3) demonstrates the tremendous distention of the intracranial vessels—of both major arteries and their smaller branches induced by raising PCO_2.

Vascular spasm of the basilar and vertebral arteries induced by the application of blood to the adventitial surface as previously described was produced in five animals, in three prior to producing CO_2-induced alteration of arterial caliber, and in two animals while a state of hypercapnic vasodilation was present. The final vasoconstriction seen was identical in degree and duration in both groups—in those animals with previously

FRASER, STEIN, POOL

Stroke, Vol. 2, May-June 1971
ADRENERGIC BLOCKADE

Reversal of hypocapnic constriction by topical phenoxylbenzamine application (1 × 10⁻⁴ molar). Despite continued hypocapnia, the previously constricted vessels seen in figure 1 are now dilated to a much larger than normal diameter (Rhesus 317). ×8

hypercapnic dilated vessels and in those with normal (baseline) arterial diameter and normal pH and P₇O₂ levels. Hypercapnic arterial dilation did not, under these conditions, in any way modify the subsequent ease of production or the degree of spasm that occurred in response to the application of blood.

Two agents were used to modify the vasoconstrictive effect of hypocapnia. (1) Dibenzylamine, shown in previous studies to remove or prevent the spastic constriction induced by blood, was irrigated into the subarachnoid space surrounding the brain stem and basilar-vertebral artery complex in the manner described above. Three cubic centimeter aliquots of varying concentrations from 1 × 10⁻⁶ molar to 1 × 10⁻² molar were applied for three minutes, while the animal was being passively hyperventilated and marked vasoconstriction associated with hypocapnia was observed. Dibenzylamine concentrations of 1 × 10⁻⁴ molar consistently resulted in a reversal of the constricted state—a markedly dilated vessel resulting (fig. 4). This significant increase in arterial caliber took place in the face of continued hypocapnia and alkalosis as measured by arterial blood gases. More concentrated solutions of this agent produced a more rapid response but the degree of vasodilation was similar to the result produced by 1 × 10⁻⁴ molar concentrations. More diluted solutions (1 × 10⁻⁵ and 1 × 10⁻⁶ molar) produced partial dilatation of shorter duration. This response was observed in all six of the animals in which this agent was used.

In each of these animals, a control experiment was performed by the application of the normothermic buffered diluent (alcohol

FIGURE 4
Reversal of hypocapnic constriction by topical phenoxylbenzamine application (1 × 10⁻⁴ molar). Despite continued hypocapnia, the previously constricted vessels seen in figure 1 are now dilated to a much larger than normal diameter (Rhesus 317). ×8

FIGURE 5
Rhesus 1028. Phenoxylbenzamine (1 × 10⁻⁴ molar) dilatation of basilar and vertebral arteries, during severe hypocapnia (P₀₂ 12 mm Hg). ×8

Stroke, Vol. 2, May-June 1971
in Ringer's solution) without the addition of dibenzylamine. The diluent exerted no effect on the caliber of the observed intracranial vessels.

(2) Tyramine was utilized in three animals in similar concentrations (1 × 10⁻² and 1 × 10⁻⁴ molar). A different response was observed. During conditions of severe hypocapnic arterial constriction, the application of the drug produced a transient vasodilation of the basilar and vertebral arteries (three to five minutes). The vessel returned to its previous constricted state while hyperventilation-induced hypocapnia was maintained. Maximum but transient dilatation was observed at 1 × 10⁻² molar concentrations. In two animals no effect was observed with concentrations up to 1 × 10⁻⁶ molar, but 1 × 10⁻⁴ molar tyramine produced a transient but significant dilatation of the vessels in all three animals.

(3) BaCl₂: It had occurred to us that the arterial dilatation observed after the application of dibenzylamine may not be simply the result of an alpha adrenergic blocking effect but may reflect poisoning of the vascular smooth muscle—a form of "vasoparalysis." In order to exclude this possibility, it was necessary to demonstrate the preservation of the capacity of the smooth muscle to constrict in response to a smooth muscle stimulus known not to require neural mediation. BaCl₂ is known to be an effective cerebral vasoconstrictor agent and to exert this effect on denervated cerebral vessels. When 3 ml of 0.2 molar BaCl₂ (pH 2.8) was applied to the subarachnoid space surrounding a dibenzylamine-dilated vessel (fig. 5) (under conditions of continued hypocapnia), immediate severe constriction of the basilar and vertebral arteries resulted (fig. 6). This marked vasoconstriction was not altered by the

FIGURE 6
*Rhesus 1028. Reversal of phenoxybenzamine dilatation by BaCl₂ application. Marked arterial constriction is observed. (Small 5-0 silk suture adjacent to basilar artery.) X8*

FIGURE 7
*Fluorescent noradrenalin-containing periarterial nerve plexus. Rhesus basilar artery. X360*

*Stroke, Vol. 2, May-June 1971*
ADRENERGIC BLOCKADE

FIGURE 8
Freeze-dried fluorescent cross section of human anterior cerebral artery, removed during hemispherectomy. Inner folded fluorescent ring represents intima. Profuse noradrenalin fluorescence is observed external to the media. ×80

subsequent application of more concentrated dibenzylene (1 × 10⁻⁷ molar).

Catecholamine Fluorescent Studies
A normal vessel treated by the fluorescent technique reveals the presence of an abundant noradrenalin-containing periarterial nerve plexus. These fibers can be demonstrated in stretch preparations (fig. 7), and in cross section after freeze drying (fig. 8). After the application of tyramine to a vessel, the catecholamine fluorescent techniques reveal a virtual total depletion of the fluorescent noradrenalin stores in the periarterial nerves (fig. 9).

In contrast, after the application of dibenzylene to the vessel, fluorescent studies reveal an essentially normal noradrenalin content of the periarterial fiber plexus. While the techniques used are not quantitative, the catecholamine depletion produced by tyramine is in striking contrast to the essentially normal nerve plexus revealed after dibenzylene application.

Discussion
Under normal circumstances two major factors are operative in the regulation of cerebral blood flow. The first of these, a myogenic response, was originally described by Bayliss and has since been observed by numerous investigators. Common to each of the above studies was the observation that a reduction in cerebral arterial perfusion pressure was accompanied by dilatation of the cerebral arteries, whereas an elevation of arterial pressure was accompanied by constriction of the cerebral arteries. The resultant effect

FIGURE 9
Severe noradrenalin depletion after tyramine application. Compare with normal example shown in figure 7. ×170

Stroke, Vol. 2, May-June 1971

225
of such a mechanism was the maintenance of a constant blood supply to the brain despite wide variations in systemic arterial pressure. It is believed that this phenomenon, now widely recognized as the Bayliss effect, results from a true myogenic stretch reflex, increased transmural tension resulting in a shortening of vascular smooth muscle with consequent arterial caliber narrowing—increased resistance to blood flow being the result. Meyer and Denny-Brown have suggested that this phenomenon is of great importance in vessels of 50 to 20 μm diameter, and that this purely myogenic response is seen immediately after a reduction in perfusion pressure, and prior to the accumulation of acid metabolites and changes in pH or P CO₂.⁴¹ These authors propose that the initial mechanism protecting the brain against ischemic changes during a pathological interruption of blood flow is provided by this myogenic phenomenon.

A second, separate, and currently regarded as the most important mechanism in the control of cerebral blood flow is a metabolic one. An increase in CO₂, an increase in H⁺ concentration (due to lactic acidosis), or a significant decrease in O₂ will each result in vasodilatation of the cerebral vessels. Since the first experimental observation by Cow in 1911³⁶ of the effect of CO₂ upon the caliber of the isolated carotid artery, it has been firmly established that variations in arterial CO₂ levels produce striking alterations in cerebral arterial size and cerebral vascular resistance. The mechanisms and mediating pathways for this response are unknown.

The possibilities may include: (1) a direct effect of CO₂ upon vascular smooth muscle, (2) alterations in plasma H⁺ levels occurring in response to P CO₂ alterations, and in turn affecting vascular smooth muscle directly, (3) secondary alterations in CSF H⁺ levels, which exert a modulating influence over a brain stem vasomotor center, and (4) H⁺ or CO₂ effects being mediated through the periarterial nerves, either by altering the rate of release of noradrenalin from the nerve terminals or by altering the responsiveness of an H⁺ receptor to released transmitter substances.

Until recently it was assumed that CO₂ in some way directly affected the contractile ability of vascular smooth muscle,¹² ³⁶ but this notion, as the only explanation for the regulation of CBF,¹³ has received increasing criticism. Current data would suggest that H⁺ concentrations in the brain extracellular compartment and in the cerebrospinal fluid (CSF) (the compartments which compose the external milieu of the cerebral vessels) are responsible for cerebral vascular reactivity to CO₂.⁴⁻¹¹ ⁴² Undefined, however, is the location of this pH-sensitive site. Lassen¹⁰ has suggested that a local mechanism might be operative, whereby a change in the intracellular H⁺ concentration of the arterial smooth muscle causes a change in vascular tone and caliber.

The role of a brain stem center sensitive to changes in P CO₂ or pH has been repeatedly studied¹⁴⁻¹⁸ since the initial series of experimental observations of Shalit and co-workers¹⁸ allowed them to suggest the existence of a mesencephalic center responsive to changes in P CO₂ of the CSF. It has also been proposed that CO₂ utilizes two mediating sites: (1) vascular smooth muscle being affected directly, and (2) the reticular activating system.⁴³ Skinhøj and Paulson²⁰ have presented contrary evidence demonstrating the total lack of influence of P CO₂ changes in the posterior circulation upon total CBF. In fact, in their study a reduction in total CBF was observed in some cases when hypercapnic blood was perfused through the vertebral-basilar system. This was attributed to the high P CO₂ influencing the brain stem respiratory centers and causing in turn hyperventilation and hypocapnic-induced cerebral vasoconstriction. These data have been recently tested and reviewed by Kogure et al.¹⁹ These workers conclude that "clearcut evidence has accumulated to support the role of the brain stem in the regulation of cerebral blood flow and metabolism." These workers' own experimental data suggested that the brain stem center which influences CBF is not directly responsive to changes in P CO₂. They also suggest that CO₂ does act "directly on cortical vessels," but that these changes are "not a function of induced cortical pH changes alone." While confirming the classical notion of a direct local action by CO₂ - pH on intracranial vascular smooth muscle, no data were accumulated in regard to the mediating site for this response.¹⁰

In a recent review of this problem, Lassen¹⁰ concluded that there existed a local action by CO₂ on the arterial wall, which in
ADRENERGIC BLOCKADE

conjunction with the extravascular HCO₃⁻-determined the pH in the smooth muscle cells and in the surrounding tissues and simultaneously controlled smooth muscle contractability. He also noted that this hypothesis did not "preclude a role for the perivascular sympathetic fibers since a pH change might alter smooth muscle sensitivity to catecholamines." It is to this hypothesis that the present study was directed.

Previous studies in our laboratory utilizing the catecholamine fluorescent technique of Falck in cats, dogs, monkeys and human specimens have demonstrated an abundant noradrenalin-containing periarterial fiber plexus on all major intracranial vessels. This confirms the findings of Neilsen and Owman in the cat. Electron microscopic characterization of these periartrial waves has been carried out by Nelson in cats. These findings amplify earlier studies of parasympathetic and sympathetic intracranial arterial innervation. The major contributions allowed by the fluorescent technique are: (1) The nervous supply to the intracranial vessels is revealed as being extremely profuse on the larger intracranial vessels, fewer fibers being seen on the smaller arteries, and none at all on arterioles of less than 15 μm diameter, and (2) The sympathetic origin (superior cervical ganglion) and noradrenalin stores contained in these nerves are confirmed. Despite this demonstrably abundant noradrenergic innervation of the intracranial vessels, the physiology of these nerves remains obscure.

At a recent Cerebral Blood Flow Symposium, Falck et al. reported that hypocapnic-induced constriction of the cerebral vessels was prevented by B-adrenergic blocking agents. These authors noted that INPEA when administered to human subjects reversed the reduction in cerebral blood flow associated with a decreased P CO₂. They also reported preliminary data showing that pial vasoconstriction associated with hyperventilation was prevented on the side of prior removal of the superior cervical ganglion.

Additional pharmacological studies in man have been reported by Korein et al. These authors report that hyperventilation-induced slow wave changes in the EEG (presumed secondary to a reduction in cerebral blood flow) are prevented by the prior administration of a β-adrenergic blocking agent.

Bloor et al. have reported data in support of an important sympathetic cerebral vascular constrictor pathway. Using dye-dilution techniques in monkeys, a 25% decrease in CBF occurred after cervical sympathetic stimulation. Similar stimulation experiments by James and co-workers have produced supporting data using baboons. These workers reported significant changes in CBF occurring after both sympathetic and parasympathetic stimulation and suggested that the sympathetic system interacts with the effect of CO₂.

In spite of this collective evidence for both sympathetic and parasympathetic participation in CO₂-induced alterations in cerebral vascular resistance, in several primate species, including man, many investigators in the field do not assign the now widely documented periarterial noradrenalin-containing nerves an important role in the control of cerebral blood flow.

The present study, by demonstrating pharmacological blockade of the hypocapnic vasoconstrictive response, would suggest that a neural pathway does exist for the mediation of CO₂-induced cerebrovascular response.

Certain observations noted in the present study deserve further comment. First, it is well established that the primary site of resistance changes in the arterial system is at an arteriolar level. This remains the strategic site for alterations of blood flow and is presumably the most significant locus of action for the CO₂ vascular response. It should be noted that significant alterations in the caliber of the larger cerebral vessels do accompany changes in arterial P CO₂. These changes are easily observed through the operating microscope.

Similar arteriographical data have been reported elsewhere, demonstrating changes in caliber of the main branches of the circle of Willis in response to alterations in arterial P CO₂ in both normal vessels and vessels in spasm, the latter being due to the presence of subarachnoid blood.

Reference to figures 1 and 3 will demonstrate that hypocapnia induced by hyperventilation (P CO₂ 11 mm Hg) results in a measurable vasoconstriction, whereas significant vasodilation occurs with hypercapnic states (P CO₂ 66 mm Hg). This hypocapnic-induced vasoconstriction may be reversed with...
dilatation of the vessel to a larger than normal caliber by the intrathecal application of an alpha adrenergic blocking agent. Dibenzyline (phenoxybenzamine) is an extensively studied, long-acting alpha adrenergic blocking agent. It is known to inhibit the functioning alpha receptors without altering the function or catecholamine content of adrenergic nerves. Nor does it alter the basic response mechanism of effector cells, in this instance the capacity of vascular smooth muscle to contract. There are few data available in regard to the ability of this drug to cross the blood-brain barrier. According to Rothballer and Nickerson very high doses of phenoxybenzamine cause CNS effects, but these are not believed due to central alpha adrenergic blockade but probably related to the effects of hydrolyzed breakdown products of the agent. Presumably this agent does not enter the subarachnoid space in significant concentration after parenteral administration.

In the current study it was necessary to demonstrate the preservation of the contractile ability of the vascular smooth muscle after the application of phenoxybenzamine in order to exclude the possibility of a toxic paralysis of the artery. This was tested by the topical application of barium chloride. This agent was used by Cow and is presumed not to require neural mediation for its effect. Its application in the present study resulted in immediate and intense vasoconstriction, even after the application of high concentrations (1 × 10⁻² molar) of phenoxybenzamine (figs. 5 and 6). Smooth muscle contractility, therefore, had been retained.

It is significant that following the application of dibenzyline, histochemical fluorescent studies of the basilar and vertebral arteries revealed a normal catecholamine content in the periarterial nerve endings. While this histological method is not a quantitative one, the possibility that exhaustion of the periarterial nerve terminal catecholamine content was responsible for the arteries’ dilated and nonreactive state was excluded.

Further pharmacological support for a receptor site locus for the effect of hypocapnia is provided by the action of tyramine. Following the application of this agent under hypocapnic conditions, only transient dilatation of the basilar and vertebral arteries was observed with a rapid return to the constricted state. At the end of the experiment, fluorescent techniques revealed a virtual total depletion of the periarterial noradrenergic stores following application of this agent (fig. 8). Hypocapnic vasoconstriction, therefore, takes place in the absence of periarterial noradrenergic stores.

If, as these data suggest, the noradrenergic periarterial system does offer a mediating site for CO₂-induced alterations in cerebral vascular tone, it is of interest to speculate on the nature of this mechanism. Since our experiment has approached this phenomenon by manipulation of the components of the neuromuscular junction at a local arterial level, we cannot provide data regarding the existence or effectiveness of a remote brain stem vasomotor center. If, as has been suggested, CO₂ exerts its effect at two foci: (1) directly in vascular smooth muscle, and (2) via a remote brain stem vasomotor center (which in turn secondarily alters cerebral vascular tone), the present study would suggest that both mechanisms are interrupted by alpha adrenergic blockade. The alpha adrenergic receptor, then, may be the final common mediating pathway in the hypocapnic vasoconstrictive response.

Electron microscopic characterization of the intracranial vascular nerves has provided only preliminary data regarding the anatomy of the neuromuscular junction. Dense core vesicles (presumably containing noradrenalin) have been observed in periarterial nerve terminals as close as 980 Å from the vascular smooth muscle cells. These same workers have suggested that “a morphological substrate does exist for effective nervous control of the cerebral vessels.”

The present study would suggest that the effect of hypocapnia or a decrease in H⁺ concentration is mediated through the alpha adrenergic receptor, possibly by altering its responsiveness to circulating or local transmitter substances. CO₂ or H⁺ would not appear to act by causing a release of periarterial noradrenalin stores, as pharmacological depletion of these stores does not prevent vascular responses occurring in response to P₀₂ alterations. Alpha adrenergic blockade would, of course, prevent the transmission of a normally mediated constrictive effect by functionally removing the receptor site.
**ADRENERGIC BLOCKADE**

It is readily apparent, however, that the present data are derived solely from experiments with large caliber vessels. Further, the major site of cerebral vascular resistance is at an arteriolar level for which no data from this study have been obtained. It is conceivable that the periarterial noradrenergic system exerts little influence over the caliber of these smaller vessels, which remain the strategic site for alterations in CBF. Some support for this thesis is suggested by the diminution in the number of periarterial fibers demonstrated by the fluorescent technique in the smaller cerebral vessels, no fibers at all being seen in vessels of 15 to 20 μm in size.81

It would seem unlikely that the smaller cerebral vessels have markedly different pharmacological properties from their larger parent vessels. However, this issue is not settled by our present study. Previously cited data61-64 would suggest, however, that neural influences do extend to the resistance vessels of the cerebral vascular tree.

In order to provide further data regarding this aspect, studies using a cortical window and a similar protocol are being utilized in current experiments. Certain technical problems are introduced with this technique because of the limited dimensions and accessibility of the cortical subarachnoid space.

**Summary**

The previous demonstration of the presence of an abundant adrenergic periarterial fiber plexus and the apparent reversal of blood-induced cerebral arterial spasm by alpha adrenergic blockade formed the initiative for the present study.

As part of the study of the reactive properties of the cerebral vessels, the basilar and vertebral arteries of nine Rhesus monkeys were exposed by the transclival route.

Under carefully controlled conditions, alternate hypercapnic and hypocapnic states were produced. Marked enlargement of the basilar and vertebral arteries was observed with hypercapnia. Hypocapnia caused severe constriction of these same vessels. All vessel caliber changes were documented by photographs through the operating microscope.

By the introduction of sympathetic agents into the subarachnoid space, we attempted to modify arterial constriction associated with low Pco₂ levels.

The present study has shown the following: (1) Alterations in Pco₂ are associated with changes in caliber of the major intracranial vessels. (2) Mere depletion of the periarterial noradrenalin stores does not prevent hypocapnic-induced alterations of cerebral arterial caliber. (3) Alpha adrenergic receptor blockade removes hypocapnic arterial constriction. (4) Vascular smooth muscle contractility was shown to be preserved under these conditions.

We have suggested that the alpha adrenergic receptor may be a mediating site for hypocapnic constriction of the intracranial vessels.

We have also proposed that the alpha adrenergic receptor may be H⁺ sensitive, so that changes in pH (caused by changes in CO₂ levels) alter the responsiveness of the receptor to transmitter substances.

**References**

ADRENERGIC BLOCKADE

Adrenergic Blockade of Hypocapnic Cerebral Arterial Constriction
RICHARD A. R. FRASER, BENNETT M. STEIN and J. LAWRENCE POOL

Stroke. 1971;2:219-231
doi: 10.1161/01.STR.2.3.219

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1971 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

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/2/3/219

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/