

Parasympathetic Cholinergic Control of Cerebral Blood Flow in Dogs

LOUIS G. D'ALECY AND CYNTHIA J. ROSE

SUMMARY We studied the effects of cholinergic receptor activation on cerebral blood flow in dogs anesthetized with chloralose. Continuous measurements of cerebral blood flow, arterial and cerebral spinal fluid pressure, heart rate, and respiratory carbon dioxide tension were made during parasympathetic nerve stimulation and during intra-arterial infusion of acetylcholine. Multiple samples of arterial and cerebral venous blood were taken before, during, and after cholinergic vasodilation and analyzed for oxygen tension, carbon dioxide tension, and pH. Dose-response curves obtained by intra-arterial infusion of acetylcholine at 0.27–1,080 $\mu\text{g}/\text{min}$ and stimulation frequency-response curves obtained by excitation of the major petrosal nerve at 2–40 Hz demonstrated a dose or frequency-dependent cerebral vasodilation. The maximum cerebral vasodilation (171% of control flow) was obtained with an acetylcholine infusion of 1,080 $\mu\text{g}/\text{min}$. During infusion of 27 μg of acetylcholine/min arterial blood gases showed little or no change and thus could not have produced the observed change in cerebral blood flow. The changes in cerebral venous blood were all consistent with the observed increase in cerebral blood flow; oxygen tension rose from 30.4 to 36.0 mm Hg, carbon dioxide tension fell from 45.7 to 42.3 mm Hg, and pH rose from 7.342 to 7.360. Ipsilateral stimulation of the major petrosal nerve at 10 Hz, with a 3-msec pulse duration and 60-second stimulation period, produced an increase in cerebral blood flow to 111% of control flow. Cholinergic receptor blockade with atropine (1 mg/kg, i.v.) completely eliminated the cerebral vasodilation produced by acetylcholine infusion at 27 $\mu\text{g}/\text{min}$ and significantly reduced the vasodilation resulting from major petrosal nerve stimulation. We conclude that the cerebral circulation has the capacity for significant cholinergic vasodilation.

THE ABUNDANT anatomical evidence demonstrating the existence of nerve fibers on the cerebral vessels has recently been reviewed.¹ The sympathetic autonomic component of this innervation has been shown to have the capacity for marked cerebral vasoconstriction via an α -adrenergic receptor mechanism.^{2,3} A parasympathetic cerebral vasodilator mechanism was suggested by early workers when they observed pial vessel dilation in response to cranial nerve stimulation.⁴ More recently, intravertebral atropine was shown to suppress the dilation resulting from inhalation of 5% CO_2 ,⁵ and intravertebral neostigmine enhanced cerebral vasodilator reactivity to CO_2 .⁶ Atropine was also shown to eliminate "autoregulatory dilation" in unanesthetized rabbits.⁷ Each of these observations is consistent with a parasympathetic cholinergic vasodilator mechanism for the cerebral circulation. None of these studies demonstrates a parasympathetic cholinergic increase in cerebral blood flow. A cholinergic vasodilator mechanism to complement the adrenergic vasoconstrictor mechanism has, therefore, not been clearly defined for the control of cerebral blood flow. In the present study, parasympathetic nerve stimulation and intra-arterial infusion of a cholinergic agonist, acetylcholine, were used to test for a possible parasympathetic cholinergic cerebral vasodilator mechanism.

Methods

GENERAL PREPARATION

Adult male dogs weighing between 17.0 and 30.9 kg were anesthetized with α -chloralose (100–120 mg/kg of

body weight, iv), and anesthesia was maintained by continuous infusion (40 mg/kg per hour) or by hourly supplements as required. Each dog was mechanically ventilated (Harvard 607 respiration pump) via an intratracheal tube at 15 breaths/min. The tidal volume was adjusted to give an end-expiratory carbon dioxide tension between 4.5% and 5.0%, as monitored by an infrared analyzer (Beckman LB-2). Esophageal temperature at the level of the heart was monitored and used to control body temperature at 39°C with a heating pad and proportional controller. Arterial pH was adjusted by intravenous infusion of 1.5% sodium bicarbonate to approximately 7.41. Central arterial pressure was measured in the arch of the aorta through a 75-cm polyethylene (PE 260 Intramedic) cannula passed from the femoral artery. Mean arterial pressure and heart rate were electronically determined from the arterial pressure pulse. Cerebrospinal fluid pressure was measured through a 30-cm section of saline-filled polyethylene tubing (PE 90 Intramedic) inserted into the cisterna magna through a puncture of the atlantooccipital membrane. Continuous recordings of cerebral blood flow, mean arterial pressure, respiratory carbon dioxide tension, cerebrospinal fluid pressure, heart rate, and pulsatile arterial pressure were made on a six-channel oscillograph (Gould Brush 200).

CEREBRAL BLOOD FLOW PREPARATION

The cerebral venous outflow preparation used in this study has been described for previous studies of the autonomic control of the cerebral circulation.^{2,3} An uncontaminated measurement of cerebral blood flow is obtained from the dorsal (cerebral) drainage system of the head without compromising the ventral (extracerebral) drainage system. The sigmoid sinuses are occluded within the occipital bone with heparinized cotton pellets without opening the cranium. The occipital emissary vein is cau-

From the Department of Physiology, University of Michigan, Ann Arbor, Michigan.

Supported by American and Michigan Heart Associations and U.S. Public Health Service Grants 5R01-NS12386-02.

Received November 10, 1976; accepted for publication January 31, 1977.

terized during the exposure of the bone over the sigmoid sinuses. The right temporal sinus is occluded downstream with pellets and cannulated toward the midline for withdrawal of cerebral venous blood samples. The left temporal sinus is exposed for transducer placement by thinning the bone, with a No. 1 rotary bone file, at the junction of the superior nuchal ridge and the dorsal margin of the zygomatic arch. At this location the temporal sinus is completely surrounded by bone as it forms the lateral extension of the transverse sinus. We currently use an ultrasonic flowmeter (American Ultrasonics Laboratories, model 1012) rather than the electromagnetic flowmeter used in previous studies. Doppler transmit and receive crystals, mounted on a plastic block, are positioned over the sinus and secured with stainless steel screws and suture wire. Quick-cure acrylic is coated over the screws, suture, and transducer. This is a unique application of Doppler technology inasmuch as the angular relationships between transmit-receive crystals and the axial flow of blood is fixed in the bone. The dural lining of the sinus adheres to the bone in the same way as periosteum. Thus there is no dimensional instability in this configuration; this allows calibration of each preparation at the end of the experiment by cannulation of the left temporal sinus and collection of a volume of blood for timed intervals. Four representative calibration curves are presented in Figure 1, to show the relationship between collected cerebral blood flow (milliliters per minute) and the visually averaged oscillograph record of flowmeter output (millivolts). Least square regression analysis for each series of points was used to compute the calibration factor, as the slope of the regression line. The average r^2 for 13 calibration lines was 0.992 ± 0.003 SEM.

The retrograde infusion of acrylic is the final verification procedure which determines the completeness of the dor-

sal-ventral venous sinus separation and tests for the presence of the anastomotic branch of the dorsal petrosal sinus. At the end of each experiment the dog was heparinized (750 U/kg of body weight) for the calibration procedure and then killed by iv infusion of 50 ml of saturated potassium chloride. A thin mix (monomer to polymer ratio, 1:1 by volume) of acrylic (Stratford Cookson Tramix) was infused into the cerebral venous drainage system through the calibration cannula in the left temporal sinus, at a constant pressure of 25 mm Hg. The acrylic mixture continued to flow for 15–20 minutes and was left to harden for 30–40 minutes. Vessels as small as 125 μ m regularly were found to have filled when dissected post mortem. The head was removed from the atlas and the occlusions of the right and left sigmoid and temporal sinuses were examined. The cranium was opened and the brain removed for examination of the uniformity of perfusion. The perfused brain was weighed for calculation of cerebral blood flow in ml/min per 100 g. The cavernous sinuses were opened to check for the presence of acrylic in the area of the sella turcica; this would indicate the existence of the anastomotic branch of the dorsal petrosal sinus. In previous studies it was noted that one out of three of the preparations had to be discarded because of the existence of the anastomotic branch of the dorsal petrosal sinus which communicates with extracerebral flow channels.² It was noted at that time that these dogs also had "dew" claws on their hindlegs. In the present study dogs with "dew" claws were avoided and none of the preparations was discarded because of the presence of anastomotic veins. In each dog the dura and bone in the area of the sigmoid and temporal sinuses were removed from the inside, to check for the adequacy of the occlusions in each area. Data from dogs with incomplete occlusions (two dogs) or anastomotic veins (none) were excluded from this study because in these cases cerebral blood flow may have been contaminated.

INTRA-ARTERIAL INFUSION PREPARATION

The aortic arch was exposed by using a rib-spreader placed in a left thoracotomy at the 3rd intercostal space. The left subclavian artery was exposed by blunt dissection and occluded with a bulldog clamp. The roots of the right subclavian and common carotid arteries were exposed at their origin from the brachiocephalic artery with the aid of a thermal cautery and blunt dissection. The right subclavian artery was occluded with a bulldog clamp so that only the common carotid arteries received blood from the brachiocephalic artery (Fig. 2). Any small branches of the brachiocephalic artery were ligated. An 18-gauge needle, bent into a fishhook shape, was connected to a 35-cm section of polyethylene tubing (PE 100 Intramedic). The needle, tubing, and connectors were siliconized (Siliclad, 1:100 dilution) to minimize clot formation. The fishhook needle was inserted in the proximal portion of the brachiocephalic artery and secured in place with a ligature to maintain the needle parallel to the long axis of the vessel and with the opening of the needle facing craniad. A loop of the tubing was secured to the chest wall to minimize disruption of the needle placement by manipulation of the pump end of the tubing. The rib-spreader was then removed and the lungs were reinflated. At the end of the

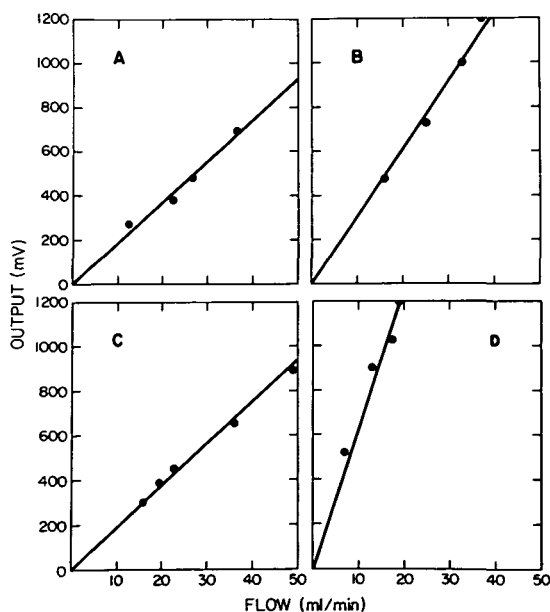


FIGURE 1 Four individual plots of blood flow (ml/min) vs. flowmeter output (mV); the lines represent a linear least squares regression analysis through zero. A calibration, plot, and regression analysis were made for each dog from 4 or 5 data points taken just before death, by collecting cerebral venous outflow for timed intervals and noting flowmeter output in millivolts.

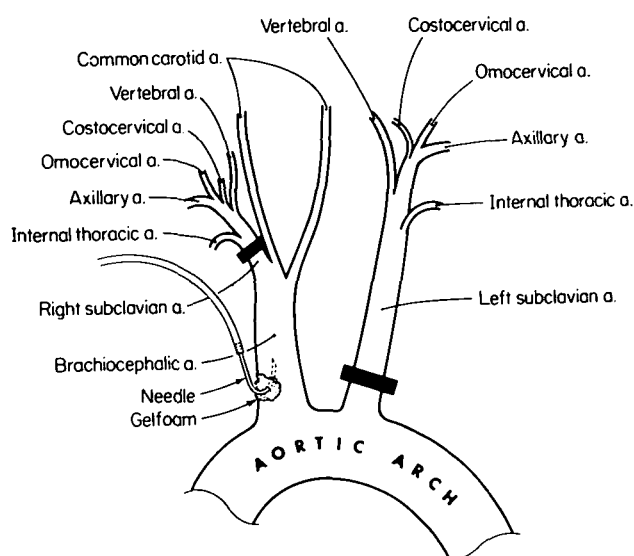


FIGURE 2 A schematic representation of the arch of the aorta and its major branches in the dog. The black bars on the roots of the right and left subclavian arteries indicate the points of occlusion. The right and left common carotid arteries were the only remaining source of blood to the head. Infusate entered the circulation via needle in brachiocephalic artery and was distributed to common carotid arteries, thus avoiding dilution in general systemic circulation and dilution by the vertebral arteries.

experiment the arterial occlusions were checked by dissection.

This preparation restricts the distribution of the infusate to the head and neck and the infusate is not diluted by an unpredictable fraction of blood from the vertebral or other arteries. Minimal systemic effects of the infusate are thus obtained. The infusion line was connected through a three-way stopcock to a 10-ml glass syringe, mounted in an infusion pump (Harvard 600). The infusion rates were 1.09 or 4.35 ml/min. Control infusions of saline at these two infusion rates had no effect on cerebral blood flow. Four different concentrations (0.25, 2.5, 25, 250 $\mu\text{g}/\text{ml}$) of acetylcholine were used, thus providing infusion doses of 0.271, 1.08, 2.71, 10.8, 27.1, 108, 271, and 1,080 $\mu\text{g}/\text{min}$. A stock solution of acetylcholine chloride (1,000 $\mu\text{g}/\text{ml}$) (Sigma) in normal saline was prepared by rapidly weighing out the crystalline salt which had been stored desiccated and below 0°C . The stock solution was stored at 0°C and samples were diluted daily for the required concentrations which in turn were kept in an ice bath until placed in the infusion pump.

PARASYMPATHETIC NERVE STIMULATION PREPARATION

The left major petrosal (greater superficial petrosal in man) nerve was approached for stimulation by dissecting along the course of the facial nerve (VII) through the petrous portion of the temporal bone, to the medial aspect of the middle ear. At the genu of the facial nerve the major petrosal nerve courses anteromedially within the bone is free from the facial nerve. The central and peripheral ends of the genu were sectioned thus leaving only the genu connected to the major petrosal nerve. This was

done to localize the stimulation pulses to the major petrosal nerve. It was necessary to remove the stapedius muscle and the tensor tympani muscle in order to gain access to the nerve. This often resulted in bleeding which was difficult to control with pressure or thermal cautery. Great care was required to avoid fracture of the petrous bone into the cranial cavity. These surgical complexities often resulted in failure at this stage of the preparation.

Trains of rectangular stimuli, 3 msec in duration, at frequencies of 2, 5, 10, 20, and 40 Hz and lasting 90 seconds were delivered by unipolar or bipolar concentric platinum electrodes to the major petrosal nerve. The voltage was selected to give maximum response at a given frequency (20 Hz) and then the same voltage was used for all frequencies. Local bleeding or spinal fluid leakage along the facial nerve often produced unacceptable current spread as indicated by muscular twitching. Only responses with no twitching were analyzed.

BLOOD GAS DETERMINATIONS

Arterial and cerebral venous blood samples were analyzed at 39°C for oxygen and carbon dioxide tension and for pH to determine whether blood gas changes could account for the observed cerebral vasodilation. An acetylcholine infusion dose of 27 $\mu\text{g}/\text{min}$ was selected for use in the experiments in which blood gases were measured because preliminary dose-response data indicated this produced a near maximum dilation. A 1-minute infusion was used for the arterial sampling sequence and a 2-minute infusion was used for the cerebral venous sampling sequence. The longer infusion time for the venous sampling sequence was chosen to facilitate obtaining a representative cerebral venous sample, by allowing for vascular transit time through the brain. Six infusion sequences, alternating between the arterial and cerebral venous sequences, were performed approximately 11 minutes apart. For each sequence, samples were taken approximately 3 minutes before infusion, 30 seconds before the end of the infusion, and 3 minutes after the infusion.

A total of nine arterial and nine cerebral venous samples were obtained from each dog. To facilitate arterial sampling, a 3-cm section of heavy-walled silicone tubing was substituted for a section of the femoral artery. The cerebral venous sample was taken from the right temporal sinus by drawing blood through a 15-cm section of polyethylene tubing (PE 260 Intramedic) into a 3-cm section of heavy-walled silicone tubing. For each sample a 12-mm, 26-gauge needle was used to puncture the silicone tubing and draw a 1.5-ml blood sample into a heparinized 2-ml glass syringe. Within 10 seconds after sampling, the blood was within the analysis cuvette. The oxygen, carbon dioxide, and pH determinations were completed within 2.5–3 minutes. Calibration of the blood gas electrodes was performed just before, and checked immediately after, the experimental sequences with two analyzed gases (10.00% carbon dioxide in nitrogen and 5.00% carbon dioxide with 12.00% oxygen in nitrogen). The pH electrode was calibrated with buffers prepared in accordance with the directives of the National Bureau of Standards at 6.84 and 7.381. Within 1 minute after calibration the first sample

was introduced; after flushing, the calibration was checked, and the test was accepted if the calibrations changed by no more than ± 1.5 mm Hg for oxygen, ± 1.0 mm Hg for carbon dioxide, and ± 0.005 pH units.

DATA ANALYSIS

Blood Flow and Stimulation Frequency Curve

Analog records of mean cerebral blood flow were visually averaged by taking the value of a straight line through the 1-minute period of the oscillograph record prior to each stimulation and for approximately 1 minute during the plateau of the response to stimulation. The visual averaging procedure is shown to be accurate by the average r^2 value of 0.992 for the regression analysis done for each calibration *in vivo*. The response to each stimulation frequency was expressed as percent of the control flow taken just prior to each stimulation.

Dose-Response Curve

Analog records of mean cerebral blood flow were visually averaged for the 1-minute period prior to each infusion and for approximately 30 seconds during the plateau of the response. The calibration for each flow transducer placement and the brain weight from the anterior cranial fossa was used to express each flow in ml/min per 100 g. Flow was also expressed as the percent of the corresponding control flow for each infusion. Three tests were made for each dose in each of five dogs (only four dogs were used for the infusion doses of 271 and 1,080 $\mu\text{g}/\text{min}$). Two composite dose-response curves were computed by averaging 15 observations (12 for the two highest doses), three observations per dose in each of five dogs, and expressing the results as percent of control flow and as flow in ml/min per 100 g.

Cholinergic Blockade

Analog records of mean cerebral blood flow were read as described and cerebral blood flow expressed in ml/min per 100 g. Control flow was compared to flow during stimulation or infusion of acetylcholine at 27 $\mu\text{g}/\text{min}$ before and after atropine sulfate, 1.0 mg/kg, *iv*.

Blood Gas Experiments

Analog records of cerebral blood flow, mean arterial blood pressure, heart rate, and cerebrospinal fluid pressure were read for 1 minute prior to infusion, the last 30 seconds during the infusion, and 1 minute following the completion of the response. The average response for each variable to six infusions in each dog was computed and expressed in ml/min per 100 g, mm Hg, and beats/min for each dog. A total of 30 observations (27 for cerebrospinal fluid pressure) were made in five dogs for the preinfusion period, the infusion period, and the postinfusion period. During three of the infusion periods arterial samples were taken and during the alternate three infusion periods cerebral venous samples were taken. A total of 15 arterial and 15 cerebral venous observations were made in five dogs for the preinfusion period, the infusion period, and the postinfusion period. Each arterial or cerebral venous sam-

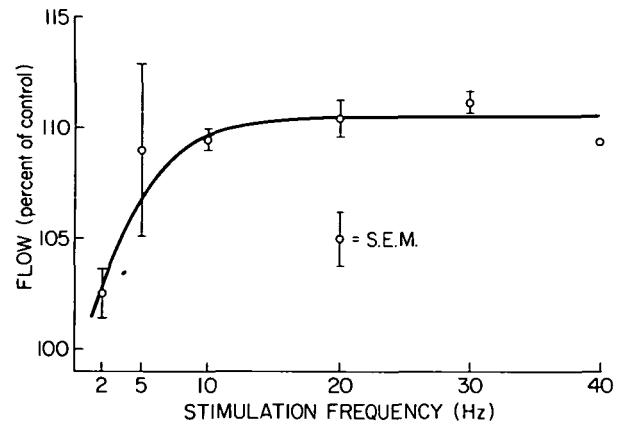


FIGURE 3 A graded vasodilation was observed with parasympathetic stimulation. The vasodilation increased with the increase in the frequency of stimulation from 0 to 40 Hz. The voltage, duration, and train duration were held constant for each of the six stimulation frequencies (3–5 V, 3 msec, 90 seconds). Each point is the average of four observations in three dogs (except 40 Hz, only one observation). Dashes indicate standard error of the mean based on $n=3$.

ple was analyzed for oxygen tension, carbon dioxide tension, and pH. The preinfusion and infusion periods were compared by paired *t*-test with $n = 5$ and 4 degrees of freedom.

Results

MAJOR PETROSAL NERVE STIMULATION

Stimulation of the major petrosal nerve produced a frequency-dependent increase in cerebral blood flow that reached a maximum of approximately an 11% increase in flow (Fig. 3). The increase in cerebral blood flow was statistically significant ($n = 3$) using the paired *t*-test ($P < 0.009$) at 10, 20, and 30 Hz. No change in respiratory carbon dioxide tension was observed before, during, or after stimulations.

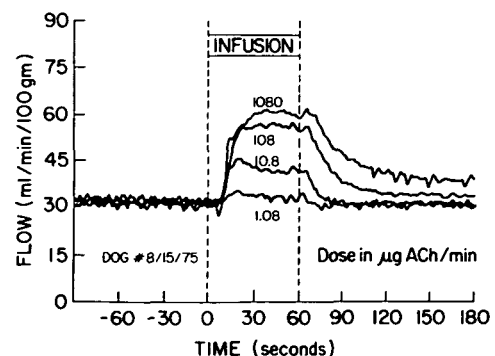


FIGURE 4 Intra-arterial infusion of acetylcholine (ACh) produced a dose-dependent increase in cerebral blood flow. The figure shows a composite oscillograph tracing of the cerebral blood flow response to four of the eight acetylcholine infusion doses given in this experiment. After an initial delay cerebral blood flow increased rapidly to a plateau and, when the infusion was stopped, the flow slowly returned to control levels.

ACETYLCHOLINE DOSE-RESPONSE CURVE

Intra-arterial infusion of acetylcholine in doses of 0.271–1,080 $\mu\text{g}/\text{min}$ produced a statistically significant dose-dependent increase in cerebral blood flow that reached a maximum of 60% above control flow. The average of all the control flow determinations for all doses for all dogs was 34.48 ± 2.66 (SEM) ml/min per 100 g, and the average maximum response to 1,080 $\mu\text{g}/\text{min}$ was 64.6 ± 4.27 ml/min per 100 g. A composite oscillograph record from one dog is shown in Figure 4. Dose-response curves in ml/min/100 g are shown in Figure 5, and on the basis of percent of control flow in Figure 6.

CHOLINERGIC BLOCKADE WITH ATROPINE

The average control flow for 10 observations in five dogs before atropine was 33.16 ± 2.54 (SEM) ml/min per 100 g. During infusion of acetylcholine (27 $\mu\text{g}/\text{min}$) cerebral blood flow increased to 48.76 ± 2.94 ml/min per 100 g (10 observations in five dogs). After the administration of atropine (1.0 mg/kg iv) control flow was essentially unchanged at 34.12 ± 2.88 ml/min per 100 g. During infusion of acetylcholine in the presence of cholinergic blockade cerebral blood flow averaged 34.12 ± 2.88 (SEM) ml/min per 100 g and indicated complete blockade (Fig. 7). Six stimulations (four at 20 Hz and two at 30 Hz) of the major petrosal nerve in three dogs produced an increase of $8.95 \pm 1.08\%$ in cerebral blood flow prior to atropine and an increase of $2.30 \pm 1.97\%$ after atropine; these results indicate a statistically significant (paired *t*-test, $P < 0.014$) blockade of the response to nerve stimulation ($n = 3$).

BLOOD GAS RESULTS

The average of 30 observations (27 for cerebrospinal fluid pressure), six in each of five dogs, of cerebral blood flow, mean arterial pressure, heart rate, and cerebrospinal fluid pressure before, during, and after infusion of acetylcholine (27 $\mu\text{g}/\text{min}$) are presented in Table 1. Cerebral blood flow increased 54.8%, from 34.43 ± 4.66 (SEM)

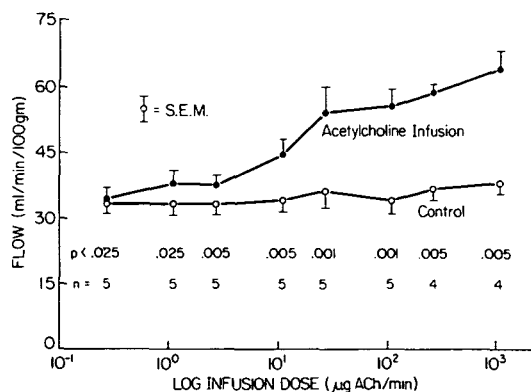


FIGURE 5 A graded cerebral vasodilation was observed with intra-arterial infusion of acetylcholine. A statistically significant increase ($P < 0.005$) over control flow was observed for acetylcholine infusions of from 2.71 to 1,080 $\mu\text{g}/\text{min}$. Cerebral blood flow was normalized to ml/min per 100 gm, using individual calibration curves and brain weight from the anterior cranial fossa. Dashes indicate standard error of the mean.

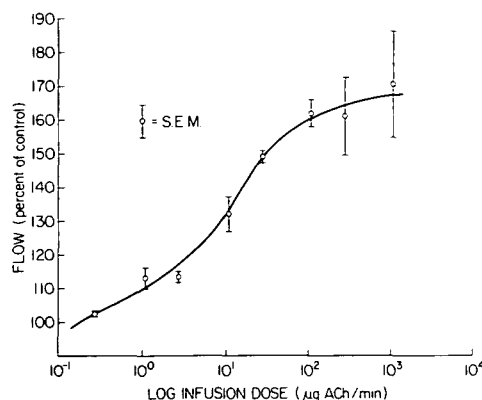


FIGURE 6 The data from Figure 5 are replotted by normalizing cerebral blood flow to percent of control. The control flow was computed as the average of the flow for 1 minute just before the start of each infusion. Dashes indicate standard error of the mean.

ml/min per 100 g to 52.15 ± 5.51 ml/min per 100 g. Mean arterial pressure and cerebrospinal fluid pressure increased very slightly (2.6 and 3.1 mm Hg, respectively), and heart rate increased by 17 beats/min. Arterial oxygen tension, carbon dioxide tension, and pH were essentially unchanged. Cerebral venous oxygen tension increased by 18.4% (from 30.4 to 36.0 mm Hg), carbon dioxide tension decreased by 7.5% (45.7 to 42.3 mm Hg), and pH increased from 7.342 to 7.360 units. The average of three observations for each dog on arterial and cerebral venous

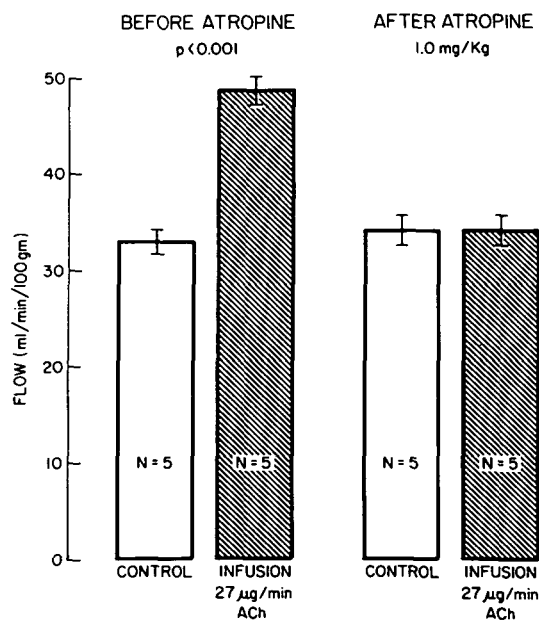


FIGURE 7 Each bar represents the average of five observations (one each in five dogs). The open bars represent the control flow taken just before each infusion. The cross-hatched bars represent cerebral blood flow during infusion of acetylcholine (27 $\mu\text{g}/\text{min}$). The two bars on the left show a statistically significant increase ($P < 0.001$) in cerebral blood flow during infusion, before atropine. The two bars on the right show no change in control flow (before or after atropine) or in flow during infusion after the administration of atropine (1.0 mg/kg).

TABLE 1 Overall Status in Blood Gas Experiments

	<i>n</i>	No. of observations	Preinfusion	Acetylcholine infusion	Differences (infusion - preinfusion)	Postinfusion
Cerebral blood flow (ml/min per 100 g)	5	30	34.43 ± 4.66	52.15 ± 5.51	17.72*	34.06 ± 4.44
Mean arterial blood pressure (mm Hg)	5	30	130.1 ± 5.2	132.6 ± 3.3	2.6	128.8 ± 5.6
Heart rate (beats/min)	5	30	157.5 ± 7.7	174.6 ± 6.1	17.1†	159.3 ± 7.4
Cerebral spinal fluid pressure (mm Hg)	5	27	5.8 ± 2.2	8.9 ± 3.3	3.1	5.9 ± 2.2
Arterial Po ₂ (mm Hg)	5	15	80.3 ± 3.6	83.5 ± 3.7	3.33†	82.1 ± 3.5
Arterial Pco ₂ (mm Hg)	5	15	34.6 ± 0.6	34.2 ± 0.8	-0.406	33.9 ± 0.6
Arterial pH	5	15	7.417 ± 0.017	7.415 ± 0.018	0.00140	7.418 ± 0.016
Cerebral venous Po ₂ (mm Hg)	5	15	30.4 ± 1.7	36.0 ± 2.9	5.58‡	28.7 ± 1.6
Cerebral venous Pco ₂ (mm Hg)	5	15	45.7 ± 0.9	42.3 ± 0.6	-3.426*	45.7 ± 1.2
Cerebral venous pH	5	15	7.342 ± 0.017	7.360 ± 0.014	-0.01728†	7.345 ± 0.016

Results are expressed as mean ± SEM; *n* = number of dogs.

The averaged cardiovascular variables include the observations from both the arterial and cerebral venous sampling sequences (six observations in each of five dogs) (except cerebrospinal fluid pressure, three observations in one dog missing). All standard errors were computed with 4 degrees of freedom (*n* = 5). Statistical significance of differences was tested with the paired *t*-test. Unsymbolized differences were not statistically significant (*P* > 0.06). Infusion of acetylcholine (27 μg/min) produced a 54.8% increase in cerebral blood flow with little or no change in arterial blood gases.

* *P* < 0.01.

† *P* < 0.03.

‡ *P* < 0.06.

oxygen tension, carbon dioxide tension, and pH for the blood samples taken before, during, and after infusion of the acetylcholine (27 μg/min) are presented in Table 2.

Discussion

The existence of an autonomic sympathetic vasoconstrictor mechanism for the cerebral circulation has been demonstrated by several laboratories.^{2, 8-10} In the coronary vascular bed there exists, in addition to sympathetic control, a parasympathetic control mechanism^{11, 12} which has the opposite effect of the sympathetics on blood flow. The present study clearly demonstrates the existence of cholinergic receptors in the cerebrovascular tree. The existence of these receptors is a necessary prerequisite for the operation of a parasympathetic cholinergic cerebral vasodilator mechanism.

Acetylcholine infusion produced a dose-dependent increase in cerebral blood flow that occurred with little or no change in arterial blood pressure, oxygen tension, carbon dioxide tension, or pH. The increase in heart rate and arterial blood pressure most likely is attributable to relaxation of the walls of the carotid sinus by the action of acetylcholine (decreasing stretch) which would induce a baroreceptor reflex to increase heart rate and blood pressure. This response, if anything, would attenuate the observed cerebral vasodilation by activating sympathetic vasoconstrictor fibers which have been shown to decrease cerebral blood flow.² The cerebral vasodilation produced by acetylcholine or nerve stimulation was blocked by atropine (1.0 mg/kg); this defined the response as a muscarinic cholinergic vasodilation and not a nonspecific dilation due to choline or acetic acid.

TABLE 2 Individual Blood Gases and pH

	Po ₂ (mm Hg)			Pco ₂ (mm Hg)			pH units		
	Pre	Inf	Post	Pre	Inf	Post	Pre	Inf	Post
Dog 1									
Arterial	82.0	86.0	85.5	32.8	32.0	32.2	7.432	7.441	7.435
Cerebral venous	29.3	29.7	27.7	43.6	41.3	42.3	7.362	7.378	7.377
Dog 2									
Arterial	80.9	86.2	81.5	33.3	32.5	32.9	7.473	7.467	7.471
Cerebral venous	25.8	28.2	24.7	44.0	42.0	44.0	7.394	7.407	7.387
Dog 3									
Arterial	76.2	80.7	80.0	35.4	35.7	34.8	7.418	7.417	7.418
Cerebral venous	30.7	39.0	27.7	48.7	44.0	49.3	7.344	7.347	7.338
Dog 4									
Arterial	92.0	93.3	92.4	35.8	35.2	34.9	7.380	7.371	7.384
Cerebral venous	36.3	40.8	34.7	46.5	43.5	47.0	7.307	7.340	7.317
Dog 5									
Arterial	70.3	71.2	71.0	35.3	35.3	34.4	7.383	7.378	7.382
Cerebral venous	29.8	42.2	28.7	45.8	40.7	45.7	7.305	7.330	7.305

Arterial and cerebral venous values are the average of three observations per dog. Samples were taken 3 minutes before acetylcholine (27 μg/min) infusion (Pre), 30 seconds before the end of the infusion (Inf), and 3 minutes after the end of the infusion (Post).

The extent to which the cholinergic receptors on the cerebral vessels are innervated by the parasympathetic nervous system is more difficult to evaluate. Stimulation of the left major petrosal nerve produced a maximal cerebral vasodilation that was approximately one-sixth (10% vs. 60% increase in flow) that produced by acetylcholine. This discrepancy is attributed, at least in part, to the ipsilateral distribution of the left major petrosal nerve. There are, however, three cranial nerves which distribute parasympathetic fibers to the head (oculomotor, facial, and glossopharyngeal). If one speculates that the total parasympathetic innervation of the cerebral vessels is evenly distributed among the right and left oculomotor, right and left facial (major petrosal), and right and left glossopharyngeal nerves, then the stimulation used in this study activated only one-sixth the total parasympathetic effector mechanisms on the cerebral vessels. If this estimate of one-sixth for the parasympathetic contribution of the left major petrosal nerve is accurate, then nearly all of the cholinergic effectors are innervated. Early studies would suggest, however, that the stimulation of the left major petrosal nerve represented activation of half (right plus left) the total innervation (i.e., no contribution from the 3rd and 9th cranial nerves).^{4, 13} If this is the case then the majority (four-sixths or 66%) of the cholinergic effectors in this bed are not innervated. This study can give direct evidence only for the left major petrosal nerve as contributing to an innervated cholinergic cerebral vasodilator mechanism. Therefore, at least 33% of the cholinergic receptors are innervated and as many as 100% of the receptors could be innervated.

The observed vasodilation during cholinergic receptor activation appears to be an active dilation rather than the release of tonic vasoconstriction or facilitation of tonic vasodilation. In a previous study using the same preparation, α -receptor blockade with dibozane (1 or 2 mg/kg) or phentolamine (2 mg/kg) produced no change in resting cerebral blood flow.² This indicates that in this preparation there is little or no tonic α -receptor vasoconstriction of the cerebral vessels to be released by α -blockade. Likewise there appears to be little parasympathetic tone to the cerebral vessels. Cholinergic receptor blockade with atropine (1 mg/kg) in this study produced no change in the resting cerebral blood flow (Fig. 7), indicating a lack of tonic cerebral vasodilator influence. In preliminary studies (four observations in two dogs) using physostigmine for reversal of atropine-induced cholinergic blockade of parasympathetic vasodilation (left major petrosal stimulation), the resting flow stayed at control values and the response to stimulation returned to preblockade levels. Although preliminary, this observation is consistent with the idea that there is, in this anesthetized preparation, little or no resting parasympathetic vasodilator tone. It is, therefore, concluded that the increase in cerebral blood flow seen in this study was caused by an active cerebral vasodilation.

Alterations in cerebral metabolism and arterial blood gas composition are known determinants of cerebral blood flow. The observations made in this study of arterial and cerebral venous oxygen tension, carbon dioxide tension, and pH do not support the contention that the observed increase in cerebral blood flow was produced by either

changes in cerebral metabolism or arterial blood gas composition. There was little or no change in arterial blood gas composition and pH before, during, or after cholinergic cerebral vasodilation. Because there was little or no change in arterial blood gas or pH these factors could not have contributed to the increase in cerebral blood flow. If cerebral blood flow was increased to a level above the metabolic demands of the tissue one would predict that the venous blood would contain more oxygen, less carbon dioxide, and less hydrogen ion. In this study a 54.8% increase in cerebral blood flow was accompanied by an increase in venous oxygen tension (30.4 to 36.0 mm Hg), a decrease in carbon dioxide tension (45.7 to 42.3 mm Hg), and an increase in pH (7.342 to 7.360 units). These observations are consistent with an increase in cerebral blood flow in excess of cerebral metabolic demands. An increase in cerebral metabolism, if anything, would have produced the opposite effect on cerebral venous blood gases and pH. For example, if the cerebral metabolism increased, then cerebral venous oxygen tension would either remain the same (if the flow exactly matched the metabolism) or would decrease (if the increased metabolism exceeded the increase in flow). Recent studies of intra-arterial infusion of atropine⁵ and neostigmine⁶ indicate that cholinergic blockade or cholinesterase inhibition do not alter cerebral metabolic rate for oxygen. It is therefore unlikely that intra-arterial acetylcholine infusion would increase the cerebral metabolic rate for oxygen. If the acetylcholine caused a decrease in cerebral metabolism, then this would indirectly cause a decrease in cerebral blood flow rather than the observed increase in flow. In the absence of oxygen content measurements it cannot be ruled out that cerebral metabolism changed slightly. If cerebral metabolism increased, the change was not sufficient to either decrease cerebral venous oxygen tension or eliminate the observed increase in cerebral venous oxygen tension. It is therefore concluded that the increase in cerebral blood flow observed in this study was not caused either by an increase in cerebral metabolism or by changes in arterial blood gas and pH.

The basic argument and the essential observations in this study were first presented nearly 50 years ago. The evolution of a sealed, airtight cranial window¹⁴ and the unexplained dilation of pial vessels in response to stimulation of the cephalic end of the sectioned vagus nerve¹⁵ prompted Cobb and Finesinger⁴ to explore the possibility of a reflex vasodilator system for the pial vessels. In a systematic study⁴ of the pial vessel diameters in response to stimulation of the cranial nerves (III, V, VII, VIII, IX, X, XI, XII) they observed that a dilator pathway traveled in the facial nerve. Chorobski and Penfield,¹³ in an elegant series of anatomical studies, traced the vasodilator fibers from the medulla along the facial nerve to the greater superficial petrosal nerve which ultimately formed a plexus on the internal carotid artery. Although their arguments were strong and their observations sound, their conclusions were not widely accepted. Pial vessel diameters do not necessarily reflect cerebral blood flow, and arterial carbon dioxide tension was not measured.

During the 1940s and 1950s most of the work on the control of the cerebral circulation was restricted to studies

in man, thus eliminating the possibility of direct electrical activation of the vasodilator mechanisms. More recently, electrical stimulation of the bulbar vasomotor center in cats produced either an increase or decrease in cerebral blood flow.¹⁶ Again no blood gas measurements were made and cerebral blood flow was only qualitatively determined by a heated thermistor technique. In a study of cerebral autoregulation, stimulation of the brainstem in monkeys with sectioned spinal cords produced a 40% increase in cerebral blood flow.¹⁷ Blood gases were monitored and changes in arterial pressure were considered. Blood flow, however, was measured in the common carotid arteries (with external carotid circulation eliminated) and possible alterations in cerebral metabolism resulting from the brainstem stimulation, although not likely, were not completely ruled out. Stimulation of the distal end of the sectioned 7th cranial nerve in two cats elicited an increase in cerebral blood flow as indicated by ¹⁴C-antipyrine autoradiography.¹⁸ The hydrogen clearance technique was used in rats to demonstrate an increase in local blood flow in response to topical application of cholinomimetic drugs.¹⁹ In a microapplication study of pial vessel diameters a carbachol-induced dilation was competitively antagonized by atropine.²⁰ Intra-arterial infusions of acetylcholine produce dilation in the cerebral²¹ and renal²² vascular beds. A weak vasodilation in response to intra-arterial methacholine was observed with a venous outflow technique in the dog.²³ The abundant anatomical evidence for cholinergic receptors has recently been reviewed.²⁴ Although each of these studies suggests a parasympathetic vasodilator mechanism, the indirect methods used for measurement of cerebral blood flow or the lack of control studies of blood gases or cerebral metabolism precluded a definitive conclusion concerning the existence of a parasympathetic cholinergic cerebral vasodilator mechanism.

The current demonstration of a parasympathetic cerebral vasodilator mechanism, in addition to supporting the observation of several earlier workers in the field, more clearly defines a reciprocal autonomic control capability for the cerebral circulation. Previous studies from this laboratory have demonstrated a sympathetic vasoconstrictor mechanism, and the present study describes a counterbalancing parasympathetic vasodilator mechanism. A similar dual autonomic control capability has been documented for the coronary circulation.^{11, 12} The reciprocal nature of this autonomic control in both the cerebral and coronary beds is analogous to the well documented reciprocal control of heart rate by the autonomic nervous system. The extent to which this autonomic control capability is involved in the normal and pathological regulation of the cerebral circulation has not yet been defined. Application of cholinergic blockade in pathological cerebral vasodilation should be examined and the possibility of a therapeutic use for cholinergic cerebral vasodilators could be considered. This study only demonstrates the capacity for a cholinergic cerebral vasodilation and gives no evidence

for the role of this mechanism in the regulation of cerebral blood flow.

Acknowledgments

The heparin used in this study was graciously supplied by Upjohn Company, Kalamazoo, Michigan.

References

1. Nelson E, Rennels M: Innervation of intracranial arteries. *Brain* **93**: 475-490, 1970
2. D'Alecy LG: Sympathetic cerebral vasoconstriction blocked by adrenergic alpha receptor antagonists. *Stroke* **4**: 30-37, 1973
3. D'Alecy LG, Feigl EO: Sympathetic control of cerebral blood flow in dogs. *Circ Res* **31**: 267-283, 1972
4. Cobb S, Finesinger JE: Cerebral circulation. XIX. The vagal pathway of the vasodilator impulses. *Arch Neurol Psychiatr* **28**: 1243-1256, 1932
5. Kawamura Y, Meyer JS, Hiromoto H, Aoyagi M, Tagashira Y, Ott EO: Neurogenic control of cerebral blood flow in the baboon; effects of the cholinergic inhibitory agent, atropine, on cerebral autoregulation and vasomotor reactivity to changes in PaCO₂. *J Neurosurg* **43**: 676-688, 1975
6. Aoyagi M, Meyer JS, Deshmukh VD, Ott EO, Tagashira Y, Kawamura Y, Matsuda M, Achari AN, Chee ANC: Central cholinergic control of cerebral blood flow in the baboon; effect of cholinesterase inhibition with neostigmine on autoregulation and CO₂ responsiveness. *J Neurosurg* **43**: 689-705, 1975
7. Mchedlishvili GI, Nikolaishvili LS: Evidence of a cholinergic nervous mechanism mediating the autoregulatory dilatation of the cerebral blood vessels. *Pfluegers Arch* **315**: 27-37, 1970
8. James IM, Millar RA, Purves MJ: Observations on the extrinsic neural control of cerebral blood flow in the baboon. *Circ Res* **25**: 77-93, 1969
9. Kobayashi S, Rhoton Al Jr, Waltz AG: Effects of stimulation of cervical sympathetic nerves on cortical blood flow and vascular reactivity. *Neurology (Minneapolis)* **21**: 297-302, 1971
10. Meyer JS, Yoshida K, Sakamoto K: Autonomic control of cerebral blood flow measured by the electromagnetic flowmeters. *Neurology (Minneapolis)* **17**: 638-648, 1967
11. Feigl EO: Sympathetic control of coronary circulation. *Circ Res* **20**: 262-271, 1967
12. Feigl EO: Parasympathetic control of coronary blood flow in dogs. *Circ Res* **25**: 509-519, 1969
13. Chorobski J, Penfield W: Cerebral vasodilator nerves and their pathway from the medulla oblongata. *Arch Neurol Psychiatr* **28**: 1257-1289, 1932
14. Forbes HS: The cerebral circulation. I. Observation and measurement of pial vessels. *Arch Neurol Psychiatr* **19**: 751-761, 1928
15. Forbes HS, Wolff HG: Cerebral Circulation. III. The vasomotor control of cerebral vessels. *Arch Neurol Psychiatr* **19**: 1057-1086, 1928
16. Molnár L, Szántó J: The effect of electrical stimulation of the bulbar vasomotor centre on the cerebral blood flow. *J Exp Physiol* **49**: 184-193, 1963
17. Langfitt TW, Kassell NF: Cerebral vasodilatation produced by brainstem stimulation; neurogenic control vs. autoregulation. *Am J Physiol* **215**: 90-97, 1968
18. Salanga VD, Waltz AG: Regional cerebral blood flow during stimulation of seventh cranial nerve. *Stroke* **4**: 213-217, 1973
19. Scremin OU, Rovere AA, Raynald AC, Giardini A: Cholinergic control of blood flow in the cerebral cortex of the rat. *Stroke* **4**: 232-239, 1973
20. Kuschinsky W, Wahl M, Neiss A: Evidence for cholinergic dilatatory receptors in pial arteries of cats. *Pfluegers Arch* **347**: 199-208, 1974
21. Lowe RF, Gilboe DD: Canine cerebrovascular response to nitroglycerin, acetylcholine, 5-hydroxytryptamine, and angiotensin. *Am J Physiol* **225**: 1333-1338, 1973
22. Vander AJ: Effects of acetylcholine, atropine, and physostigmine on renal function in the dog. *Am J Physiol* **206**: 492-498, 1964
23. McClure C Jr, Green HD: Cerebral venous outflow in the dog; influence of adrenergic and cholinergic substances. *Am J Physiol* **197**: 1183-1190, 1959
24. Edvinsson L: Neurogenic mechanisms in the cerebrovascular bed. *Acta Physiol Scand (suppl 427)*: 1-35, 1975