EFFECTS OF INCREASED CEREBROSPINAL FLUID ADENOSINE CONCENTRATION OR CARBON DIOXIDE TENSION ALTERATIONS ON CEREBRAL HEMODYNAMICS IN THE DOG

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ABSTRACT

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JoAnn C. Havran

It has been suggested that adenosine and/or carbon dioxide may be involved in local regulation of cerebral blood flow. This study was designed to determine whether increasing the cerebrospinal fluid concentration of adenosine or increasing or decreasing the cerebrospinal fluid carbon dioxide tension, influences cerebral hemodynamics. Cerebral venous outflow was measured from the cannulated sinus confluence after occluding the transverse canals with bone wax. The cerebrospinal fluid system was perfused with artificial cerebrospinal fluid via a needle inserted into the right lateral ventricle; outflow was via a needle in the cisterna magna. Cerebrospinal fluid infusion rate was 4 ml/min, which produced adequate cerebrospinal fluid pressure to force the perfusate around the entire brain surface, as demonstrated by India ink added to the perfusate, and permitted rapid saturation of the cerebrospinal fluid system with the test solution. Cerebrospinal fluid solutions containing: 1) no adenosine (control solution); 2) 10 or 100 μg/ml adenosine; 3) high or low carbon dioxide tension with no adenosine were perfused for 10-12 minutes. Inflow and outflow cerebrospinal fluid

carbon dioxide tension, oxygen tension and pH were determined systematically. Cerebral arterial carbon dioxide tension was increased by ventilating the animal with 10% CO_2-21% O_2-69% N_2 for 5 min to test the reactivity of the cerebral vasculature. Results indicate that increasing the cerebrospinal fluid concentration of adenosine or increasing or decreasing cerebrospinal fluid carbon dioxide tension does not appreciably influence cerebral vascular resistance or cerebral blood flow. However, comparable increases in cerebral arterial carbon dioxide tension resulted in a 104% increase in cerebral blood flow and a 45% decrease in cerebral vascular resistance. Our data do not support the hypotheses that adenosine or carbon dioxide in cerebrospinal fluid are involved to a major extent in local cerebral blood flow regulation. But our results do suggest that a type of reciprocating response may exist between the intra- and extra-parenchymal brain vasculature.

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INTRODUCTION

It has been suggested that adenosine and/or carbon dioxide may be involved in the local regulation of cerebral blood flow. The hypothesis has been put forth on the basis of the following observations.

The presence of adenosine in normal brain tissue and cerebrospinal fluid was first demonstrated by Deuticke and Gerlach (20). Although subsequent studies have revealed that bilateral intracarotid infusion of adenosine is without affect on cerebral blood flow (6,14) it has been shown that adenosine does not cross the blood brain barrier (6). In addition, the concentration of adenosine in cerebrospinal fluid has been found to increase during ischemia (6) and direct application of adenosine to pial vessels reportedly causes vasodilation (6,96). These results lend support to the hypothesis that adenosine is involved in the local regulation of cerebral blood flow.

Studies by Wolff and Lennox (99) in 1930 revealed that changes in arterial carbon dioxide tension dramatically influenced pial artery diameter. More recently, direct measurements of cerebral blood flow by various techniques have shown that cerebral blood flow closely follows changes in arterial carbon dioxide tension (40,47,78) and that the effect of arterial carbon dioxide tension is independent of corresponding changes in arterial blood pH (38,82). Furthermore, it has been postulated that local plasma and/or cerebrospinal fluid variations in carbon dioxide tension may alter cerebral vascular resistance (38).

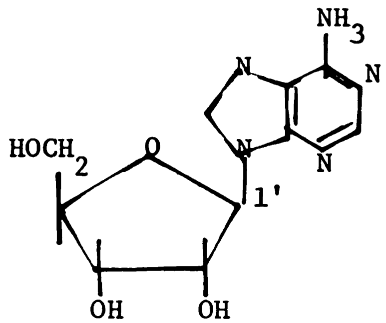
These studies of local vascular regulation in the brain have indicated possible roles for adenosine and carbon dioxide in cerebral blood flow regulation. Coupled with the facts that the cerebrospinal fluid bathes the entire brain surface and has fluctuating concentrations of carbon dioxide and adenosine, the above observations have prompted speculation that the concentration of these vasoactive substances in the cerebrospinal fluid may locally regulate cerebral blood flow. The present study was designed to test the hypothesis that adenosine and/or carbon dioxide tension in the cerebrospinal fluid are involved in the local regulation of cerebral blood flow.

LITERATURE REVIEW

Adenosine

Adenosine is one agent which may be involved in the regulation of cerebral blood flow. In 1966, Deuticke and Gerlach (20) reported that adenosine formation is one step in the degradation of ATP in the brain. The usual breakdown of ATP in organs other than the brain and heart takes place through the formation of vasoinactive inosine monophosphate (IMP) and therefore by-passes the formation of vasoactive adenosine. Because of these findings and studies that show adenosine elicits marked reactivity in vascular smooth muscle in other regional circulations (4,79), adenosine may conceivably participate in the regulation of cerebral blood flow.

Chemically, adenosine is a purine nucleoside. Purine nucleosides are N-glycosyl derivatives of a heterocyclic base, conjugated to a sugar of the furanose configuration. They have a β-glycosidic linkage from C^1 of the sugar to N^9 of the base. The conformation of the glycol bond in isolated nucleosides is predominantly ANTI and the conformation of the furanose ring is co-planar for 4 atoms with the fifth carbon atom displaced from the plane by 0.5 Å. The displacement is on the same side as the protruding fifth C atom and the resultant conformation is designated as ENDO (Drawn Figure). Characteristically, purines are stable in alkali and readily hydrolyzed by acid.



Adenosine effects on coronary vasculature

Coronary ischemia has been shown to increase purine nucleoside concentration in coronary venous blood (42). Likewise, decreasing coronary blood flow may enhance the vasoactivity of the coronary sinus blood (83) and decrease both myocardial oxygen consumption (31) and cardiac contractile force (2). Studies by Berne et al. (5) in open chest dogs, show that decreasing arterial oxygen tension elicits an increase in coronary blood flow.

However, in an earlier study Nelemans (70) observed an increase in pulmonary vascular resistance in the heart lung preparation when the lungs were perfused with normal venous blood other than fresh coronary venous blood. He suggested that a vasodilator substance is released by the heart and is present in coronary sinus blood. Additional studies by Berne (4) have suggested that parameters affecting myocardial oxygen tension (i.e., increased myocardial metabolism; decreased coronary arterial resistance) may cause the release of a vasodilator substance originating in the myocardium.

Considering the spectrum of possible vasodilator substances found in the myocardium, adenosine was selected as the most probable because: 1) it is a potent vasodilator; 2) it readily crosses the myocardial cell membrane; 3) it is readily inactivated in the blood; and 4) it occurs in the heart in the form of adenine nucleotides. As a mechanism, Berne suggests that a decrease in myocardial oxygen tension depresses the rate of oxidative phosphorylization allowing degradation of intra-cellular nucleotides to adenosine. Diffusing out of the myocardial cell into the interstitial space, adenosine induces vasodilation of the local arterioles and thereby increased coronary blood flow. The increase in myocardial blood flow is thought to wash out the adenosine causing coronary blood flow to return to normal.

Subsequent studies were designed to test this hypothesis. To determine whether the adenosine concentration actually increases during hypoxia, Rubio et al. (80) briefly occluded the left coronary artery in open chest dogs. Adenosine was detectable in coronary sinus blood after occlusion whereas it was not detectable in the control. Because of the rapid uptake of adenosine by red cells, they speculated that the amount of adenosine recovered in the serum would account for only 20% of the increase in coronary blood flow. After correcting for the amount of adenosine taken up by red blood cells (half the concentration within 10 sec.), they concluded that sufficient amounts of adenosine were being produced to effect the change in coronary blood flow.

An important consideration of the possible role of adenosine in the regulation of coronary blood flow was whether the normal heart contained adenosine. Studies initiated by Rubio and Berne (81) measured directly the concentration of adenosine in normal pericardial fluid. The average concentration of adenosine in the normal heart was reported as .22 n moles/g of myocardial tissue. Furthermore, under moderate degrees of hypoxia (up to 25 sec.) the adenosine concentration continued to increase. These values corresponded closely with those found by Olsson (71) who directly measured adenosine concentration in the heart muscle.

Berne, collaborating with Rubio and Duling (7), published his concept of how adenosine formation and release may operate in the regulation of coronary blood flow. Within the muscle cell the enzymes adenosine deaminase and adenylic acid deaminase are found as is ATP. During hypoxia or increased myocardial metabolism ATP is broken down to ADP and subsequently to AMP. AMP can be as deaminated to IMP or dephosphorylated to adenosine by 5' nucleotidase at the cell wall. Some of the adenosine formed reaches the interstitial space where it acts locally to induce arteriolar dilation and therefore increase coronary blood flow. Once in the interstitial space, adenosine can enter the capillaries where it may remain intact and active in the plasma phase, or be inactivated by adenosine deaminase as it enters a red cell. However, Berne suggests that the majority of the adenosine formed probably reenters the myocardial cells and is rephosphorylated to adenine nucleotides.

The latest studies in this area are of a histochemical nature (79) and were designed to determine whether the actual site of adenosine production in the heart muscle is diffuse or localized. Using dog, guinea pig, and rat hearts, this group associated the 5' nucleotidase activity with the membranes lining the compartments (T tubules, etc.) open to extracellular space. Adenosine, therefore, is formed throughout the entire myocardium.

Adenosine effects on skeletal vasculature

Blood flow in exercising skeletal muscle, as in cardiac muscle, is proportional to the oxygen requirements of the tissue. In both, increased metabolic rate or decreased oxygen supply is associated with the breakdown of adenyl nucleotides (79). ATP is reportedly released from contracting frog sartorius muscle (24) and exercising human forearm (24). However, it has been stated that ATP as such does not cross the cell membrane (44,60). Therefore, whether ATP produced intracellularly reaches extracellular fluid in sufficient concentration to contribute to local blood flow regulation remains controversial. Yet, the fact remains that close intra-arterial injections of adenosine, ATP or AMP into skeletal muscle have been demonstrated to decrease vascular resistance (83).

Adenosine, specifically, has been reported to be present in resting skeletal muscle (24), and increases more than five times in the venous plasma of dogs after ischemic contraction (24). Under identical conditions, no change in venous blood ATP or AMP concentration was found (24). Adenosine also apparently accumulates in both intact contracting and isolated, ischemic contracting rat muscle (79). Berne and colleagues (4) suggest that the concept proposed for adenosine mediated regulation of coronary blood flow may also apply to skeletal muscle.

One basic difference, however, does exist between heart and skeletal muscle with respect to the formation of adenosine. In skeletal muscle adenine nucleotide degradation occurs primarily via IMP formation with minimal production of adenosine whereas adenosine is the major degradation production in the heart. Histochemical studies (24) identical to those done in the heart determined adenosine was produced in localized zones within muscle cells in close proximity to blood vessels. Elsewhere in skeletal muscle, adenine nucleotide degradation proceeded via IMP formation.

Adenosine effects on the cerebral vasculature

One step in the degradation of ATP in the rat brain observed by Deuticke and Gerlach was the formation of adenosine (20). To ascertain a possible direct affect of adenosine on the cerebral vasculature, Buyanski and Rapela (14) injected adenosine intravenously and intra-arterially in doses up to 100 μg/kg. Only a minimal dilator effect was noted in both studies, even when potentiated with intravenous injections of dipyridamole (deaminase inhibitor). On this basis it was concluded that endogenous adenosine probably does not serve a major role as a regulator of cerebral blood flow.

In 1973, Berne's group (6) confirmed Buyanski and Rapela's (14) report but attributed the results to the fact that adenosine does not appreciably cross the blood brain barrier. Experiments were conducted in which ^{14}Carbon-Adenosine was infused into both internal carotid arteries but cerebrospinal fluid samples yielded no radioactivity. Yet adenosine induced a two to threefold increase in the diameter of a pial when applied directly to that vessel through a cranial window. Hence, it was concluded that adenosine does not cross the blood brain barrier at a rate that permits effective vasodilator concentrations to reach the cerebral vascular smooth muscle.

Earlier studies (20) established the presence of adenosine in normal brain tissue and cerebrospinal fluid. Like the heart, the brain is capable of avidly incorporating adenosine into adenine nucleotides. Berne, Rudio and Curnish (6) proved this point by injecting Uranium-^{14}Carbon-Adenosine into the cerebrospinal fluid. Eighty-four to 87% of the radioactivity recovered in the cerebral tissue was in the form of ATP, ADP, AMP and IMP. Little nucleotide was found in the cerebrospinal fluid. They concluded that adenosine can be released from the ischemic brain into the cerebrospinal fluid and be reincorporated from the cerebrospinal fluid into brain nucleotides. This closed system contrasts with that in tissues like the heart and skeletal muscle in which some of the adenosine and its degradative products are washed away by perfusing blood (6).

Carbon dioxide effects on the renal vasculature

The reported effects of altering systemic carbon dioxide tension on renal vasculature vary with experimental conditions and the laboratory animal studied. Bohr et al. (11) reported that breathing 10-30% carbon dioxide increased renal vascular resistance and decreased both mean arterial pressure and renal blood flow in anesthetized dogs. Renal vascular resistance in unanesthetized dogs reportedly is not affected by the inhalation of carbon dioxide (92).

In other animals such as the harbour seal (12) and the rabbit (28), increased systemic arterial carbon dioxide tension raised renal vascular resistance. In contrast, Takács and Kállay (93) have shown that breathing 5-20% carbon dioxide decreases renal vascular resistance in anesthetized rats. Studies by Simmons and Oliver (89) found that renal vascular resistance was inversely related to changes in normal systemic arterial carbon dioxide tension.

Local, constant pressure perfusion of vascularly isolated kidneys as reported by Lockett (59) demonstrated that doubling carbon dioxide tension decreased renal vascular resistance (25%) and thereby increased renal blood flow. However, halving the carbon dioxide tension resulted in a 13% increase in renal vascular resistance and a decrease in flow.

Daugherty et al. (17), employing an extracorporeal lung circuit directly, perfused the intact kidney with hypercapnic blood. The effect of an abrupt local increase in Pa_{CO_2} at constant flow was a steady decrease in renal artery perfusion pressure, reflecting a decrease in renal vascular resistance.

Thus, it seems that the local effect of high Pa_{CO_2} is to induce vasodilation of the renal vasculature. However, the effect of systemically increasing arterial carbon dioxide tension is not yet definitive.

Carbon dioxide effects on cutaneous and muscle vasculature

Early studies on the local effect of carbon dioxide on isolated limbs failed to separate muscle and skin vascular responses. The response of the human cutaneous vasculature to carbon dioxide has been studied by measuring heat loss as an indicator of vasodilation or constriction. Diji and Greenfield (23) reported an increase in heat loss (interpreted as vasodilation) when carbon dioxide was injected subcutaneously or when the hand (22), or whole body (58) was immersed in carbon dioxide rich water. Measuring total hindlimb blood flow in the cat, Fleisch et al. (26) found that elevating the perfusing arterial carbon dioxide tension locally produced a transient decrease followed by a prolonged increase in flow. Subsequent studies in humans (51) and dogs (19) confirm these observations.

Radawski and colleagues (76) in 1972 were the first to discriminate between skin and muscle blood flow. Arterial blood was pumped through an isolated lung and then into the brachial artery. The isolated lung was ventilated with gas mixtures containing progressively increasing concentrations of carbon dioxide. To isolate the response of the skin from muscle vasculature, pressures from small and large skin and muscle arteries were measured, as well as flows from the cephalic (mainly skin) and brachial (mainly muscle) veins. The results indicated that total limb resistance decreased as a function of brachial artery carbon dioxide tension due primarily to a decrease in skin vessel resistance; muscle resistance was not consistently affected. The results also showed that local, graded changes in blood carbon dioxide tension perfusing the muscle at a constant pressure do little to change total limb blood flow. However, both muscle and skin vasculature exhibit a comparable depression in response to severe hypercapnia.

<u>Carbon dioxide effects on mesenteric circulation</u>

The effect of systemic carbon dioxide tension has received little attention in connection with the mesenteric circulation. Bernthal (8), Mohamed and Bean (66), and Brickner et al. (13) reported that blood flow in the intact intestinal bed varied directly with systemic carbon dioxide levels while perfusion pressure decreased or remained relatively constant during hypercapnia or hypocapnia, respectively. The study by Mohamed and Bean (66) also contrasted the systemic and local effects of increased carbon dioxide tension on the mesenteric vasculature and found the response to be in agreement, i.e.: mesenteric flow increased, perfusion pressure remained relatively constant, and mesenteric vascular resistance decreased. McGinn and associates (64), while ventilating cats with a constant volume respirator to prevent the respiratory effects of carbon dioxide, altered end tidal carbon dioxide by increasing the carbon dioxide concentration in the gas mixture from 1 to 8%. Their results were in agreement with those described above in that flow through the intact mesenteric bed increased as systemic arterial carbon dioxide tension increased and perfusion pressure remained constant, thus indicating a reduction in resistance with raised arterial carbon dioxide tension.

The systemic effect of carbon dioxide on the mesenteric bed seems to be well-established. However, a conclusion with respect to the local effects of carbon dioxide tension on mesenteric vasculature cannot be drawn.

Effects of carbon dioxide on heart function and the coronary vasculature

The systemic effects of hypercapnia on heart function has been studied by Nahas et al. (68). They reported that heart rate and cardiac output were initially depressed by increased levels of carbon dioxide. After this initial response compensation was attributed to increased levels of catecholamines (released by stimulation of the sympatho-adrenal system).

In isolated preparations hypercapnia reportedly increased coronary blood flow. Hilton and Eichholtz (41) showed that in the heart-lung preparation an increase in coronary blood flow occurred during perfusion with hypercapnic blood. Similar results were published by Gremels and Starling (32). In the constant pressure perfused guinea pig heart, coronary blood flow varied directly with the carbon dioxide tension of the blood, indicating again that resistance decreases in response to increased carbon dioxide levels (32). In support of the above observations, isolated perfused preparations in the rabbit (94) and the dog (75) defined the total affect of carbon dioxide tension on the heart as a decrease in the frequency and force of contraction and a decrease in the rate of conduction even in the face of increased coronary blood flow.

The effects of a large sudden decrease in pH of the perfusing blood, evoked by raising the carbon dioxide tension, on coronary vascular resistance was published by Daugherty et al. (17). Using the extracorporeal lung circuit described previously, carbon dioxide tension in the blood perfusing the heart was elevated. A cannula was inserted into the aorta via the left subclavian artery and manipulated into the mouth of the left common coronary artery. Perfusion pressure decreased as did left ventricular contractile force. These findings indicate that carbon dioxide tension is locally vasoactive in the coronary vascular bed and also reduces cardiac contractility. The direct effect of increased levels of carbon dioxide tension was to depress heart function, decrease coronary resistance and increase coronary artery flow in both systemic and local perfusion studies. In the systemic studies, however, a compensatory response due to increased catecholamines (released by stimulation of the sympatho-adrenal system) followed the initial depression.

<u>Carbon dioxide effects on the cerebral vasculature</u>

Hypercapnia produced by carbon dioxide inhalation causes marked dilation of the cerebral resistance vessels and cerebral blood flow increases (45,46,78). Hypocapnia due to hyperventilation results in intense cerebral vasoconstriction and decreased cerebral blood flow (40). The change in cerebral blood flow approximates 3.5% per mm Hg change in Pa_{CO_2} (40,47,78). Vascular response curves to Pa_{CO_2} have been established for a limited range in the dog (40), and baboon (45), and over a much wider range in the monkey (78); more restrictive curves are available for man (47).

Typically, the relation between cerebral blood flow and Pa_{CO_2} is sigmoid within a range of 15 to 150 mm Hg (78), so that above 100 mm Hg and below 20 mm Hg changes in flow are considerably reduced. If the Pa_{CO_2} decreases below 20 mm Hg the cerebral blood flow does not continue to decrease. This may be due to a slight tissue acidosis elicited from a slight but manifest tissue hypoxia resulting from the already marked cerebral blood flow reduction and from the Bohr effect (11), also the limits of maximum vessel constriction may prevent further decreases in flow. According to Agnoli (1) and Severinghaus et al. (84), when hypercapnia or hypocapnia is maintained for hours or days, adaptation occurs and the cerebral blood flow returns slowly to normal.

Pressure effects on cerebral blood flow have been studied from two aspects: these aspects are intravascular pressure and extravascular (cerebrospinal fluid) pressure.

Direct observations of the pial circulation during hypertension and hypotension were made by Fog in 1937 (27). He observed constriction of the pial vessels during hypertension and dilation during hypotension. More extensive studies of this relationship have been reported by Harper and Glass (40) and Haggendaal and Johansson (34). Their experiments revealed that at 50 mm Hg arterial pressure, the vascular response to changes in carbon dioxide tension is abolished (probably because the cerebral vessels are maximally dilated at this low arterial pressure).

The mechanism for this relationship between the vascular response to carbon dioxide tension and cerebral arterial pressure at this time is still unclear. Two theories, tissue metabolism and the Bayliss effect have been proposed but neither have been adequately substantiated.

Increased levels of carbon dioxide dilate the cerebral bed and increase cerebrospinal fluid pressure (91). However, independent changes in cerebrospinal fluid pressure over a wide range have little effect on cerebral blood flow (35). Specifically, in the dog, increases up to 50 mm Hg cerebrospinal fluid pressure can be tolerated without influencing cerebral blood flow (35).

The assumption cannot be made that cerebral vessels in different regions of the brain respond to increases in Pa_{CO_2} equally or even in the same direction. It is well-established that there are marked regional differences in resting flow (53) and local differences in response to other pharmacological agents (29). Using autoradiography Hansen et al. (36) found that changes in gray matter blood flow in response to inhalation of 5% carbon dioxide tension were proportionately greater than white matter blood flow. In fact, within a limited range of Pa_{CO_2}, 35-65 mm Hg, gray matter flow rate increased 150% more than white matter flow.

As discussed above, the site of action of carbon dioxide is of a regional nature within the cerebral vascular bed. Furthermore, it has been proposed that centers in the brainstem influence cortical blood flow either directly or indirectly (86). Investigators supporting this hypothesis have shown that: 1) carbon dioxide gas infused into the lumbar subarachnoid space or cisterna magna increase overall cerebral blood flow (85); 2) reversible lesions placed in the mesencephalon of experimental animals reversibly diminished the responsiveness of cerebral blood flow to inhaled carbon dioxide (85); 3) stimulation of pontotubular centers can increase or decrease cerebral blood flow independent of carbon dioxide concentration (67).

On the other hand, this thesis has been challenged by other groups employing more direct physiological techniques. Skinhoj and Paulson (90) altered the Pa_{CO_2} in unanesthetized human subjects by infusing blood with varying levels of carbon dioxide into the carotid or vertebral artery. Similar studies were performed on dogs by Kogure et al. (49) who infused the middle cerebral artery and the vertebral artery. Both groups reported that regional cerebral blood flow corresponded directly with Pa_{CO_2} in internal carotid or middle cerebral artery whereas cerebral blood flow was unaffected by infusion of high levels of carbon dioxide in the vertebral-basilar system.

Some investigators have suggested that the effect of carbon dioxide tension on the vasomotor function is mediated through pH changes in the arteriolar wall. (The pH changes inversely with the carbon dioxide concentration but H ions cross the blood brain barrier very slowly.) However, if the arterial carbon dioxide tension is held constant, acute changes of blood pH, induced by i.v. infusion of ammonia chloride or sodium bicarbonate, do not influence the cerebral blood flow (38,82). Kety and associates (46) were also unable to show any increase in cerebral blood flow in patients with diabetic acidosis and no change in cerebral blood flow in man could be found during non-respiratory acidosis or alkalosis (82). More recently, it has been shown in dogs that cerebral blood flow is independent of plasma pH when arterial pH is altered over the range of 6.7 to 7.6 pH units by infusing lactic acid or sodium bicarbonate intravenously at a constant Pa_{CO_2} (38). In a further series of experiments, also in dogs, McDowall and Harper (63) confirmed that non-respiratory acidosis induced at a constant Pa_{CO_2} resulted in no significant change in cortical blood flow.

Hence, it has been suggested that intracellular pH changes rather than the pH of the blood is the basis for reactivity of the cerebral arterioles.

The effects of pH on cerebral blood flow

In 1949 Elliott and Jasper (25) published the first study which examined cerebrospinal fluid ions. They found an increase in pial vessel diameter when a bicarbonate free (unbuffered) solution (pH 6.2) was infused over the cortex. Vasoconstriction was observed 30 sec after switching to a bicarbonate containing fluid which assumed a physiological pH of 7.4. Comparable results were obtained by Gotoh et al. (30) when the cerebrospinal fluid pH was altered by blowing carbon dioxide across the surface of the cortex. Measuring cortical blood flow by the heat clearance method, Betz and Kozak (10) found changing blood flow closely correlated with changes in the cortical fluid pH during carbon dioxide alterations. Similarly, McDowall and Harper (63) observed that the pH of fluid overlying the cortex fell not only during inhalation of carbon dioxide but also during intravenous infusion of lactic acid. Unfortunately, in the latter two experiments, exposure of the brain was extensive or prolonged and the experimental results may have been due to surgical trauma.

Siesjo et al. (88) altered the ionic concentration of the cerebrospinal fluid in dogs while perfusing the ventriculo-cisternal system. Pa_{CO_2} was held constant and cerebral blood flow was measured by xenon clearance. The pH of the cerebrospinal fluid was changed by varying the bicarbonate concentration of the artificial cerebrospinal fluid. Their results indicated that cerebral blood flow closely follows changes in bicarbonate and/or possible hydrogen concentration in the cerebrospinal fluid. Recent studies by Wahl et al. (95) in cats and rats concur with these results. Using the micropipette technique (95) solutions with different bicarbonate concentrations were infused into the adventitia of the pial arterioles. This technique was designed to alter directly the ions in the extracellular fluid. When low bicarbonate concentrations were used local vasodilation was observed while solutions containing high concentrations of bicarbonate elicited local vasoconstriction. These results were confirmed by Cameron and Segal (15) who conducted similar experiments in rabbits. However, neither group measured the artificial cerebrospinal fluid pH nor was the possible direct effect of bicarbonate considered.

In mechanically ventilated cats, Wei et al. (98) inserted pial windows to observe selected pial vessels. Photographic methods were used to measure changes in vessel caliber. Artificial cerebrospinal fluid was bubbled with carbon dioxide to alter the carbon dioxide tension and pH. In one series of experiments the artificial cerebrospinal fluid was equilibrated with different carbon dioxide tensions. In another series, artificial cerebrospinal fluid was prepared with different concentrations of bicarbonate and equilibrated with different carbon dioxide tensions so that the pH and carbon dioxide tension could be independently manipulated. The results showed that marked increases in carbon dioxide tension or marked decreases in carbon dioxide tensions had no influence on pial vessel diameter provided the pH of the artificial cerebrospinal fluid was maintained constant. Therefore, their data indicate that the local effect of carbon dioxide is mediated through changes in cerebrospinal fluid or extracellular fluid pH.

All the investigators reviewed above have concluded that pH is the primary factor regulating the diameter of the cerebral arterioles. The mechanism of action and the pH sensitive site, however, remain controversial. Two hypotheses have been put forth.

Thurau's group (52) suggested that the extracellular fluid pH is the important parameter. Since the extracellular fluid surrounds the brain tissue cells they appear to directly control the extracellular fluid pH. As a plausible mechanism, they proposed that the smooth muscle cells of the arterioles may be selectively permeable to hydrogen ion thus changing transmembrane potentials. Another possibility suggested by Kuschinsky et al. (52) is that the pH effect may be mediated by the local calcium ion concentration. This is supported by studies in which EDTA was included in their physiologically normal artificial cerebrospinal fluid solution. It is important to note that micropipette studies by Kuschinsky et al. (52) and studies by Betz's group (9) suggest that local potassium ion concentrations may also be an important parameter along with pH.

Gotoh et al. (30) theorized that the true stimulus of cerebral vasomotor activity was the intracellular smooth muscle pH. According to this hypothesis the arterioles act like carbon dioxide electrodes, i.e., carbon dioxide diffuses freely through the endothelial membrane, whereas hydrogen and bicarbonate ion cannot freely cross this membrane. Hence the intravascular carbon dioxide tension and the extravascular bicarbonate determine the pH around and presumably inside the smooth muscle cell. Additional support for this hypothesis came from studies where dilation was observed while blowing carbon dioxide across the cortex surface after intravenous infusion of acetazolamine (carbonic anhydrase inhibitor) (30).

Posner, Plum and Zee (74) repeated the ventricular perfusion experiments of Siesjo et al. (88) in which pH of the artificial cerebrospinal fluid was altered by changing the bicarbonate concentration. However, cerebral blood flow was measured by the technique of Rapela and Green (77) instead of by xenon clearance. The perfusion rate was 1 cc/min lasting 20 min. to 2 hours and the Pa_{CO_2} was held constant. Their results showed that cerebral blood flow did not change, when the pH of artificial cerebrospinal fluid was altered.

Leusen et al. (56) performed similar ventricular perfusion studies in cats, measuring blood flow in the caudate nucleus and hemispheric blood flow by heat clearance and xenon clearance, respectively. Blood flow in the caudate nucleus changed with the bicarbonate concentration, i.e., the pH of artificial cerebrospinal fluid, but the hemispheric blood flow did not change.

The observations of these two groups suggest that alterations of bicarbonate concentration or pH of the entire ventriculo-cisternal system has little affect on total cerebral blood flow. Both groups have proposed that the pial vessels and parenchymal vessels may elicit reciprocal responses. Another possibility suggested by Posner, Plum and Zee (74) is that hydrogen ion may be actively transported out of the nervous system before sufficiently penetrating to the deep lying cerebral vessels. Under these conditions only the most superficial vessels of the ventricular and subarachnoid spaces would respond to pH changes.

MATERIALS AND METHODS

Adult mongrel dogs of either sex, averaging 20 kg, were induced (50 mg/kg) and maintained under anesthesia with sodium pentobarbital. Each dog was mechanically ventilated with a Harvard constant volume respirator via a cuffed endotracheal tube.

Blood flow to the cerebral tissues is supplied primarily by the anterior, middle and posterior cerebral arteries (Figure 1) which arise from the Circle of Willis (Figure 2). Secondary and tertiary arteries arising from the three major arteries are interconnected by anastomoses. A rich capillary network is found in the cortex whereas the white matter of the brain has a less abundant vascular supply.

The cerebral veins and the veins of the encasing bone are drained via large, sometimes osseous, venous sinuses (Figure 3). The dorsal sagittal sinus receives venous blood from the dorsal cerebral veins which run along the sulci and gyri draining nearly the entire cortex of the cerebrum. The straight sinus receives venous blood from the great cerebral vein, the occipital cerebral vein, the thalamostraite vein, the vein of the corpus callosum and the internal cerebral vein thus draining the deep areas (thalamus and corpus striatum) as well as the pia of the cerebrum. The paired transverse sinuses merge with the dorsal sagittal sinus and the straight sinus to form the confluence of sinuses within the dorsal part of the occipital bone. Lateral to the confluence, the transverse sinuses receive the posterior cerebral vein and the occipital emissary vein thus draining the cortex of the temporal lobe and the deep muscles of the neck.

Further along, the transverse canal bifurcates forming the temporal and sigmoid sinuses, feeding venous blood into the vertebral and internal and external jugular veins. In the dog the external jugular veins are the main channel for return of venous blood from the head.

In the present study, the cerebral venous outflow (cerebral blood flow) was measured from a cannula inserted into the confluence of the sinuses after the transverse sinuses had been occluded (Figure 4). Therefore, cerebral blood flow represents the veins supplying the dorsal sagittal sinus and straight sinus excluding the venous blood supplied by the transverse sinuses.

The skin and dorsal neck muscles were reflected from the occipital bone to expose the boney area over the sigmoid sinuses. A burr hole was drilled in each transverse canal and the canals were occluded with warm bone wax. A larger hold was drilled through the skull into the sinus confluence and a tapered, stainless steel tube of 5 mm in diameter was inserted into and seated in the hole. Rubber tubing was fixed onto the cannula and its tip placed at approximately head level. Blood flowed by gravity into a reservoir (50 ml volume) and was returned continuously to a cannulated femoral vein via a Holter roller-type blood pump. Reservoir level was maintained constant with an electronic level controller device. Outflow was determined with a cylinder and stopwatch. This allowed approximately two-thirds of the cerebral blood flow to be monitored, uncontaminated by extracranial sources (77).

Completeness of the separation of the intra- and extra-cranial circulation was tested by briefly occluding the cerebral venous outflow tubing. An increase in cerebral venous pressure exceeding 39 mm Hg was considered an acceptable preparation. If the preparation is essentially collateral free, the venous pressure will rapidly approach arterial pressure and therefore exceed 40 mm Hg. However, if occlusion of the transverse sinuses is incomplete or there are anastomosis between the sagittal or straight sinuses and extracranial sources, the venous pressure will be less than 40 mm Hg. The level of communication determines the level of venous pressure during this test, i.e.: when the transverse sinuses are patent, the venous pressure increases by less than 5 mm Hg. Heparin was administered in a dose of 10 mg/kg to prevent coagulation.

To perfuse the ventriculo-cisternal system the temporal muscles were reflected laterally from the midline to expose the parietal bone over the area of the lateral ventricles. A 2 mm in diameter trephine hole was made in the cranium above the right lateral ventricle while care was taken not to tear the dura. With the aid of a micro-manipulator, a 20 gauge bevel needle was inserted into the right lateral ventricle. To allow continuous flow through the ventricular system an 18 gauge needle was inserted into the cisterna magna.

The artificial cerebrospinal fluid used in this study was prepared fresh daily from a stock solution of 34 mg $Na_2HOP_4 \cdot H_2O$ 224 mg KCl, 2.1 g $NaHCO_3$, 7.25 g NaCl dissolved in distilled water to one liter. To prevent the formation of a precipitate 20.33 gm of $CaCl_2$

and 20.33 gm of $MgCl_2 \cdot 6H_2O$ each dissolved in distilled water to 100 ml were prepared separately. The complete solution was prepared by adding 0.10 ml each of the $CaCl_2$ and $MgCl_2 \cdot 6H_2O$ solution to 100 ml of the stock solution.

Cerebrospinal fluid perfusion was initiated with artificial cerebrospinal fluid, pH 7.4, until all measured parameters stabilized. This solution was also interposed between test solutions to wash out the ventricular system and establish new control values. Test solutions were introduced into the ventricular system by changing to a perfusion syringe containing the desired test agent. Perfusion time for each test solution was 10-12 min. and sometimes 20 min. Artificial cerebrospinal fluid and cerebral venous outflow samples were taken at the end of each perfusion period. Carbon dioxide tension, oxygen tension and pH determinations were made immediately using a Beckman radiometer. The test solutions were prepared from artificial cerebrospinal fluid and were as follows: 1) 10 μg adenosine/liter artificial cerebrospinal fluid, 2) 100 μg adenosine/liter artificial cerebrospinal fluid, 3) CO_2 = 87 mm Hg, 4) CO_2 = 160 mm Hg, 5) CO_2 = 18 mm Hg. The carbon dioxide tension and hence the pH of the perfusates was altered by bubbling with 10% carbon dioxide.

The system was perfused with artificial cerebrospinal fluid at a rate of 4 ml/min using a Harvard constant volume infusion pump. The perfusion rate was selected to provide a cerebrospinal fluid pressure of approximately 12 mm Hg. This pressure was adequate to force the artificial cerebrospinal fluid around the entire brain surface but not high enough to significantly impair cerebral blood flow (35). To test the distribution of the artificial cerebrospinal fluid, India ink was added to the perfusate at the end of each experiment. After 3 min. the dog was sacrificed and the brain removed and examined for staining.

To assure the validity of the preparation and confirm the responsiveness of the cerebral bed to changes in arterial carbon dioxide tension, each dog was challenged with 10% carbon dioxide for 5 min. sometime during the experimental period. The resultant increase in arterial carbon dioxide tension was expected to elicit the classical cerebral hemodynamic response, i.e., a large decrease in cerebral vascular resistance and a large increase in cerebral blood flow.

Mean systemic pressure, central venous pressure and cerebrospinal fluid pressure were continuously recorded via Statham pressure transducers and a Sanborn direct writing recorder. Systemic pressure was recorded from a catheter placed in the aorta via the right femoral artery. Cerebral venous outflow pressure was recorded from a needle inserted into the cerebral venous outflow tubing. Cerebrospinal fluid pressure was monitored from the needle inserted into the right lateral ventricle. Cerebral vascular resistance was calculated by dividing the cerebral perfusion pressure (aortic minus cerebral venous pressure) by cerebral blood flow.

Statistical analysis of the data was performed using the Student's "t" test modified for paired replicates. A "p" value less than 0.05 was considered significant.

Figure 1. Distribution of the major cerebral arteries to general brain areas.

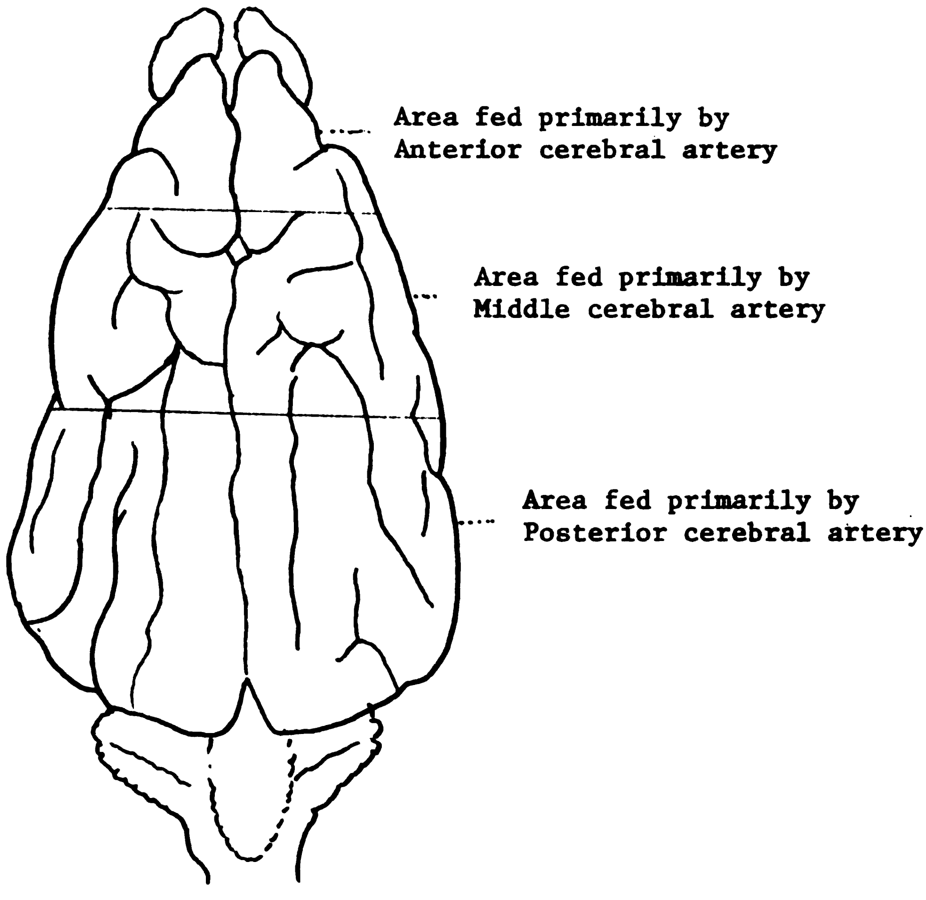

Area fed primarily by
Anterior cerebral artery
Area fed primarily by
Middle cerebral artery
Area fed primarily by
Posterior cerebral artery

Figure 1

Figure 2. The Circle of Willis.

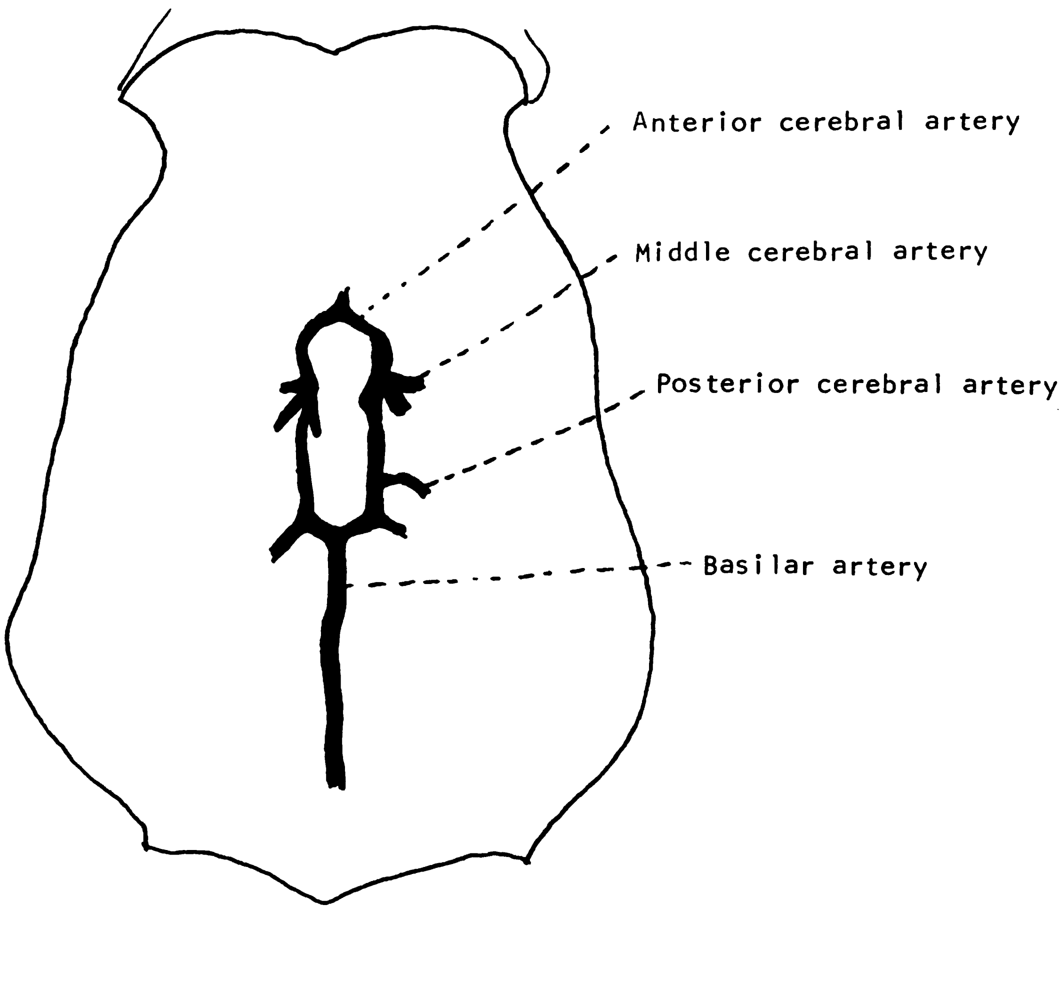

Anterior cerebral artery
Middle cerebral artery
Posterior cerebral artery
Basilar artery

Figure 2

Figure 3. Lateral view of the cranial venous sinuses and related veins.

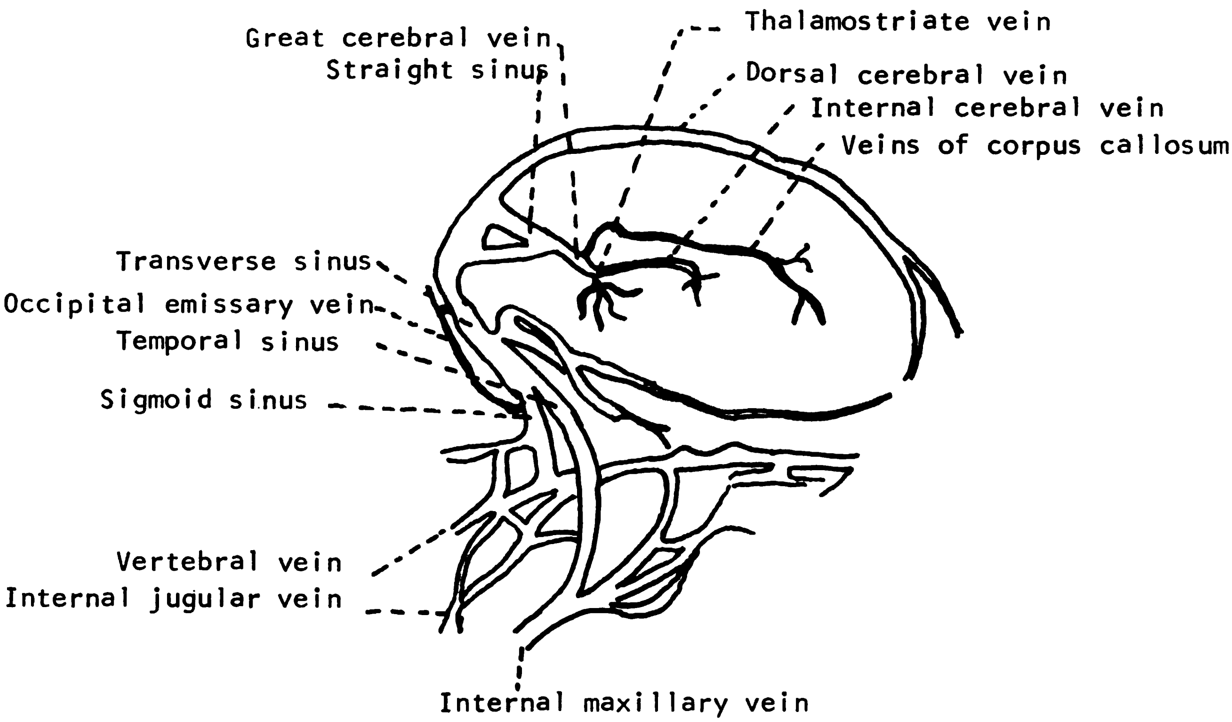
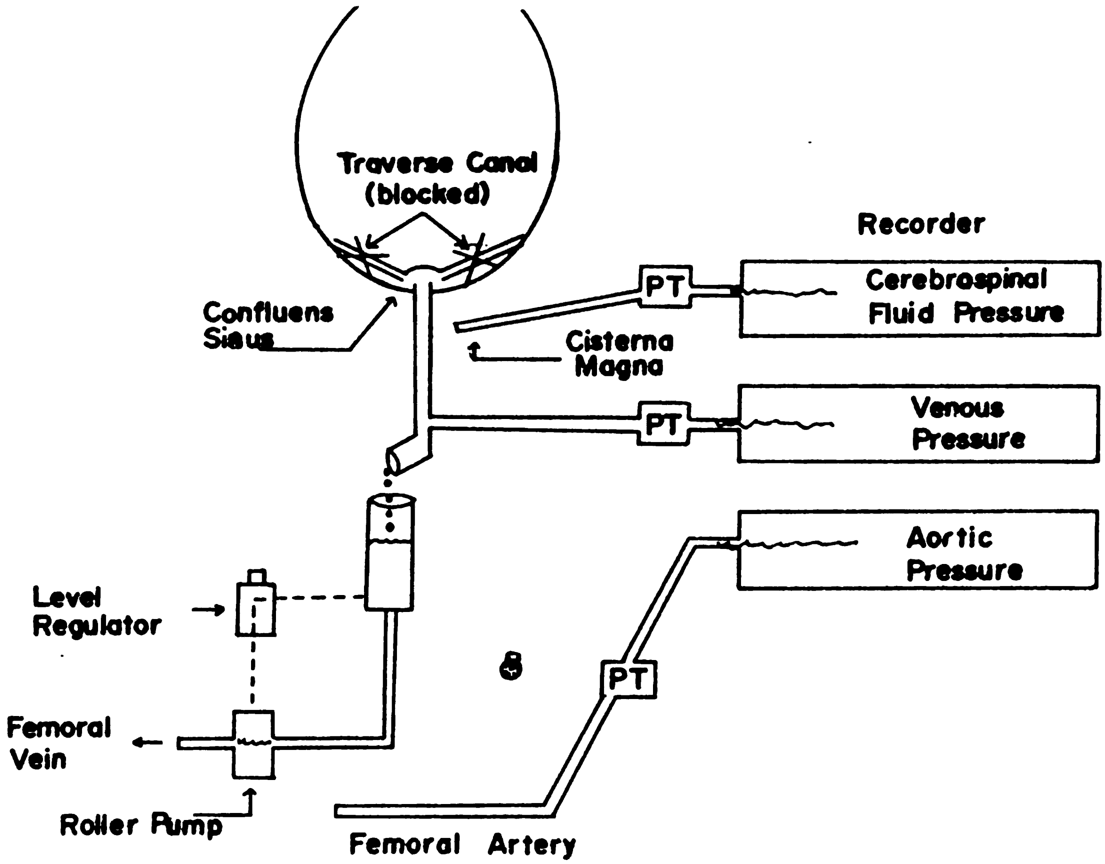

Great cerebral vein
Straight sinus
Thalamostriate vein
Dorsal cerebral vein
Internal cerebral vein
Veins of corpus callosum
Transverse sinus
Occipital emissary vein
Temporal sinus
Sigmoid sinus
Vertebral vein
Internal jugular vein
Internal maxillary vein

Figure 3

Figure 4. Preparation used to measure cerebral blood flow. Pressure transducer (PT).



Traverse Canal
(blocked)
Recorder
Cerebrospinal
Fluid Pressure
Confluens
Sinus
Cisterna
Magna
PT
PT
Venous
Pressure
Aortic
Pressure
Level
Regulator
PT
Femoral
Vein
Roller Pump
Femoral Artery

Figure 4

RESULTS

India ink perfusion

India ink was added to the artificial cerebrospinal fluid at the end of each experiment to determine whether the perfusion rate was adequate to saturate the entire brain surface, sulci and ventricles with articial cerebrospinal fluid. After 3 min the dog was sacrificed and the brain removed and examined for staining. Figure 5 shows a typical brain after such a procedure. Note that the entire brain surface as well as the sulci and ventricular system including the Circle of Willis are deeply stained.

Perfusion with 10 μg/ml adenosine

Increasing the artificial cerebrospinal fluid concentration of adenosine from 0 to 10 μg/ml had no significant affect on cerebral hemodynamic parameters: cerebral perfusion pressure (Δ P), cerebral blood flow (CBF), cerebral vascular resistance (CVR), as shown in Figure 6a. The clear bar represents values in the control period during which the artificial cerebrospinal fluid perfusate contained no adenosine. The slotted bar represents values from the experimental period when the perfusate contained 10 μg/ml adenosine. Figure 6b shows the gas and pH values of the effluent perfusate.

Figure 5. Brain after perfusing ventriculo-cisternal system with artificial cerebrospinal fluid containing India ink. Perfusion rate, 4 cc/min; average cerebrospinal fluid pressure 12 mm Hg.

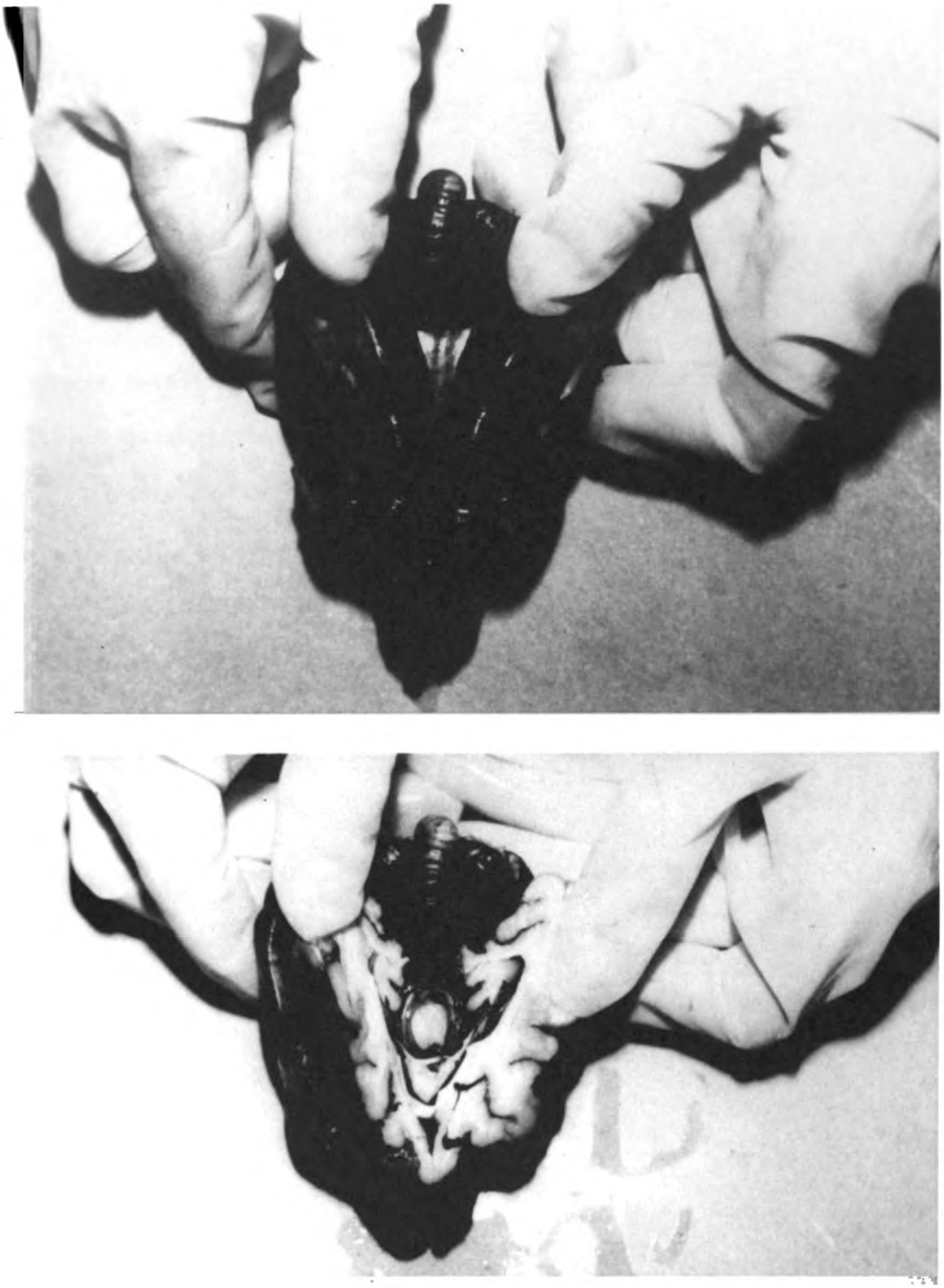
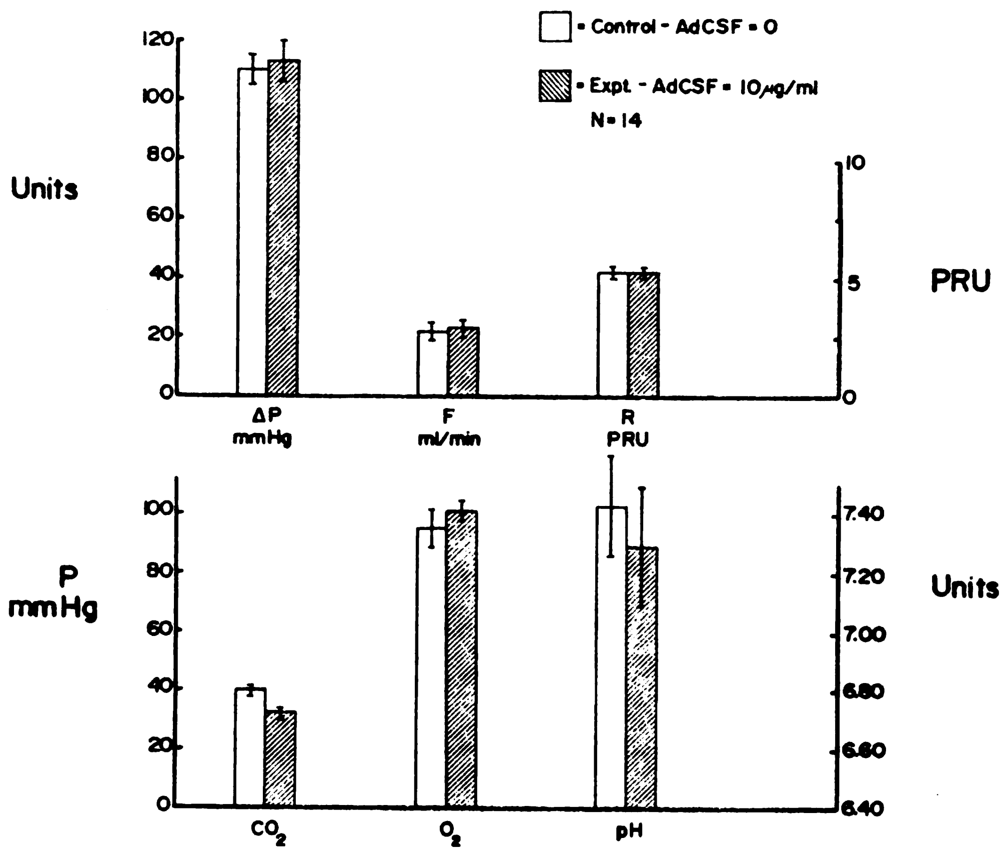


Figure 5

Figure 6a. Effects of increasing and artificial cerebrospinal fluid adenosine concentration from 0 to 10 μg/ml on cerebral hemodynamics. N = number of experiments; P = cerebral perfusion pressure; F = cerebral blood flow; R = cerebral vascular resistance (PRU = peripheral resistance units, mm Hg/mm/min).

Figure 6b. Effluent artificial cerebrospinal fluid gas and pH values for control and test solution (10 μg adenosine/ml).



= Control - AdCSF = 0
= Expt. - AdCSF = 10µg/ml
N = 14
Units
120
100
80
60
40
20
0
10
5
0
PRU
ΔP
mmHg
F
ml/min
R
PRU
P
mmHg
100
80
60
40
20
0
7.40
7.20
7.00
6.80
6.60
6.40
Units
CO2
O2
pH

Figure 6

Perfusion with 100 μg/ml adenosine

In five dogs of the preceding group the artificial cerebrospinal fluid concentration of adenosine was increased sequentially from 0 to 10 to 100 μg/ml artificial cerebrospinal fluid. Figure 7a depicts the statistically insignificant ($p < 0.05$) changes in cerebral hemodynamics.

Gas tensions and pH values for each of these perfusates are shown in Figure 7b.

Increased artificial cerebrospinal fluid carbon dioxide

Figure 8a shows the effects of a sudden increase in carbon dioxide tension in the perfusing artificial cerebrospinal fluid on vascular hemodynamics of the cerebral bed. The effluent artificial cerebrospinal fluid carbon dioxide tension was increased from 40 to 88 mm Hg. At the end of the 12 minute perfusion period, cerebral perfusion pressure and cerebral blood flow had increased significantly while cerebral vascular resistance had decreased significantly from control values.

As shown in Figure 8b, the artificial cerebrospinal fluid carbon dioxide tension was increased on the average of 47 mm Hg. Oxygen tension and pH were reduced 18 mm Hg and 0.27 units, respectively.

Perfusion with very high artificial cerebrospinal fluid carbon dioxide

To further test the effect of carbon dioxide tension on cerebral hemodynamics a very large increase in the artificial cerebrospinal fluid carbon dioxide tension was elicited. Increasing the artificial cerebrospinal fluid carbon dioxide tension to enormous levels resulted

Figure 7a. Effects of increasing the artificial cerebrospinal fluid adenosine concentration from 0 to 10 to 100 μg/ml on cerebral hemodynamics.

Figure 7b. Effluent artificial cerebrospinal fluid gas and pH values for control and test solutions.

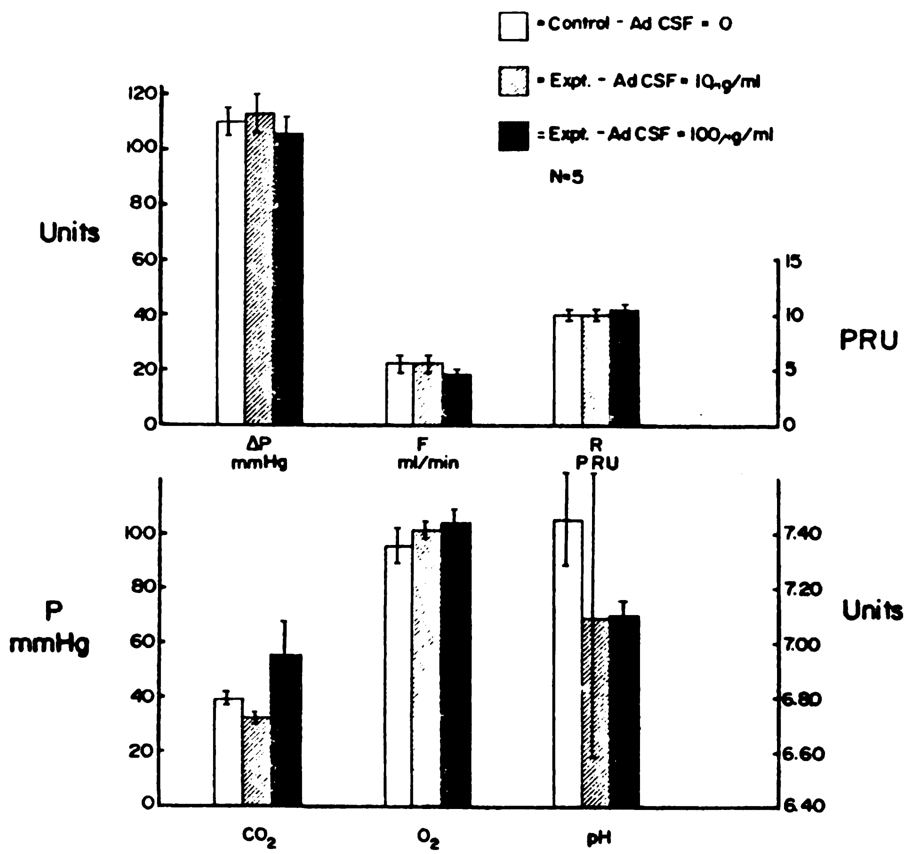
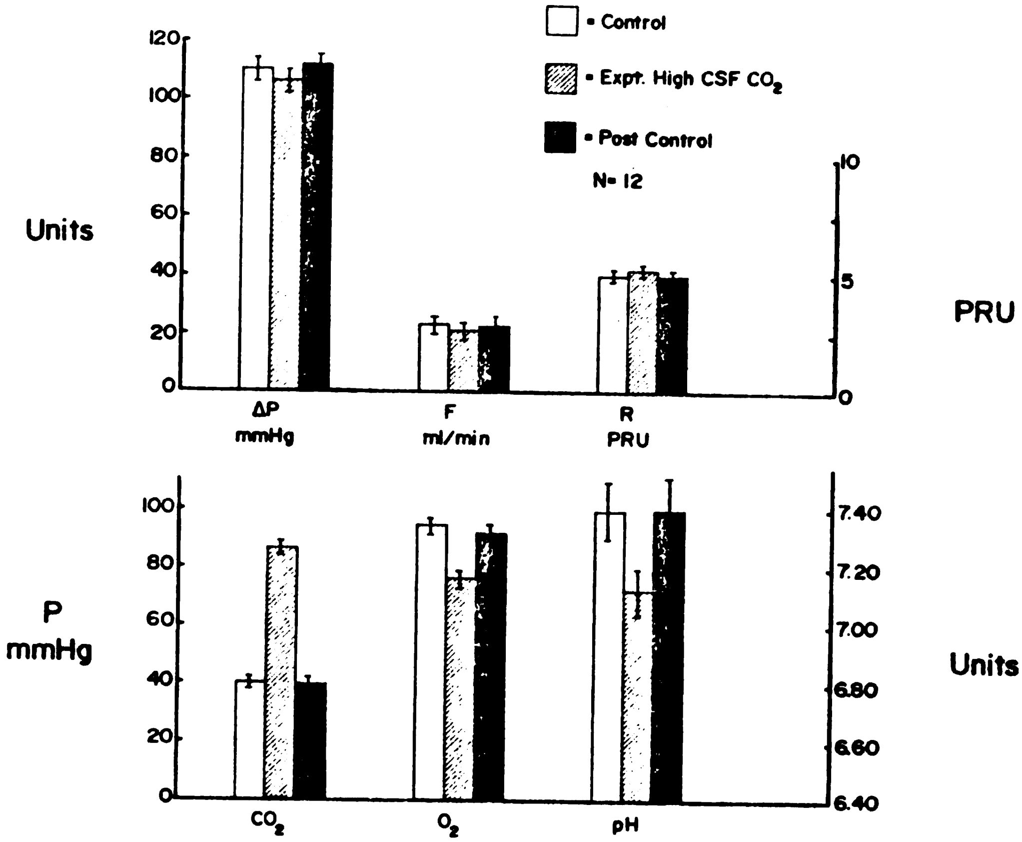

= Control - Ad CSF = 0
= Expt. - Ad CSF = 10μg/ml
= Expt. - Ad CSF = 100μg/ml
N=5
Units
120
100
80
60
40
20
0
15
10
5
0
PRU
ΔP
mmHg
F
ml/min
R
PRU
P
mmHg
100
80
60
40
20
0
7.40
7.20
7.00
6.80
6.60
6.40
Units
CO_2
O_2
pH

Figure 7

Figure 8a. Effects of a moderate increase in artificial cerebrospinal fluid carbon dioxide tension on cerebral hemodynamics.

Figure 8b. Effluent artificial cerebrospinal fluid gas and pH values for control, experimental and post-control solutions.



= Control
= Expt. High CSF CO_2
= Post Control
N= 12
Units
PRU
120
100
80
60
40
20
0
10
5
0
ΔP mmHg
F ml/min
R PRU
P mmHg
Units
100
80
60
40
20
0
7.40
7.20
7.00
6.80
6.60
6.40
CO_2
O_2
pH

Figure 8

in statistically insignificant changes in cerebral hemodynamics as shown in Figure 9a.

Figure 9b depicts changes in effluent artificial cerebrospinal fluid gas tensions and pH. In this test the control carbon dioxide tension was 39.4 as compared to a carbon dioxide tension test value in excess of 140 mm Hg. (Some experimental values exceeded the maximum carbon dioxide tension reading of 160 mm Hg on the radiometer.) The control oxygen tension was 54.5 as compared to a test value of 61.5 mm Hg and the control pH was 7.37 as compared to 6.57 pH units in artificial cerebrospinal fluid containing very high carbon dioxide tension.

Comparison of artificial cerebrospinal fluid and cerebral venous blood gas tensions and pH while perfusing with very high artificial cerebrospinal fluid carbon dioxide tension

To determine whether an increase in artificial cerebrospinal fluid carbon dioxide concentration is reflected in the cerebral venous blood, artificial cerebrospinal fluid and cerebral venous blood samples were taken simultaneously at the end of the control, experimental and post-control periods for immediate analyzation. Figure 10 illustrates the change in cerebral venous carbon dioxide tension when the artificial cerebrospinal fluid carbon dioxide tension was increased from 40 to 145 mm Hg. Cerebral venous blood carbon dioxide tension increased 14 mm Hg (39%). Associated with this was a 0.11 unit fall in cerebral venous blood pH. Neither artificial cerebrospinal fluid nor cerebral venous blood oxygen tension was significantly altered.

Figure 9a. Effects of a large increase in artificial cerebrospinal fluid carbon dioxide tension on cerebral hemodynamics.

Figure 9b. Effluent artificial cerebrospinal fluid gas and pH values for control, experimental and post-control solutions.

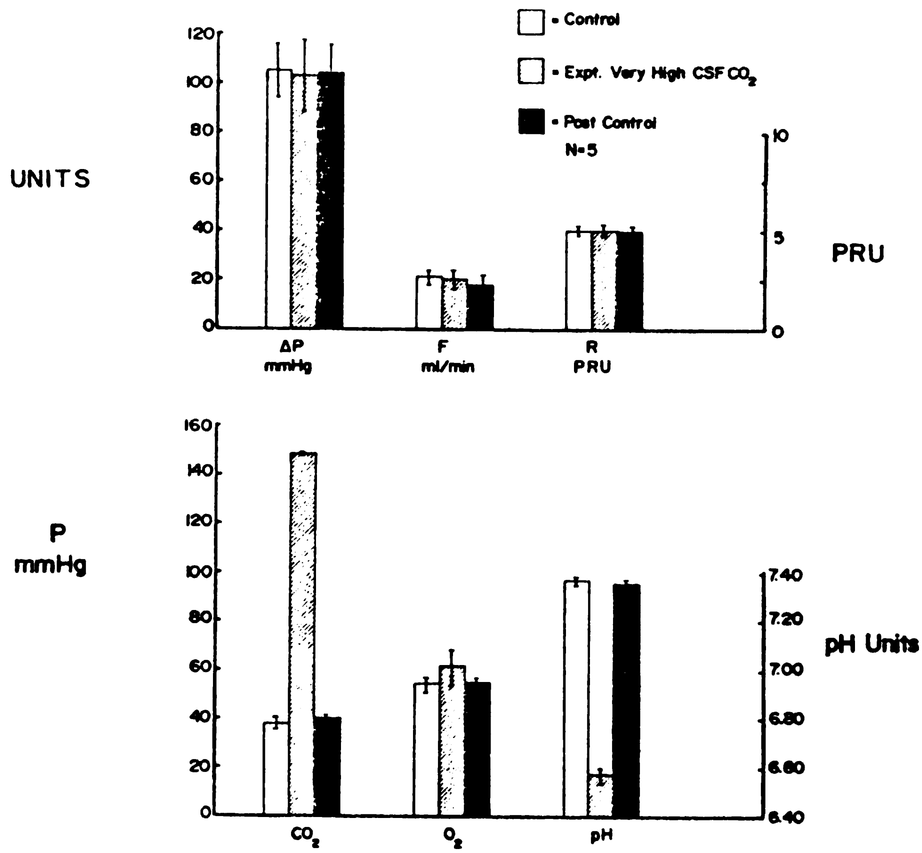
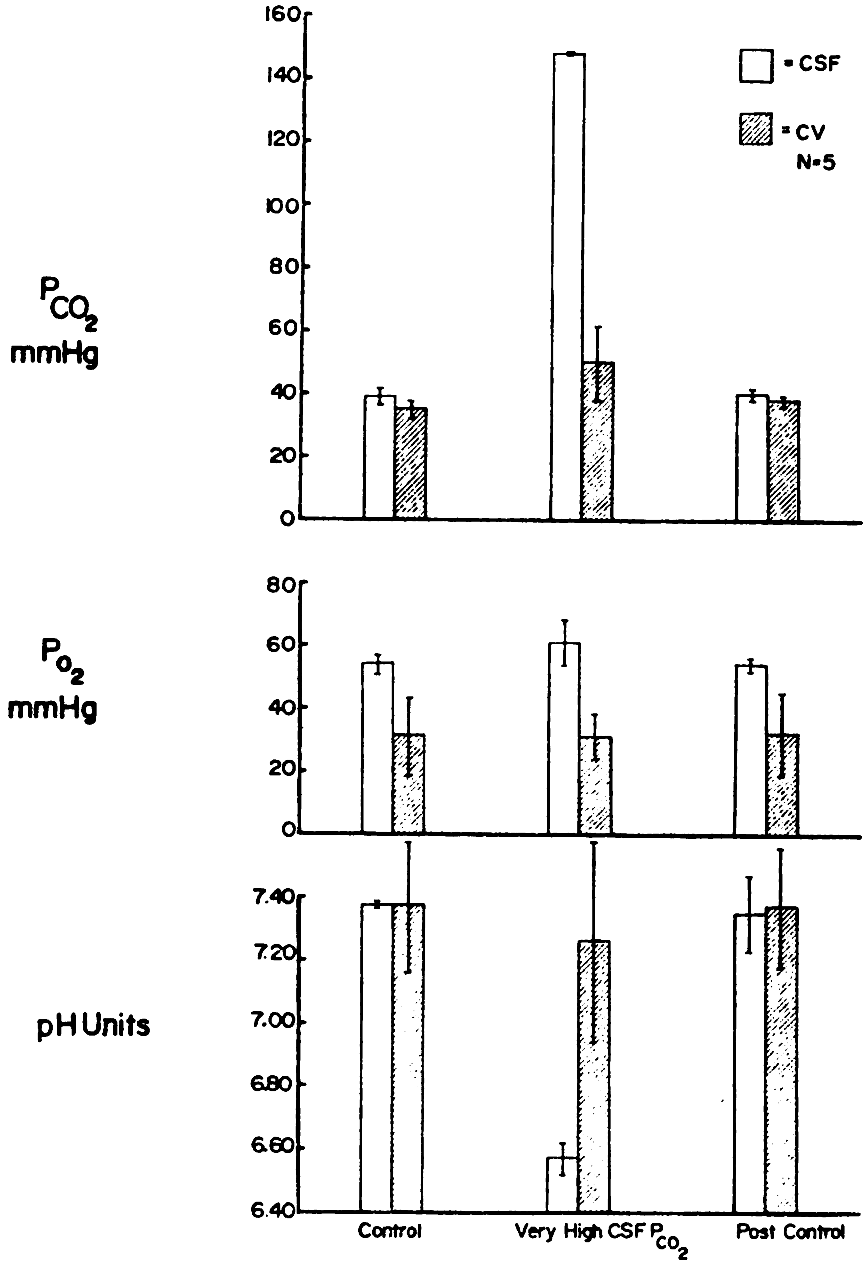

UNITS
120
100
80
60
40
20
0
= Control
= Expt. Very High CSF CO_2
= Post Control
N=5
10
5
0
PRU
ΔP
mmHg
F
ml/min
R
PRU
P
mmHg
160
140
120
100
80
60
40
20
0
7.40
7.20
7.00
6.80
6.60
6.40
pH Units
CO_2
O_2
pH

Figure 9

Figure 10. Comparison of effluent artificial cerebrospinal fluid and cerebral venous blood outflow gas and pH values for control, experimental (a large increase in artificial cerebrospinal fluid carbon dioxide tension) and post-control periods.



160
140
120
100
80
60
40
20
0
P_{CO_2} mmHg
= CSF
= CV
N=5
80
60
40
20
0
P_{O_2} mmHg
7.40
7.20
7.00
6.80
6.60
6.40
pH Units
Control
Very High CSF P_{CO_2}
Post Control

Figure 10

Perfusion with low artificial cerebrospinal fluid carbon dioxide

The effect on cerebral hemodynamics of perfusing the ventriculo-cisternal system with an artificial cerebrospinal fluid solution deficient in carbon dioxide is illustrated in Figure 11a. As the graph indicates, a reduction in artificial cerebrospinal fluid carbon dioxide tension from 40 to 20 mm Hg did not significantly ($P < 0.05$) affect cerebral perfusion pressure, cerebral blood flow or cerebral vascular resistance.

The gas tensions and pH values for the control, experimental and post-control periods are shown in Figure 11b.

Increased arterial carbon dioxide tension

Systemic arterial carbon dioxide tension was increased from 38 to 48 mm Hg, eliciting the classical change in cerebral hemodynamic parameters, Figure 12a. During this test period, ventriculo-cisternal perfusion was maintained with artificial cerebrospinal fluid containing normal gas and pH values, Figure 12b.

Comparison of artificial cerebrospinal fluid and cerebral venous gas tensions and pH during ventilation with 10% carbon dioxide

Figure 13 compares the control, experimental and post-control gas and pH values of the effluent artificial cerebrospinal fluid to those of the cerebral venous blood. This experiment was designed to determine whether an increase in arterial carbon dioxide tension is reflected in the artificial cerebrospinal fluid.

Figure 11a. Effects of decreasing the artificial cerebrospinal fluid carbon dioxide tension on cerebral hemodynamics.

Figure 11b. Effluent artificial cerebrospinal fluid gas and pH values for control, experimental and post-control periods.

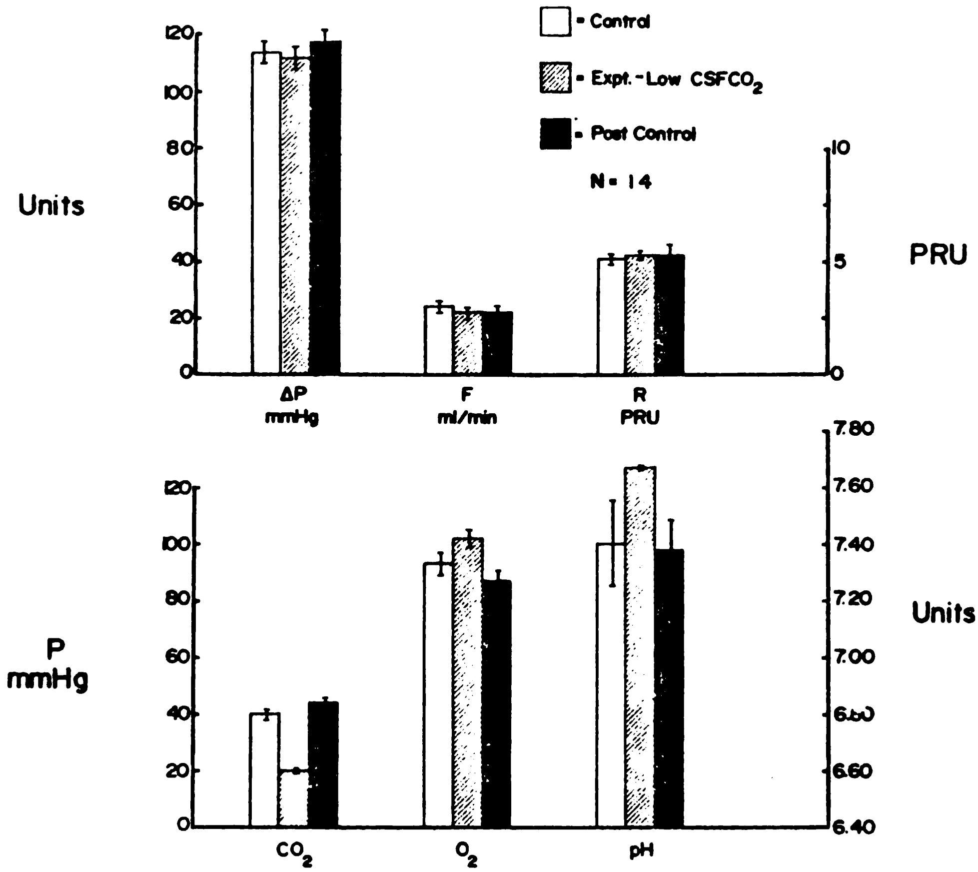

Control
Expt.-Low CSFCO2
Post Control
N = 14
Units
PRU
ΔP mmHg
F ml/min
R PRU
P mmHg
Units
CO2
O2
pH

Figure 11

Figure 12a. Effects of increasing the arterial carbon dioxide concentration on cerebral hemodynamics.

Figure 12b. Effluent artificial cerebrospinal fluid gas and pH values for control, experimental and post-control solutions.

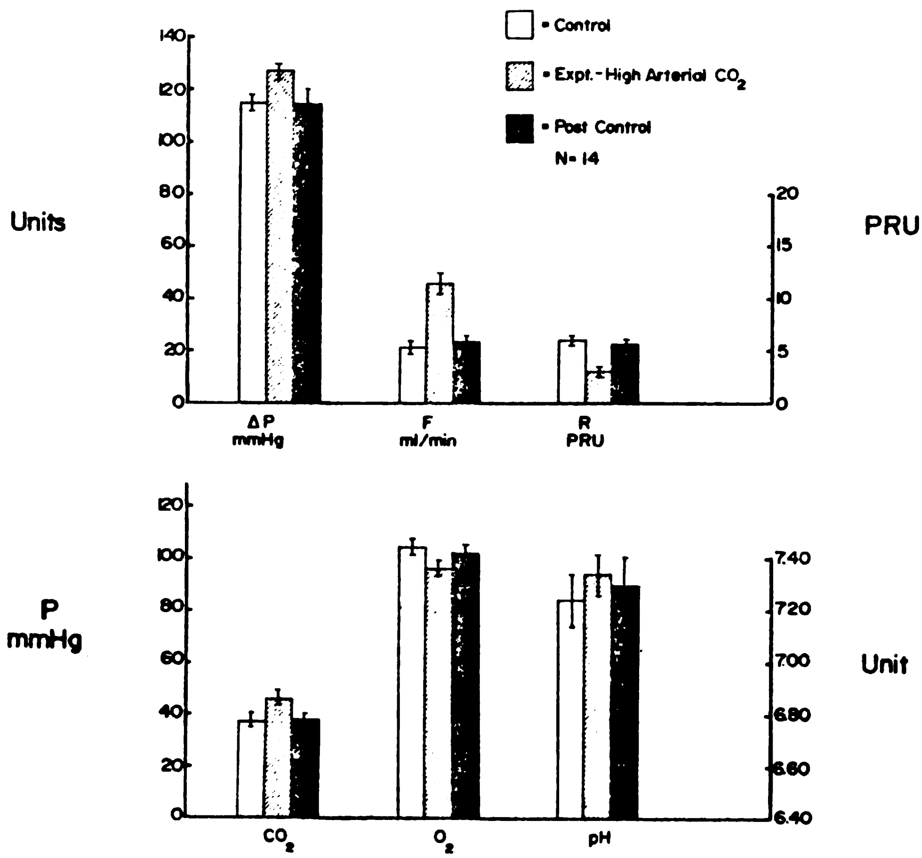
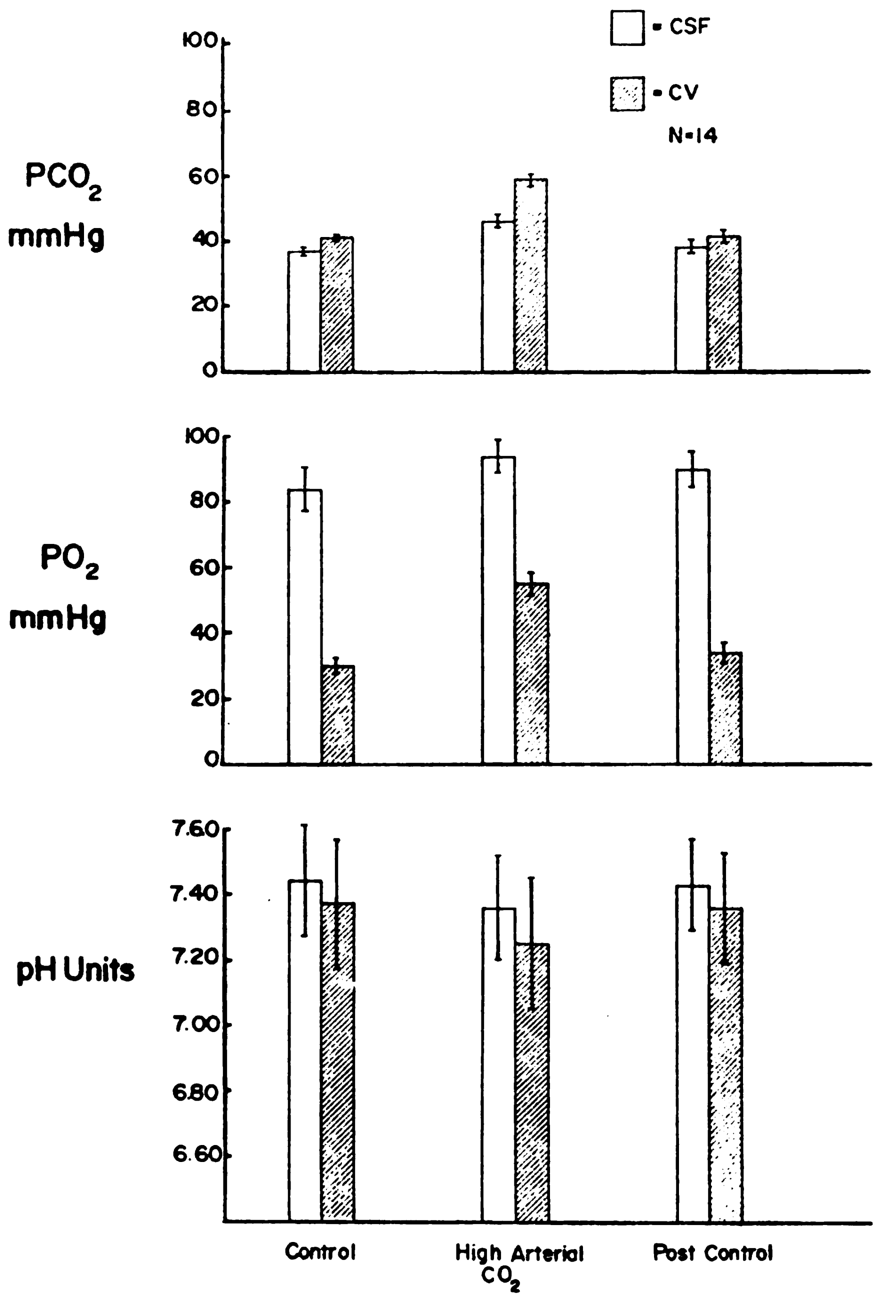

□ = Control
= Expt.-High Arterial CO_2
= Post Control
N= 14
Units
PRU
140
120
100
80
60
40
20
0
20
15
10
5
0
ΔP mmHg
F ml/min
R PRU
P mmHg
Unit
120
100
80
60
40
20
0
7.40
7.20
7.00
6.80
6.60
6.40
CO_2
O_2
pH

Figure 12

Figure 13. Comparison of effluent artificial cerebrospinal fluid and cerebral venous blood outflow gas and pH values for control, experimental (increased arterial carbon dioxide tension) and post-control periods.



= CSF
= CV
N=14
PCO2 mmHg
100
80
60
40
20
0
PO2 mmHg
100
80
60
40
20
0
pH Units
7.60
7.40
7.20
7.00
6.80
6.60
Control
High Arterial CO2
Post Control

Figure 13

An 18 mm Hg increase in cerebral venous carbon dioxide tension was induced by ventilation with 10% carbon dioxide for five minutes. The increase in arterial carbon dioxide tension seems to be reflected in the artificial cerebrospinal fluid which rose 9 mm Hg (17%). Associated with this increase was a fall in artificial cerebrospinal fluid pH (0.80 units), and a 0.25 mm Hg increase in artificial cerebrospinal fluid oxygen tension.

DISCUSSION

These results show that altering the adenosine concentration and/or carbon dioxide tension of the artificial cerebrospinal fluid has no consistent effect on cerebral blood flow in the dog. The artificial cerebrospinal fluid concentration of adenosine was increased to 10 and subsequently to 100 μg/ml without eliciting a significant change in cerebral perfusion pressure, cerebral blood flow, or cerebral vascular resistance.

However, Berne et al. (6) and more recently Wahl and Kuschinsky (96) reported dilation of pial vessels during local application of artificial cerebrospinal fluid containing adenosine. Also, the degree of pial vessel dilation was directly related to the concentration of adenosine.

Artificial cerebrospinal fluid carbon dioxide tensions of 18, 87, or 160 mm Hg also did not consistently influence cerebral blood flow when perfused through the ventriculo-cisternal system. This is particularly significant when compared to the classical decrease in cerebral vascular resistance and increase in cerebral blood flow elicited when arterial carbon dioxide tension was increased in the same preparations. In addition to this well-established response to increased arterial carbon dioxide tension, other investigators (50,95) have shown that pial vessel diameter increases when carbon dioxide tension and hydrogen ion concentration are increased locally in cranial window studies.

Therefore, the present study seems to suggest that pial vessels are unresponsive to increases in the artificial cerebrospinal fluid concentration of adenosine or carbon dioxide. However, in view of the cranial window studies referred to above, the present study may suggest a reciprocal type of responsiveness between cerebral extra- and intra-parenchymal vessels. This hypothesis implies that the pial vessels, which are well innervated (43) and apparently sensitive to vasoactive agents in cerebrospinal fluid (6,47,95,96), may respond to these stimuli by increasing or decreasing diameter, i.e., altering pial blood flow, without causing a similar change in total cerebral blood flow. Hence, the intra-parenchymal vessels would be expected to respond reciprocally to the change in extra-parenchymal blood flow in order to maintain constant total cerebral blood flow. Essentially, this is intra-parenchymal autoregulation.

Recent studies provide support for the reciprocal response hypothesis. Posner, Plum and Zee (74) measured cerebral blood flow using the Rapela technique. They observed no change in cerebral blood flow when the pH or carbon dioxide tension of the cerebral ventricular system was altered indirectly by varying the cerebrospinal fluid bicarbonate concentration in the dog. Using a similar experimental approach, Leusen (56) demonstrated that blood flow in the caudate nucleus varied directly with the pH of the cerebrospinal fluid perfusate while hemispheric blood flow did not change. It should be noted, however, that these studies and the present study do not agree with Siesjo et al. (88), who used similar experimental procedures but measured cerebral blood flow indirectly by Xe clearance.

As proposed, the reciprocal response hypothesis offers a possible explanation to the observations that sympathetic stimulation does not appreciably alter cerebral blood flow (39). This hypothesis thus offers to reconcile two apparently contradictory observations: pial vessel response to catecholamines and the lack of response in cerebral blood flow to catecholamines in cerebrospinal fluid. It is well-known that the pial vessels are highly innervated with adrenergic fibers (43) and that local application of epinephrine to pial arteries elicits vasoconstriction (97). However, ventriculo-cisternal perfusion with artificial cerebrospinal fluid containing epinephrine does not significantly alter cerebral blood flow (unpublished observation).

Other vasoactive agents besides adenosine, carbon dioxide and epinephrine may also trigger a reciprocal response. The data of Martin and Rapela (62) suggest that while serotonin may elicit an increase in extra-parenchymal vascular resistance, total cerebral blood flow may not be affected.

Therefore, the present study, considered alone, indicates that neither the adenosine concentration nor carbon dioxide tension of the artificial cerebrospinal fluid has an effect on total cerebral blood flow. However, in view of the cranial window studies, one can assume that the pial vessels dilate in response to increased artificial cerebrospinal fluid adenosine concentration or carbon dioxide tension. Therefore, it is likely that a type of reciprocal responsiveness exists between the extra- and intra-parenchymal vessels which function to maintain total cerebral blood flow constant.

It is well-established that carbon dioxide is highly permeable to most tissues and that arterial hypercapnia decreases cerebrovascular resistance and increases cerebral blood flow. Therefore, one would expect that increasing the artificial cerebrospinal fluid carbon dioxide tension should increase arterial carbon dioxide locally and increase cerebral blood flow. The present study, as well as others (37,72,74), have shown that increased artificial cerebrospinal fluid carbon dioxide tension does not increase cerebral blood flow and the reciprocal response hypothesis was proposed (37,74). However, in view of the classical cerebral response elicited by increased arterial carbon dioxide tension, the reciprocal response hypothesis does not fully explain why carbon dioxide, which is presumably taken up by the cerebral arteries from the cerebrospinal fluid, fails to increase cerebral blood flow.

The present study shows that the cerebral blood carbon dioxide tension influences the cerebrospinal fluid carbon dioxide tension and visa versa. While breathing 10% carbon dioxide, cerebral venous outflow and effluent cerebrospinal fluid carbon dioxide tension increased 50% and 17%, respectively (Figure 13). In comparison, a 250% increase in effluent artificial cerebrospinal fluid elicited a 39% increase in cerebral venous outflow carbon dioxide tension (Figure 10). This difference may be partially accounted for by the large buffering capacity of the blood and the low buffering capacity of the cerebrospinal fluid.

Yet, it is important to note that a 39% increase in cerebral venous outflow carbon dioxide tension should be indicative of a similar increase in cerebral arterial carbon dioxide tension. (Several major arteries supplying the brain, including the Circle of Willis and major tributaries, are bathed by artificial cerebrospinal fluid. Thus changes in carbon dioxide concentration in the cerebrospinal fluid should be reflected by parallel alterations in arterial carbon dioxide concentration since this gas diffuses readily through tissues.) A 10% increase in cerebral arterial carbon dioxide tension is more than adequate to elicit the classical decrease in cerebrovascular resistance and increase in cerebral blood flow. It is possible that the large increase in carbon dioxide concentration is accomplished primarily in the cerebral venous system and therefore does not increase arterial carbon dioxide tension sufficiently to elicit the classical response. However, in view of the cranial window studies by Levin, Sepulveda and Yudelevich (57), this is unlikely. This group perfused the exposed pia with tritiated water (which is also highly diffusable) and within 30 sec. radioactivity was detectable in the cerebral venous outflow. Also, in the present study the entire ventricular system, Circle of Willis and brain surface, were perfused for 12-20 min. (Figure 4), which should have provided adequate exposure to sufficiently increase the carbon dioxide tension of the cerebral resistance vessels. However, no change was seen in cerebrovascular resistance or cerebral blood flow.

As discussed previously, the mechanism by which carbon dioxide influences cerebral vascular resistance is speculative. The present study does not explain this phenomena. Experiments differentiating between the cerebral arterial, capillary and venous beds and their characteristics are necessary before this mechanism can be explained.

CONCLUSION

In conclusion, the results indicate that increasing the cerebrospinal fluid concentration of adenosine or increasing or decreasing the cerebrospinal fluid carbon dioxide tension does not appreciably influence cerebral vascular resistance or cerebral blood flow. However, comparable increases in cerebral arterial carbon dioxide tension results in a 104% increase in cerebral blood flow and a 45% decrease in cerebral vascular resistance. The present study does not support the hypothesis that the adenosine concentration or carbon dioxide tension in the cerebrospinal fluid are involved to a major extent in local cerebral blood flow regulation. Yet, in view of the large body of evidence demonstrating that local, topical application of adenosine or carbon dioxide dilates pial arteries, it has been suggested that a type of reciprocal response may exist between the intra- and extra-parenchymal vessels.

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