Step Hypocapnia to Separate Arterial from Tissue PCO\(_2\) in the Regulation of Cerebral Blood Flow

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ABSTRACT

The change in cerebral blood flow was determined after a step decrease in the PCO\(_2\) of arterial blood from 40 to 25 mm Hg in awake man. Subjects monitored their own end-tidal PCO\(_2\) (infrared analyzer) and adjusted their voluntary ventilation to produce the step change, which they maintained for at least 1 hour. Cerebral blood flow relative to control was determined from the arterial-jugular venous oxygen saturation differences. After the step change, arterial PCO\(_2\) fell in less than 30 sec to a plateau, cerebral blood flow fell with a time constant (to 1/e) of 0.3 min to a plateau of 68% of control, while jugular venous PCO\(_2\) fell with a time constant for the fast component of 3.5 min. Base excess rose 1.2 mEq/liter within 1 min and remained at that level. It is concluded that CO\(_2\) affects cerebral blood flow by direct diffusion into arteriolar walls, rather than by its effect on brain tissue PCO\(_2\) or pH. It is postulated that the pH of the extracellular fluid of arteriolar smooth muscle is the common controlled variable through which CO\(_2\), and possibly hypoxia and blood pressure, determine vascular tone.

ADDITIONAL KEY WORDS brain extracellular fluid hyperventilation smooth muscle tone cerebrospinal fluid pH

Shapiro, Wasserman and Patterson (1) have recently shown that cerebral blood flow during the transient induction and withdrawal of hypercapnia correlates better with jugular venous PCO\(_2\) than with arterial PCO\(_2\). They have concluded that tissue PCO\(_2\) is more important than arterial blood PCO\(_2\) in determining cerebral blood flow. A restatement of this conclusion might be that mean tissue PCO\(_2\) rather than the PCO\(_2\) of arteriolar wall or other more rapidly perfused tissue, appears to be determinant. Their method, with slight modifications, promises to contribute to a better understanding of the site and mechanism of cerebral vasomotion by CO\(_2\), and the experiments to be described, which may be regarded as an extension of theirs (although completed before their publications appeared), have led us to the opposite conclusion; namely, that CO\(_2\) exerts its action directly upon the arteriolar wall, flow being largely independent of brain tissue PCO\(_2\). However, as will be discussed later, their findings in hypocapnia are similar to ours, and both sites may contribute under certain circumstances.

Method

We have induced a step decrease in the PCO\(_2\) of arterial blood (Paco\(_2\)) and utilized the succeeding washout period to observe the jugular venous PCO\(_2\) and the arterio-venous (A–V) O\(_2\) saturation difference, the former as an index of tissue PCO\(_2\), the latter as an index of cerebral blood flow. The justification of the assumptions required to calculate changes in blood flow from A–V O\(_2\) saturation differences have been carefully discussed by Shapiro, Wasserman and Patterson (1). We studied seven healthy adult male subjects in semisupine position, with about 20° head-up tilt. The jugular bulb was cannulated percutaneously with an 18–20 gauge, 2.5-inch needle and the...
Arterial and jugular venous $P_{CO_2}$ following a step reduction in alveolar $P_{CO_2}$. Arterial blood $P_{CO_2}$ was reduced in less than 0.5 min to a plateau level while jugular venous blood $P_{CO_2}$ fell slowly, reflecting the washout of the tissue $CO_2$ stores.

Brachial artery was catheterized by the Seldinger technique. The sampling catheter of an infrared $CO_2$ analyzer was inserted into a nostril extension (10 cm long, 1 cm diam), the mouth, or a mouthpiece. The subjects were taught to monitor their own end-tidal $P_{CO_2}$ ($P_{ACO_2}$) using the panel meter, and to hyperventilate suddenly, sufficiently to reduce their $P_{ACO_2}$ from control to 20 mm Hg with two or three fast deep breaths, and then to hold it at this level as constantly as possible for 1 to 2 hours. Ventilation was thus maximum at first and fell off gradually as body $CO_2$ stores were excreted. The $CO_2$ analyzer signal was continuously recorded in four subjects, together with arterial pressure, transduced by a strain gauge.

Paired 2-ml arterial and jugular venous blood samples were drawn two or three times in the control period, and at 0.5, 1.0, 1.5, 2.5, 4.0, 7.0, 15, 30, 60, and in three cases, at 90 and 120 min. $pH$, $P_{CO_2}$ and $P_{O_2}$ of samples were determined in suitable electrodes at 37°C. Oxygen saturation was determined by reflectance oximetry, using the Kipp hemorefractor for the three subjects studied in Copenhagen and the American Optical reflectance oximeter for the four subjects studied in San Francisco. Relative cerebral blood flow was calculated from the reciprocal of the $A-V O_2$ saturation difference. Hematocrit was determined on control and final samples in four instances.

**Results**

Although the subjects had no previous experience with breathing apparatus, after about 10 min of training, all were able to control their end-expiratory $P_{ACO_2}$ and to lower it suddenly from normal to about 20 mm Hg. The variations in the resulting $P_{ACO_2}$ are depicted in Figure 1. Subject 3 was primed by a control period of breathing 3% $CO_2$ in 25% $O_2$, in order to obtain a greater tissue—arterial $P_{CO_2}$ separation during hypocapnia; he was instructed to hold his $P_{ACO_2}$ at 25 mm $Hg$, since his air-breathing $P_{ACO_2}$ was 45 mm $Hg$. For the subjects in whom $P_{ACO_2}$ was recorded, the arterial—end-tidal $CO_2$ difference was 2 mm $Hg$ during the control period and rose to 4 to 5 mm $Hg$ during hypocapnia. No subject experienced tetany, although several developed a positive Chvostek's sign and most noted paresthesias in the extremities.

In general, subjects were successful in con-
trolling Paco₂, although there was a tendency to undershoot at first, then to hunt during the first few minutes, and to permit a rise in Paco₂ as time progressed, probably due to lack of attention. The individually calculated cerebral blood flow curves (Fig. 2) show similar variations, with a tendency to rise as Paco₂ rose (see Fig. 1).

Jugular venous Pco₂ fell slowly, reaching a plateau between 4 and 15 min after hyperventilation began. Its reapproach to an equilibrium A–V PCO₂ difference was calculated as

\[ 1 - \left[ \frac{\Delta_{t} - \Delta_{0}}{\Delta_{0} - \Delta_{en}} \right] \]

where \( \Delta \) is PvcO₂ minus Paco₂ at the time indicated in minutes. This time course is plotted semilogarithmically in Figure 3. The time constant of the initial linear portion, calculated as the time required to fall to \( 1/e \), or \( 0.368 \), is 3.5 min. This cannot be interpreted as a compartmental time constant dependent upon the ratio of tissue volume to blood flow for several reasons: 1) Flow was changing during the initial phase. 2) Venous Pco₂ is not a linear function of tissue CO₂ content because the tissue dissociation curve is not linear, and because venous Pco₂ is not equal to tissue Pco₂ (2). 3) The washout curve does not appear to be a single exponential. 4) The range of the individual data is too great to assign significance to the final (15 min) point in Figure 3, which is the only evidence of a slow phase. In view of these limitations, we have not attempted to compute compartmental flow-volume ratios.

The average Pco₂ and flow curves are plotted in Figure 4. For this purpose, subject 3 at zero time was assigned his air-breathing control values, which were Paco₂ = 46.3, PvcO₂ = 57.6, rather than the hypercapnic values shown in Figures 1 and 2. Figure 4 suggests that cerebral blood flow reached a plateau of 68% of the control at 2.5 min. At 0.5 min, flow had fallen to 74% of the control. This point is plotted on Figure 3, and indicates a time constant for cerebral blood flow of 0.3 min, about 10 times faster than that found for jugular venous Pco₂.

The average control base excess was +0.1 mEq/liter and this rose to +1.3 at 1 min, remaining within ±0.2 mEq/liter of this level for the subsequent hour. A rise due to a shift of bicarbonate from extracellular fluid to blood is to be expected with hyperventilation.

**Discussion**

This method of obtaining semicontinuous information regarding cerebral blood flow in the transient state following a step reduction of Paco₂ requires that cerebral metabolic rate be constant, that blood O₂ capacity remain constant, that the O₂ saturation in jugular venous blood reflect capillary O₂ saturation, and that the O₂ capacity of tissue and capillary blood introduce a negligible delay.
CEREBRAL BLOOD FLOW AFTER STEP HYPOCAPNIA

Changes in Paco₂, Pvc₀₂ and cerebral blood flow following a step reduction in alveolar Paco₂. Cerebral blood flow closely followed the arterial Paco₂ rather than the jugular venous blood Paco₂ washout curve.

The constancy of cerebral metabolic rate during hypocapnia was established most recently by Wasserman and Patterson (3), and accords with previous work reviewed by Lassen (4). We observed no change in hematocrit during 1 hour of hyperventilation. We estimated the time lag for an oxygen saturation change in the capillaries to reach the internal jugular bulb using values for blood flow and for cerebral venous blood volume (which approximates 3 ml/100 g brain or 5% of the cerebral blood volume). During hypocapnia, the flow is 68% of normal, or about 30 ml/min per 100 g brain. Neglecting tissue O₂ stores, the time lag is then about 3/30 min, or 6 sec. The venous samples were drawn 6 sec after the arterial samples. Under these circumstances, the reciprocal of the changes in A-V oxygen saturation difference across the brain should be a valid index of cerebral blood flow changes in awake man during hypocapnia.

It is generally assumed that the reduction of cerebrovascular tone produced by hypercapnia is caused by a local reaction in the brain tissue rather than by a reflex. Experimental evidence supporting this assumption has been published by Gotoh, Tazaki, and Meyer (5). These authors demonstrated increased blood flow in the cortex of anesthetized cats after local application of CO₂ to the exposed cortex.

The locus of action of CO₂ on cerebral resistance vessels could lie either in the wall of the vessels themselves or in the tissue served by the vessels, with some hypothetic feedback of information from the tissue to the arterioles. For example, arteriolar muscles might be sufficiently close to metabolically active cells that their Pco₂ could be determined more by their surroundings than by their luminal blood. We have attempted to separate these two possibilities by a step decrease in Paco₂, assuming that the Pco₂ of the arterial wall will fall much faster than that of the brain tissue Pco₂. We have no indicator of the rate of equilibration of Pco₂ between arteriolar wall and blood in the lumen, but have some indication from the jugular venous blood of the rate of tissue CO₂ clearance. Cortical tissue Pco₂, determined by a tissue CO₂ electrode, is about 0.5 to 1.0 mm Hg higher than the average of arterial and sagittal sinus venous Pco₂ in anesthetized cats, according to Ponten and Siesjo (2). The mean washout time for brain tissue cannot be widely different from the observed venous washout. However, the Pco₂ falls first in tissue nearest the arteriolar end of the capillary. The speed of response of the circulation—about 10 times faster than tissue washout—suggests that the arteriolar smooth muscle or some other tissue very near the arteriolar end of the capillary is the site of action of CO₂.

Thus far, we have considered the significance of our observations in terms of a uniformly perfused brain. In fact, the gray matter, forming about half the brain, has at
least 80% of the flow. Is it possible that the different time constants of gray and white matter might produce the observed washout pattern even if the cerebral resistance vessels were responding to the Pco2 of their respective tissues, rather than to arterial Pco2? A quantitative answer to this question is beyond our reach at present, requiring nonlinear equations, the time constant of each compartment changing continuously as tissue Pco2 changes. However, two limiting conditions appear to exclude this possibility without requiring a complete solution. 1) Gray-matter flow approximates 1 ml/g per min, which at this flow rate would wash out initially with a time constant of 1 min, increasing with vasoconstriction to perhaps 2 min as tissue Pco2 fell. The smaller contribution from white matter would show a time constant of about 5 min. The observed time constant for flow was 0.3 min. 2) Flow appears to plateau after 2.5 min, while tissue Pco2, particularly in the slowly perfused white matter, falls for 15 min. During this period, if, as we assume, gray matter flow is already greatly reduced, the jugular venous blood should be relatively more representative of white matter, and some evidence of gradually falling flow in white matter should be evident from the oxygen saturation changes if indeed flow were linked to tissue Pco2. Since no changes in flow can be seen after 2.5 min, we conclude that our observations cannot be attributed to heterogeneous perfusion and metabolism, and that tissue Pco2 is less important than arterial Pco2 in determining arteriolar resistance.

The contrary evidence suggesting that tissue Pco2 is the important controlling factor has been reviewed and restated by Shapiro, Wasserman and Patterson (6). They induced hypocapnia and followed A–V O2 saturation difference as an index of flow. The Pco2 was not elevated stepwise to a plateau, but slowly, by inspiring a constant CO2 concentration. They noted that the flow appeared to correlate somewhat better with the jugular venous than with arterial Pco2. During a return to control, the fall of Pco2 was more rapid because of the hyperventilation already present, and they noted that flow stabilized 8 sec after arterial but 18 sec before jugular Pco2 stabilized. They concluded that “the tissue tension of this gas may be a major, if not the principal factor, regulating the state of the cerebral vessels.” They also cautioned, “It is still not clear whether the critically important ‘tissue’ tension of this gas is that of the vascular smooth muscle or that present in neuronal tissue” (1).

Likewise, prior evidence implicating the Pco2 of arterial blood as the flow-controlling factor is somewhat inferential. Lambertsen et al. (7) showed that inhalation of O2 at 3.5 atm total pressure resulted in a rise in jugular venous Pco2 of 3 mm Hg (attributed to the effect of hyperoxia on the CO2 transport of blood), a fall in Pao2 of 5 mm Hg due to the resulting respiratory stimulation and a fall of cerebral blood flow equivalent to that expected from hyperventilation when breathing air at 1 atm pressure to the same Pco2. They concluded that arterial blood rather than tissue Pco2 controlled flow, while noting that a vasoconstriction produced by O2 could have masked a dilation associated with tissue hypcapnia. They reaffirmed this in a subsequent study during exercise (8). Ledingham, Harper and McDowall (9) have shown that if Pco2 is held constant, the administration of O2 to dogs resulted in no significant change in cerebral cortical blood flow determined by krypton clearance. The same alternative interpretation may be applied to their work, namely, cancellation of effects of high O2 and high tissue Pco2.

The effect of Pco2 is probably due to its control of pH beyond the blood-brain barrier. In a steady state of moderate hypocapnia (high altitude), cerebral blood flow correlates with CSF (cerebrospinal fluid) pH rather than with arterial Pco2 (10). The present experiments suggest that the effect is directly on arterioles rather than via tissue Pco2 and tissue ECF (extracellular fluid) pH. If so, pH in ECF of the arteriolar smooth muscle must be rapidly altered by changes in Pco2 but unaffected by changes in blood pH which have little effect on cerebral blood flow (11-13).
This implies that an ion-impermeable barrier separates the blood stream from arteriolar wall extracellular fluid. The other factors controlling the pH of cerebral arteriolar ECF are probably related to the active transport regulation of CSF pH. Betz et al. (14), using a flat glass pH electrode on the cortex, have confirmed the dependent relationship of flow to pH of cortical ECF, and Marshall et al. (15) have shown that flow does not return to normal during several hours of hyperventilation of goats, although CSF pH does return to normal. A.M. Harper (personal communication) was unable to show any return of cortical flow during sustained hyperventilation in anesthetized dogs, between the first measurements at about 5 min and several hours. Severinghaus, Ledingham, Harper and McDowall (unpublished) measured cortical blood flow (krypton clearance), cortical surface pH (determined by a flat pH glass electrode on the surface), cortical and arterial PCO₂, both during hyperbaric oxygenation and after inhibition of carbonic anhydrase (acetazolamide) in 4 dogs. They observed a fall in cortical pH at constant cortical PCO₂ with acetazolamide, and a large increase in flow (+60 to +100%).

Severinghaus (16) showed that pH, recorded by a flat electrode on the rabbit cortical surface, is restored to normal within 10 to 30 min during sustained hyperventilation and alkalemia. The possibility that the relatively large fluid layer under the electrode was regulated more slowly than the intimate ECF of cortical cells led him to suspect that very rapid pH regulation might occur in the cortex, and that cortical flow might either show no drop or a very transient drop.

A discrepancy is apparent among the above observations: with several hours of hyperventilation, flow remains low while CSF and ECF (cortical surface) pH have been observed to return toward normal. Perhaps neither CSF nor surface ECF adequately reflects the pH of arteriolar smooth muscle. Or perhaps cortical ECF pH is very rapidly, but incompletely, altered toward normal during hypocapnic alkalosis, and the residual alkalosis of the arteriolar smooth muscle ECF maintained the vasoconstriction.

The arterial pressure tends to rise with hyperventilation, particularly at first, because of the mechanical effects of vigorous hyperventilation on intrathoracic pressure and venous return. However, at constant PCO₂, hypertension does not increase cerebral blood flow (17). Thus, one may not assume that the decrease in blood flow with hypocapnia was partially offset by the associated hypertension.

In an autoregulated circulatory bed such as the brain, a factor such as PCO₂ or ECF pH, may be presumed to operate homeostatically in conjunction with other factors—perhaps tissue oxygen supply—to control flow by influencing smooth muscle tone. It is interesting to speculate that the pH of the ECF of arteriolar muscle may be affected not only by CO₂ but also by hypoxia via lactic acid produced by anaerobic metabolism in nearby cells and liberated into the ECF, and by altered blood pressure (through the effect of flow on CO₂ and O₂ in the tissue). Such a unified hypothesis has been recently suggested by Betz et al. (14).

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