Local Mechanism of CO₂ Action on Cat Pial Arterioles

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SUMMARY The effects of local hypercapnic acidosis or local hypocapnic alkalosis on pial arterioles were studied in anesthetized cats equipped with a cranial window for the direct observation of the pial microcirculation of the parietal cortex. Changes in PCO₂ and pH of the extracellular fluid were induced by perfusing the space under the cranial window with artificial cerebrospinal fluid equilibrated with different concentrations of CO₂, while P_{aCO₂} was maintained constant. Hypercapnic acidosis dilated and hypocapnic alkalosis constricted pial arterioles markedly. The results indicate that a basis exists for considering CO₂ as a mediator for local regulation of brain blood flow. The vasodilation associated with arterial hypercapnia was abolished by a reduction in CSF P_{CO₂} equal in magnitude to the rise in arterial blood P_{CO₂}, suggesting that the action of CO₂ is entirely local.

Methods

Twenty-four cats were anesthetized with intravenous sodium pentobarbital (30 mg/kg). The animals were paralyzed with intravenous decamethonium (1 mg/kg) and ventilated with a positive pressure respirator connected to a tracheostomy tube. Expiratory CO₂ concentration was monitored continuously with a CO₂ analyzer. End-expiratory P_{CO₂} was maintained constant throughout the experiment, except when gases containing CO₂ were breathed. Arterial blood gas tensions and pH were determined periodically by Radiometer electrodes. The P_{CO₂}, P_{O₂} and pH of artificial cerebrospinal fluid (CSF) used to perfuse the space under the cranial window were similarly determined. Arterial blood pressure was monitored continuously with a Statham strain-gauge connected to a catheter placed into the aorta via the femoral artery. The brain vessels were visualized through a cranial window which was located on the vertex of the skull to show arterioles in the pialateral cortex. The cranial window technique has been described in detail previously. During the experiment the outlet of the cranial window was connected to plastic tubing whose end was set at a predetermined level to give a constant intracranial pressure of 5 mm Hg. The other two openings of the window were used as inlet and outlet for perfusing the space under the window with artificial CSF. The composition of the fluid is identical to that of CSF of the cat and its composition has been given previously. The application of this fluid, when equilibrated with 6.5% CO₂, 6% O₂ and 87.5%...
nitrogen, produced no significant change in arteriolar diameter. Artificial CSF used in these experiments was equilibrated with the appropriate gases and maintained at 37°C. The space under the cranial window was perfused with this fluid at a rate of 3.8 ml/min, via a constant infusion pump. The vessels were visualized with a Leitz Ortholux reflected light microscope. Vessel diameter was measured in the first two series of experiments by a photographic technique as described previously.14 In the last series of experiments the measurements were made using a Vickers image-splitting device and closed-circuit television camera and monitor as described by Baez.14 The reproducibility of these methods and their accuracy have been examined in detail previously.10

Three series of experiments were carried out:

The first series consisted of 9 cats. Artificial CSF equilibrated with gas mixtures containing 6.5%, 25%, or 50% CO₂ was used to perfuse the space under the cranial window. The concentration of oxygen in each of these gas mixtures was 6%. Each of these fluids was perfused 4 minutes and vessel diameter measurements made in the last 2 minutes. Preliminary experiments showed that this interval was fully adequate to achieve a steady state. The order of administration of these fluids conformed to a 3 × 3 Latin square design.12

In the second series of 9 cats the same experimental design was employed, except that the artificial CSF used was equilibrated with gas mixtures containing 6.5%, 10%, or 0% CO₂. In each case the concentration of oxygen was kept at 6%. Again a 3 × 3 Latin square design was employed. The results of these two series of experiments were analyzed by analysis of variance.

The third series of experiments was used to determine to what extent the local action of CO₂ accounted for the total vasoactivity of changes in arterial blood CO₂ tension. To evaluate this contribution the following five assumptions were made:

1) That the local action of CO₂ was determined by the Pco₂ in the immediate vicinity of the vascular smooth muscle of the pial arterioles under observation. No assumption was made concerning a distinction between action exerted by extracellular Pco₂, versus that exerted by intracellular Pco₂. This does not affect the basic issues considered here. Also no attempt was made to distinguish between effects exerted by molecular CO₂, and therefore dependent on Pco₂ alone, or effects exerted through a change in local pH. Since the local pH is determined by the CO₂ tension and by the bicarbonate concentration, and the latter is not expected to change over the short duration of these experiments, Pco₂ and pH can be used interchangeably for the purposes of this experiment.

2) That the vascular smooth muscle was equidistant from the arterial blood and from the CSF. This was supported by histological studies of vessels between 35 and 70 µm in diameter, which showed that such arterioles were composed of a single layer of vascular smooth muscle bounded on the inside by a layer of endothelial cells approximately 1 µm in thickness and on the outside by the pia-arachnoid membrane approximately equal in thickness to the endothelium. A diagrammatic representation of such a vessel illustrating these relationships is shown in figure 1.

3) That the diffusion coefficient for CO₂ of the endothelium and of the pial membrane were approximately equal.

There are no direct measurements of the diffusion coefficient for CO₂ in either of these tissues. This assumption appeared justified in view of the similar histological structure of these two tissues and in view of the fact that the diffusion coefficient of CO₂ in such diverse tissues as skeletal and smooth muscle, skin and connective tissue is of the same order of magnitude.18

4) That the turnover rate of CSF in the space under the cranial window was sufficient to maintain the Pco₂ of the fluid under the cranial window equal to that of the inflowing fluid. This appeared reasonable since the space under the cranial window was equal to 0.1-0.2 ml. Therefore the turnover rate of the fluid was 19 to 38 times/min. In addition, at the completion of each of these experiments it was demonstrated that solution containing india ink filled the window completely and could be cleared from window completely within a few seconds. That is, there were no pockets of stationary fluid in the vicinity of the vessels under examination.

5) That the CO₂ tension in the arterial blood within the arteriole under observation was equal to that measured in arterial blood obtained from a large artery. The validity of this assumption is based upon the following considerations: First, the turnover rate of blood within the arteriole, based on measurements of the velocity of red cells,14 appears to be sufficient to provide a high enough turnover rate to maintain the constancy of the CO₂ tension. Duling and Berne14 described a longitudinal gradient for oxygen in the arterioles of the cheek pouch of the hamster. They ascribed this to diffusion of oxygen through the vessel wall to the surrounding tissue. If such a gradient does exist in the cerebral vessels for oxygen, it is likely that a similar one would exist for CO₂. In the presence of such a mechanism the Pco₂ of the blood in the small cerebral vessels will be higher than the measured arterial blood Pco₂, but the resulting error is likely to be small, because the difference between arterial blood and CSF...
TABLE 1 Effect of Changes in Arterial and in CSF PCO₂ on Pial Arteriolar Caliber

| PCO₂ (mm Hg) | CSF PCO₂ (mm Hg) | Pial arteriolar diameter (μ)
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>36.9 ± 2.6</td>
<td>43.5 ± 2.2</td>
<td>45.5 ± 2.8</td>
</tr>
<tr>
<td>36.9 ± 2.6</td>
<td>0</td>
<td>40.8 ± 2.2</td>
</tr>
<tr>
<td>74.1 ± 1.6</td>
<td>43.5 ± 2.2</td>
<td>57.8 ± 2.7</td>
</tr>
<tr>
<td>74.1 ± 1.6</td>
<td>0</td>
<td>48.0 ± 1.9</td>
</tr>
<tr>
<td>34.4 ± 2.8</td>
<td>43.5 ± 2.2</td>
<td>47.0 ± 2.8</td>
</tr>
</tbody>
</table>

Values are mean ± SE from 6 cats.

PCO₂ is relatively small (5–7 mm Hg) compared to about 50 mm Hg for O₂.

Given these assumptions one would predict that the effects on pial arterioles of a change in CO₂ tension in the CSF would be cancelled by the effects of change in the arterial blood PCO₂ equal in magnitude but opposite in direction to that in the CSF. Comparisons were therefore made of the vessel caliber in 6 cats under the following conditions:

1. When the animal breathed room air, while no fluid was flowing under the window.
2. When the animal breathed room air, while artificial CSF equilibrated with gas containing no CO₂ was flowing under the window.
3. When the animal breathed room air, while artificial CSF equilibrated with 6.5% CO₂ was flowing under the window.
4. When the animal breathed 7% CO₂ while the fluid equilibrated with 6.5% CO₂ was flowing under the window.
5. When the animal breathed 7% CO₂ while artificial CSF equilibrated with gas not containing CO₂ was flowing under the window.
6. When the animal breathed 7% CO₂ while no fluid was flowing under the window.

Results

In the first two series of experiments alterations in CSF PCO₂ and pH had significant effects on pial arteriolar caliber. The results of both series of experiments are shown in figures 2 and 3 which relate vessel diameter to CSF PCO₂ and pH respectively. Local hypercapnic acidosis dilated the vessels, while local hypocapnic alkalosis constricted them.

Table 1 shows the results of the last series of experiments in which the contribution of the local action of CO₂ to the total vasoactivity of changes in arterial blood PCO₂ was evaluated. It is seen that the vasodilation as a result of increased CO₂ tension is completely inhibited by a reduction in CSF PCO₂ equal to the rise in arterial blood PCO₂. This occurred despite the fact that this equivalent reduction in CSF PCO₂ when the animal breathed room air had only minimal vasoconstrictor effects.

4. When the animal breathed 7% CO₂ while the fluid equilibrated with 6.5% CO₂ was flowing under the window.
5. When the animal breathed 7% CO₂ while artificial CSF equilibrated with gas not containing CO₂ was flowing under the window.
6. When the animal breathed 7% CO₂ while no fluid was flowing under the window.
Discussion

These results show that CO₂ has a significant local action on pial arterioles. Local hypercapnic acidosis dilates the vessels and local hypocapnic alkalosis constricts them. This effect is of substantial magnitude. The results are consistent with the findings of earlier investigators that applying gaseous CO₂ or acid solutions to the surface of the brain had a dilator effect.19,20 The present results provide precise, quantitative determination of this effect of CO₂ under steady state conditions. It is of interest to compare the effects of acidosis in the present experiments with the results of other investigators in the brain as well as with the effects of acidosis on arteries in other vascular beds. Figure 4 presents such a comparison among 1) the present results, 2) the results of an earlier study from our laboratory,18 (in which the effects of hypercapnic acidosis produced by microapplication of acid solutions to surface arterioles of the rat cremaster muscle was studied), and 3) the results of the study by Kuschinsky and his colleagues.21 They studied the effects of microapplication of acid solutions on pial arterioles in an open-skull preparation in cats. The agreement over the range of pH of 7.45 to 7.00 is reasonably satisfactory in all three studies at the lower pH's though the Kuschinsky study showed considerably greater dilations than we found in the present study or in the rat cremaster muscle. The reasons for this difference are not immediately evident, but certain differences between the Kuschinsky study and the present results must be noted. For example, in their study the Kuschinsky group used transient application of acid, while our results were obtained during steady state conditions. Secondly, the decrease in pH in their solutions was induced by reduction in bicarbonate concentration while the CO₂ tension was maintained the same. In our experiments the decrease in pH was induced by a rise in CO₂ tension while bicarbonate concentration was maintained the same. Finally, their solutions had lower osmolality than ours (280 vs 315 mOsm/kg), lower PO₄ (0 vs 45 mm Hg), higher K⁺ concentration (5 vs 3 meq/liter) and higher Ca²⁺ concentration (5 vs 2.5 meq/liter). One or a combination of several of these differences may be responsible for the differences in results.

Our results also show that the local action of CO₂ can account entirely for the vasoactivity of changes in arterial blood PCO₂, at least during hypercapnia, and that no additional remote action need be postulated to explain the vasodilator action of hypercapnia.

In view of the fact that the action of CO₂ is local and sufficiently pronounced, we conclude that a basis exists for considering CO₂ as a mediator for local regulation of brain blood flow under conditions of changing metabolism or changing arterial blood pressure.

It is pertinent to examine what types of local mechanisms could account for the local effect of CO₂ on brain blood vessels. The most obvious one, and the one that is most popular, is that this represents an action exerted directly on the vascular smooth muscle cells. Sites of action might be the cell membrane or intracellular structures, such as the contractile apparatus itself or the sarcoplasmic reticulum. Another possibility is that CO₂ might be acting on cells other than the vascular smooth muscle cells which may then be either releasing other vasoactive agents or may initiate a reflex action which then results in the effect on vascular smooth muscle. Neither of these mechanisms is likely. If vasoactive agents were released and reached the vessel walls by diffusion, the application of fluid flowing at a relatively rapid rate would be expected to wash away some of these metabolic products and to severely limit their effects on vessels. The second mechanism is also unlikely. Experiments in which acid or alkaline solutions were applied by a micropipette at the very localized portion of pial arterioles vessel produced localized responses from the vessel wall in the portion of the vessel directly exposed to the solution.16,20 In these experiments the operation of a local reflex would be highly unlikely. For these reasons, we believe that the traditionally favored mechanism of action of CO₂, namely the one involving direct action on vascular smooth muscle, is the most likely one.

References

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