Analysis of Vasoactivity of Local pH, \( P_{CO_2} \) and Bicarbonate on Pial Vessels

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SUMMARY The mechanism by which the local effect of \( CO_2 \) on pial arterioles is exerted was examined in anesthetized cats equipped with a cranial window for the direct observation of the microcirculation of the parietal cortex. The dilution of pial arterioles in response to application of artificial cerebrospinal fluid with low pH was the same whether or not the \( P_{CO_2} \) of the solution was maintained in the normal range or markedly increased. The constriction of pial arterioles in response to application of artificial cerebrospinal fluid with high pH was the same whether or not the \( P_{CO_2} \) of the solution was maintained in the normal range or markedly decreased. Finally, pial arterioles did not change their caliber in response to application of cerebrospinal fluid with unchanged pH but markedly increased or decreased \( P_{CO_2} \), or bicarbonate ion concentration. These results show that the action of \( CO_2 \) on cerebral vessels is exerted via changes in extracellular fluid pH and that molecular \( CO_2 \) and bicarbonate ions do not have independent vasoactivity on these vessels.

IT HAS BEEN demonstrated that local hypercapnic acidosis, induced by exposure of the surface of the brain to gaseous carbon dioxide or by application of acid solutions, produces dilatation of cerebral vessels, in the absence of change in arterial blood \( P_{CO_2} \). Conversely, application of alkaline solutions produces vasoconstriction. This local effect of \( CO_2 \) has been traditionally considered to be the major mechanism of its action on cerebral blood vessels and it is generally believed to be mediated through change in extracellular fluid pH. It has never been demonstrated definitely, however, whether or not this local action of \( CO_2 \) is mediated exclusively through a change in local pH, or whether or not \( CO_2 \) has an additional action dependent on molecular \( CO_2 \) or bicarbonate ion concentration and independent of changes in pH. Although the view that \( CO_2 \) is acting through a change in local pH overwhelmingly predominates, the experiments supporting it, e.g., those based on application of acid or alkaline solutions to the surface of the brain, do not exclude effects dependent on molecular \( CO_2 \) or bicarbonate ions. Transient application of small amounts of acid or alkali under these circumstances is bound to alter both the pH and the \( CO_2 \) tension in the vicinity of the blood vessels, since body fluids contain bicarbonate ions which would buffer the change in \( pH \). One cannot be certain that the ensuing changes in vessel caliber or blood flow are due to the change in \( pH \) or \( CO_2 \) or to both changes.

The present experiments were designed to evaluate the independent actions of separate changes in pH and changes in \( CO_2 \) tension in the vicinity of the pial vessels.

Methods

Eighteen cats were anesthetized with intravenous sodium pentobarbital (30 mg/kg). The animals were paralyzed with intravenous decamethonium bromide (1 mg/kg) and ventilated with a positive pressure respirator connected to a tracheostomy tube. Expired \( CO_2 \) concentration was monitored continuously with a \( CO_2 \) analyzer. End-expiratory \( P_{CO_2} \) was maintained constant throughout the experiment. Arterial blood gas tensions and \( pH \) were determined periodically by Radiometer electrodes. 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 skull vertex just caudal to the coronal suture to visualize arterioles in the parietal cortex. The cranial window technique has been described in detail previously. One of the three openings of the window was connected to a Statham strain-gauge for measurement of intracranial pressure. The other two openings of the window were used as inlet and outlet for perfusing the space under the cranial window with artificial cerebrospinal fluid (CSF). 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 composition of the fluid is identical to that of CSF of the cat as described previously. During the experiment the \( P_{CO_2} \), \( P_{O_2} \) and \( pH \) of anaerobically sampled CSF coming out of the window were determined by Radiometer electrodes. The vessels on the surface of the brain were visualized with a Leitz Ortholux reflected light microscope. Vessel diameter was measured using a Vickers image splitting device and closed-circuit television camera and monitor. The method has excellent reproducibility and a high degree of accuracy.

The experiments were designed to examine the influence on the pial arterioles of artificial CSF whose pH and \( P_{CO_2} \) were independently altered. For this purpose the bicarbonate concentration of artificial CSF was altered by increasing or decreasing the amount of sodium bicarbonate used in the preparation of the fluid and correspondingly altering the amount of sodium chloride, so that the osmolality of the resulting solution remained the same. The different batches of fluid were then equilibrated with gas mixtures in which the concentration of oxygen was kept constant at 6%, while the concentration of \( CO_2 \) was zero, 6.5%, or 25%. Each fluid was used to perfuse the space under the cranial window for four minutes; vessel caliber measurements were made in the last two minutes. Preliminary experiments showed that this was ample time for the attainment of a steady state, which was invariably achieved within less than a minute. The perfusion of the space under the cranial window with the artificial CSF...
was carried out by a constant infusion pump at a rate of 3.8 ml/min. Since the space under the cranial window was less than 0.2 ml, this provided a very rapid turnover of the fluid under the window.

Three groups of 6 cats each were used. Each group was exposed to 3 different fluids. In each group one of these fluids was normal artificial CSF equilibrated with 6.5% CO₂, 6% oxygen, 87.5% nitrogen and the other two fluids were, in the first series, solutions with increased pH and varying carbon dioxide tension; in the second group solutions with low pH and varying carbon dioxide tension; and in the third group solutions with pH equal to that of normal artificial CSF and varying carbon dioxide tension. Application of different fluids was carried out according to a 3 × 3 Latin-square design. The results of each of these series of experiments were analyzed by means of analysis of variance.

**Results**

The results are summarized in table 1. Local acidosis dilated vessels markedly, and to the same extent, irrespective of whether Pco₂ was kept at the normal range or increased considerably. Local alkalosis constricted cerebral arterioles to the same extent irrespective of whether or not Pco₂ was kept at the normal range or was considerably decreased. Cerebral arterioles did not change their diameter significantly in response to marked decrease or marked increase in CSF Pco₂, as long as the pH was kept relatively constant.

**Discussion**

These results show clearly that the important variable in the mediation of changes in pial arteriolar diameter in response to alterations in the concentration of CO₂ in the CSF is the change in the extracellular fluid pH, and that neither molecular CO₂ nor the bicarbonate ion have independent vasoactivity. We deliberately chose large changes in pH and Pco₂ in the present experiment to exclude the possibility that small effects from either molecular CO₂ or bicarbonate ions might be overlooked. The possibility that in the first two experiments such effects from molecular CO₂ or from changes in bicarbonate concentration might have been present and been missed because we produced maximal changes is excluded by the results of the third series of experiments. Here large changes in CO₂ tension and bicarbonate ion concentration, of comparable magnitude to those in the other two series of experiments, did not have any significant effect on vessel diameter as long as the pH was kept constant.

These results with respect to bicarbonate ion are consistent with the findings of other investigators in other vascular beds, showing that the bicarbonate ion is not vasoactive. On the other hand, the absence of significant vasoactivity as a result of changes in Pco₂ was somewhat surprising, since it is contrary to most experience in other vascular beds. In the renal circulation of cats and in the human forearm the vasodilation associated with hypercapnic acidosis was dependent on increased Pco₂, and occurred to its full extent in the absence of a change in extracellular fluid pH. The same is true for the effects of hypercapnic acidosis on heart muscle. In isolated vein preparations, however, smooth muscle relaxation occurred as a result of decreased extracellular fluid pH in the absence of a change in Pco₂. In the pulmonary vascular bed the situation is more complex, in that a rise in Pco₂ is vasodilator while a decrease in pH is strongly vasoconstrictor. Somewhat similar circumstances prevail in the renal circulation of cats; increased Pco₂ was strongly vasodilator, while large decrease in pH produced slight vasoconstriction.

As discussed elsewhere, these divergent results may reflect differences in the mechanism of action of changes in pH and Pco₂ on vascular smooth muscle in various vascular beds. Dependence of activity on extracellular fluid pH suggests the likelihood of an effect on the cell membrane. If the action depends on changes in Pco₂, it is likely that it might be mediated through changes in intracellular fluid pH acting on intracellular sites. Likely candidates for such intracellular sites of action are the sarcoplasmic reticulum and the contractile proteins themselves. These considerations, of course, are based on the assumption that the cell membrane of vascular smooth muscle is freely permeable to molecular CO₂, but relatively impermeable to the bicarbonate ion. It is interesting to consider the possibility that the different behavior of the cerebral vessels might reflect differences in the permeability of its cell membrane to bicarbonate ions, perhaps associated with the presence of the blood-brain barrier.

**References**


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**Table 1** Effects of Independent Changes in CSF Pco₂ and pH on Pial Arteriolar Caliber

<table>
<thead>
<tr>
<th>Arteriolar diameter (µ)</th>
<th>CSF Pco₂ (mm Hg)</th>
<th>CSF pH</th>
<th>I. Effects of isocapnic and hypocapnic alkalosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>42.7 ± 5.7</td>
<td>7.37 ± 0.07</td>
<td>31.4 ± 5.4*</td>
</tr>
<tr>
<td></td>
<td>33.3 ± 2.5</td>
<td>34.3 ± 2.3</td>
<td>13.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>64.1 ± 8.2*</td>
<td>8.16 ± 0.09</td>
<td>8.14 ± 0.11</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Arteriolar diameter (µ)</th>
<th>CSF Pco₂ (mm Hg)</th>
<th>CSF pH</th>
<th>II. Effects of isocapnic and hypocapnic acidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>44.3 ± 8.5</td>
<td>64.1 ± 8.2*</td>
<td>67.6 ± 9.5*</td>
</tr>
<tr>
<td></td>
<td>41.3 ± 0.7</td>
<td>39.2 ± 2.7</td>
<td>131.8 ± 9.3</td>
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<tr>
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<td>7.33 ± 0.1</td>
<td>6.79 ± 0.17</td>
<td>6.87 ± 0.01</td>
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</table>

<table>
<thead>
<tr>
<th>Arteriolar diameter (µ)</th>
<th>CSF Pco₂ (mm Hg)</th>
<th>CSF pH</th>
<th>III. Effects of hypoxia and hypercapnia without change in pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>44.7 ± 10.1</td>
<td>42.7 ± 7.2</td>
<td>45.6 ± 10.6</td>
</tr>
<tr>
<td></td>
<td>39.9 ± 1.2</td>
<td>15.2 ± 0.8</td>
<td>150.1 ± 1.2</td>
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<td></td>
<td>7.32 ± 0.13</td>
<td>7.23 ± 0.12</td>
<td>7.25 ± 0.11</td>
</tr>
</tbody>
</table>

*All values are mean ± SE derived from 6 experiments in each series. *Diameter values significantly different from control, but not significantly different from each other. Statistical analysis based on analysis of variance.
Central Dual Innervation of Arterioles and Capillaries in the Brain

Toru Itakura, M.D., Kazumi Yamamoto, M.D., Masaya Tohyama, M.D., and Nobuo Shimizu, M.D.

SUMMARY  Fluorescence- and electron-microscopic studies were performed on nerve terminals close to intracerebral blood vessels in the rat. For the electron microscopic visualization with potassium permanganate was used. In the rat cerebral cortex deprived of the bilateral superior cavernous ganlion some aminergic terminal boutons containing large and small core vesicles were observed contiguous to blood vessels. These terminals abutted on the capillary basement membrane. Since these terminals are found in the cerebral arterioles, really innervate the cerebro-vascular system, in addition to the sympathetic supply, should be considered.

IT IS GENERALLY accepted that the cerebral arteries are innervated by both sympathetic and parasympathetic nerve fibers.1-4 Recently, Edvinsson et al. have found that central catecholaminergic (CA) fibers from the CA neurons in the brain stem, visualized by fluorescence histochemistry, closely follow the path of small blood vessels in the brain and suggest that the CA neurons play a significant role in the local microregulation of the cerebral blood flow.5 Whether or not the central CA nerve terminals, which run along the cerebral arteries, really innervate the cerebro-vascular system, in addition to the sympathetic supply, should be confirmed by electron microscopic observation.

For electron microscopic visualization of CA nerve terminals, the potassium permanganate fixation method developed by Richardson, Hökfelt, Ochi et al. has proved most suitable for the detection of granular vesicles in the sympathetic CA nerve terminals.6-8 Demonstration of CA nerve terminals in the central nervous system by potassium permanganate has been unsuccessful, presumably because of their paucity. We modified this fixation method to identify CA nerve terminals originating from the brain stem.9

To determine whether central CA neurons really take part in the control of the cerebral blood flow, we attempted to clarify the morphologic relationship of CA nerve terminals to cerebral blood vessels with electron microscopy.

Methods

Tissues from forty adult albino rats weighing 200 g, in which the superior cavernous ganlion had been excised bilaterally two weeks previously, were observed by histofluorescence and electron microscopy. For the histofluorescence study, brains were analyzed with the glyoxylic acid (GA)-formaldehyde fluorescence method10 as modified by Kimura.
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