Pial arteriolar vessel diameter and CO₂ reactivity during prolonged hyperventilation in the rabbit

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Hyperventilation reduces intracranial pressure (ICP) acutely through vasoconstriction, but its long-term effect on vessel diameter is unknown. In seven rabbits with a cranial window implanted 3 weeks earlier, the effect of prolonged hyperventilation on vessel diameter was studied. Anesthesia was maintained for 54 hours with a pentobarbital drip (1 mg/kg/hr). The pH, CO₂, and HCO₃ levels were measured in arterial blood and cisterna magna cerebrospinal fluid (CSF). The diameter of 31 pial arterioles was measured with an image splitter. After baseline measurements, pCO₂ was reduced from 38 to 25 mm Hg and allowed to return to 38 mm Hg for 10 minutes every 4 hours.

There was an initial vasoconstriction of 13%, which progressively diminished by 3% every 4 hours. Thus, by the 20th hour, vessel diameters at a pCO₂ of 25 mm Hg had returned to slightly above baseline values obtained at a pCO₂ of 38 mm Hg. The temporary return of pCO₂ to 38 mm Hg every 4 hours caused vasodilation: 12% at 4 hours, gradually increasing to 16% at 52 hours. Thus, at 52 hours, the vessel diameters were 105% of baseline at a pCO₂ of 25 mm Hg and increased to 122% at a pCO₂ of 38 mm Hg. Arterial pH had returned to baseline at 20 hours, and CSF pH had returned at 24 hours. Bicarbonate in blood and CSF remained decreased throughout the experiments. In three control experiments during which normocapnia was maintained, vessel diameter and pH and bicarbonate levels remained unaltered over the same period. The CO₂ reactivity, tested by brief periods of hyperventilation every 4 hours, also did not change.

These results indicate that hyperventilation is effective in reducing cerebral blood volume for less than 24 hours and that it should be used only during actual ICP elevations. If used preventively, its effect may have worn off by the time ICP starts to rise for other reasons, and further decreases in pCO₂ cannot be obtained. Moreover, the reduction in buffer capacity with lower bicarbonate renders the vessels more sensitive to changes in PaCO₂. This could lead to more pronounced elevations in ICP during transient rises in PaCO₂, such as during endotracheal suctioning in head-injured patients.

KEY WORDS • hyperventilation • cerebrovascular reactivity • carbon dioxide • rabbit

Hyperventilation is an effective means of controlling acute elevations of intracranial pressure (ICP). It is generally accepted that hyperventilation works by reducing cerebral blood volume through constriction of the cerebral and pial arterioles. As CO₂ readily crosses the blood-brain barrier, a decreased PaCO₂ is immediately reflected in a reduced pCO₂ in interstitial brain fluid. This, in turn, leads to a reduction in H⁺ concentration in the vicinity of the cerebral blood vessels. Because the H⁺ ion is one of the most potent relaxants of smooth muscles of cerebral arterioles, a reduction in its concentration will lead to rapid vasoconstriction. Thus, vasoconstriction is not dependent on low CO₂ levels and can be maintained only if the increased perivascular pH can be maintained. It has been shown, however, that pH in blood and cerebrospinal fluid (CSF) returns to normal during prolonged hyperventilation, despite sustained hypocapnia. The purpose of the present investigation was to examine whether the return to normal pH during prolonged hyperventilation would be accompanied by vasorelaxation. Such a finding would have important clinical implications. If this were true, prolonged profound hyperventilation to prevent ICP elevations would be ineffective and might even be counterproductive: ICP would return to baseline and acute elevations of ICP could no longer be controlled, because no further reduction of PaCO₂ could be obtained.
In rabbits with a previously implanted cranial window and maintained under constant anesthesia for 54 hours, measurements were performed of pial arteriolar diameter and of pCO2 and pH and bicarbonate levels in blood and in the cisterna magna CSF. The measurements were first made during normocapnia and then every 4 hours during hypercapnia. Moreover, CO2 reactivity was assessed by measuring the vessel diameter during brief periods of normocapnia and then every 4 hours during hypercapnia. The findings indicate that vasoconstriction is indeed correlated with high pH and not with low CO2 levels, as the vessel diameter returned to baseline in approximately 20 hours, concomitant with a return to normal values of pH in blood and CSF. Vessel reactivity to brief changes in PaCO2 was maintained throughout the experiments and, in fact, tended to increase during the course of the experiments. This was not the case in control experiments, where normocapnia was maintained for 52 hours and CO2 reactivity was tested with brief hyperventilation every 4 hours.

Materials and Methods

New Zealand White rabbits of either sex, each weighing between 2 and 4 kg, were used in this study. In order to minimize the disturbing effects of installing a (chronic) cranial window on vessel reactivity and CSF composition, this surgical procedure was carried out 3 weeks prior to the actual experiment, as described before. Pial arteriolar diameter on the parietal surface of the brain was measured with an image-splitting device connected to a microscope as described earlier. On the day of the experiment, anesthesia was induced by injection of 50 to 100 mg pentobarbital sodium into an ear vein. Next, the animal was intubated and lines were introduced into the femoral artery for measuring blood pressure with a pressure transducer and blood gases and into the femoral vein for intravenous infusion. A No. 20 intravenous needle was placed in the cisterna magna to draw CSF samples. For the duration of the experiment, the animals were infused with an intravenous solution of 5% dextrose half-normal saline, 3 ml/kg/hr, containing pentobarbital sodium for a dose of 1 mg/kg/hr and gallamine triethiodide, 2 mg/kg/hr. To avoid effusion under the cranial window and to prevent pulmonary inflammation, dexamethasone (0.02 mg/kg/hr) was also added. Every 24 hours, starting 1 day prior to the experiment, the animals received 1 ml penicillin-streptomycin (Combiotic) intramuscularly as an antibiotic prophylaxis. Between the measurements, the animals were turned on the other side every 2 hours in a further attempt to prevent any pulmonary complications. The animals were semicovered with a heating pad, and their rectal temperature was maintained at 38°C.

The rabbits were ventilated with an animal respirator in which a small amount of oxygen was bled to obtain an inspiratory pO2 of 25% to 30%. End-expiratory CO2 was measured constantly. The rate and volume of the respirator were adjusted to give an end-expiratory pCO2 of 38 mm Hg (corresponding to 38 mm Hg in the blood gas measurements) during baseline measurements and of 24 mm Hg (corresponding to a PaCO2 of 25 mm Hg) during hyperventilation. The pH, HCO3 and pCO2 levels were measured in blood and CSF every 4 hours in a microblood gas analyzer; capillary tubes were used, allowing very small samples of blood and CSF. Hematocrit was measured with a microsystem method. In two animals blood pressure tended to drop slightly and it was supported with epinephrine. It has been shown that epinephrine has no intrinsic effect on pial arteriolar diameter and that any effect on pial arteriolar diameter during epinephrine infusion is solely dependent on changes in blood pressure.

To obtain stable baseline readings, 4 hours were allowed to elapse from the beginning of anesthesia. The vessel diameters at this point, taken at a PaCO2 of 38 mm Hg, were considered 100%; all subsequent diameters of each individual vessel were calculated as a percentage of its own baseline value. Next, the animals were separated into two groups. In a control group of three rabbits, PaCO2 was maintained at 38 mm Hg for 52 hours. Every 4 hours vessel diameters and blood and CSF gas and pH levels were measured; then, CO2 reactivity was assessed by applying hyperventilation to a PaCO2 of 25 mm Hg for 10 minutes and again measuring vessel diameters. Next, PaCO2 was allowed to return to baseline for another 4 hours. The experimental group consisted of seven rabbits. After baseline measurements, the animals were hyperventilated to a PaCO2 of 25 mm Hg. Every 4 hours, vessel diameters and blood and CSF gas and pH levels were measured; then, CO2 reactivity was assessed by allowing PaCO2 to return to 38 mm Hg for 10 minutes and again measuring vessel diameters. Next, the animals were hyperventilated again for another 4 hours, for a total hyperventilation duration of 52 hours. The animals were sacrificed with an overdose of pentobarbital.

In the course of the experiment, all animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). Approval by the Institutional Animal Care Committee was also obtained.

* Image-splitting device manufactured by Vickers, Malden, Massachusetts; microscope manufactured by E. Leitz, Rockleigh, New Jersey.
† Strain-gauge transducer made by Statham Instruments, Inc., Hato Rey, Puerto Rico.
‡ Harvard ventilator manufactured by Harvard Apparatus Co., Millis, Massachusetts.
§ Capnometer, Model HP 47210A, manufactured by Hewlett-Packard, Waltham, Massachusetts.
|| pH/blood gas analyzer, Model No. 158, manufactured by Corning Medical and Scientific, Medfield, Massachusetts.
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In the text, all data are given as mean ± standard deviation, while in the figures we have used standard error of the mean.

**Results**

In the three rabbits in the control group, baseline pH in blood and CSF were 7.42 ± 0.02 and 7.36 ± 0.01, respectively. These levels remained constant and at the end of the experiment values of 7.40 ± 0.06 and 7.35 ± 0.02 were found. Baseline values of HCO₃⁻ were 24.9 ± 3.7 in blood and 26.0 ± 1.0 mmol/liter in CSF; these levels also did not change significantly, being 22.0 ± 2.8 and 25.3 ± 2.9 mmol/liter, respectively, at the end of the experiment. The PaCO₂ was maintained at 38 mm Hg. The pCO₂ in CSF was 46.3 ± 2.5 mm Hg at the beginning and 45.8 ± 7.6 mm Hg at the end of the experiment. The diameter of 16 vessels averaged 70.9 ± 16.5 μm (range 49 to 97 μm). The vessel diameter at PaCO₂ of 38 mm Hg did not change during the experiment, while constriction with temporary hyper-ventilation to PaCO₂ of 25 mm Hg remained constant around 9% of the baseline diameter (Fig. 1).

The experimental group consisted of seven rabbits. Mean arterial blood pressure remained virtually unchanged throughout the experiment (Fig. 2). The PaCO₂, pH, and HCO₃⁻ in arterial blood are shown in Fig. 3. With the aid of an accurate CO₂ analyzer, PaCO₂ could easily be held around 25 mm Hg. Baseline pH was 7.47 ± 0.05, increased to a maximum of 7.57 ± 0.06 at 30 minutes after the start of hyperventilation, then precipitously fell again, with return to baseline at 20 hours. From then on some nonsignificant variation in pH occurred, but at the end of the experiment the pH was the same as in the beginning: 7.47 ± 0.09. In contrast, arterial HCO₃⁻ remained decreased throughout the experiment. Figure 3 also shows the pCO₂, pH, and HCO₃⁻ levels in the CSF. For pCO₂ and pH the trends were similar to those in blood, albeit somewhat slower: the maximum pH and lowest CO₂ levels in the CSF were reached at 8 hours, but at 30 minutes there was already a significant change of both values. The reason for these slower changes is probably that the CSF pH and pCO₂ were measured from the cisterna magna where it takes some time for the extracellular fluid to "sink in." In CSF, HCO₃⁻ did not decrease quickly, and the minimum concentration was reached only at 24 hours.

The vessel diameter in the experimental group is shown in Fig. 4. A total of 31 pial arterioles were studied. Baseline diameter was 56.8 ± 20.5 μm (range 29 to 102 μm). Within 30 minutes, hyperventilation caused constriction of the vessels by almost 14%; after that, vessel diameter at a PaCO₂ of 25 mm Hg slowly increased, crossing the baseline level at 20 hours, concomitant with the return of the arterial blood pH to baseline. From 24 hours on, vessels were larger than at baseline, but this was not statistically significant at all 4-hour intervals; however, in cases where vessel diameter was not statistically significantly larger than baseline, p values were around 0.07 (paired t-test), so that the diameter increase was considered real. The vessel relaxation with the temporary return of PaCO₂ to 38 mm Hg ranged from 9% to 13% during the first 20 hours and from 12% to 18% after 24 hours (p < 0.01, Student’s t-test).
after the beginning of hyperventilation. The percentages of the experimental group at 30 minutes and at 4-hour intervals are significantly different from control at \( p < 0.05 \), and values lower were obtained at a \( \text{PaCO}_2 \) of 25 mm Hg, and peak values were taken during brief periods of normoventilation to a \( \text{PaCO}_2 \) of 38 mm Hg. Values marked with an asterisk are significantly different from control at \( p < 0.05 \), and values marked with a dagger at \( p < 0.1 \).

**Discussion**

These findings show that, in the rabbit, pial arteriolar vasoconstriction is not maintained with prolonged hyperventilation. This probably relates to the return of CSF pH to normal. Moreover, the responses to similar changes in \( \text{PaCO}_2 \) tended to increase in time during prolonged hyperventilation. This is probably due to the decrease in bicarbonate ion concentration in CSF, so that changes in \( \text{CO}_2 \) are buffered less adequately, resulting in larger changes in pH with equal \( \text{CO}_2 \) changes.

Several mechanisms have been implied to explain the effect of \( \text{CO}_2 \) on cerebrovascular diameter, but changes in perivascular pH with changes in \( \text{PaCO}_2 \) seem to be the most important. Low pH relaxes cerebrovascular muscle in vitro, and high pH contracts the muscle. Topical application of solution with low pH on the brain surface produces pial arteriolar dilation, and application of solution with high pH produces vasoconstriction. This response was obtained when changes in pH were induced by changes in \( \text{CO}_2 \) at a constant bicarbonate ion concentration, or when changes in bicarbonate ion concentration were made at a constant level of \( \text{CO}_2 \). Application of solutions with markedly varying pCO2 or bicarbonate ion concentration had no effect on arteriolar diameter unless the pH was allowed to change. Thus, the effect of \( \text{CO}_2 \) is mediated through changes in extracellular fluid pH. Molecular \( \text{CO}_2 \) and bicarbonate ion do not have intrinsic vasoactivity. Arteriolar dilation in response to low pH takes place rapidly, with a time constant of less than 10 seconds.

All of the above vascular responses to changes in pH only take place if the pH alteration is on the side of the CSF, and not on the intravascular side. Among the various normal constituents of the blood such as hydrogen ion, lactic acid, bicarbonate ion, and \( \text{CO}_2 \), only changes in arterial concentration of \( \text{CO}_2 \) lead to rapid cerebrovascular response. This is because only the non-ionized \( \text{CO}_2 \) molecule can rapidly cross the blood-brain barrier in either direction. Thus, only changes in \( \text{PaCO}_2 \) are rapidly reflected in changes in extracellular \( \text{CO}_2 \), and hence in extracellular pH.

In addition to its direct action on cerebrovascular smooth muscle mediated by changes in extracellular fluid pH, \( \text{CO}_2 \) may affect vessel diameter by other mechanisms. Most prominent of these are its effects on brain metabolism and sympathetic activity. However, reports on the alternative explanations for the effect of \( \text{CO}_2 \) on the cerebrovascular bed conflict and so will not be discussed further here. Suffice it to say that any effect of \( \text{CO}_2 \) on the cerebral circulation other than by influence upon extracellular pH is not nearly as important, quantitatively, as the aforementioned direct effect.

Several authors have shown that the pH of blood and CSF returns to normal during prolonged hyperventilation. In blood, this is effected mainly through renal control and, in the CSF, probably through diminished secretion of bicarbonate by the choroid plexus. The time course of these adjustments has not been determined in detail. In the present investigation in the rabbit, the pH of blood and CSF and the vessel diameter all returned to baseline between 20 and 24 hours after initiation of hyperventilation. This corresponds with data that Christensen obtained in man. It has been shown that, with prolonged hyperventilation, changes in brain pH in response to \( \text{CO}_2 \) changes are larger than those to equal \( \text{CO}_2 \) changes during normal ventilation. In our laboratory, this was found to be accompanied by increased \( \text{CO}_2 \) reactivity of pial arterioles. These findings were ascribed to diminished buffer capacity as a consequence of decreased bicarbonate concentration. Conversely, in rabbits breathing air rich in \( \text{CO}_2 \) for 6 days, an increased concentration of bicarbonate in CSF was found, accompanied by diminishing \( \text{CO}_2 \) reactivity of pial arterioles. The gradual increase in relative \( \text{CO}_2 \) reactivity in the present paper can also be ascribed to this increased magnitude of extracellular pH changes with similar \( \text{CO}_2 \) changes.

It is true that the increase in \( \text{CO}_2 \) reactivity is small (from 12% at 4 hours to 16% at 52 hours) but it corresponds to an increase in volume in these vessels of 25% and 35%, respectively. In fact, at the end of the experiment the vessel diameter at a \( \text{PaCO}_2 \) of 38 mm Hg was 122% of baseline, with an increase of 49% in blood volume in those vessels. In this study, ICP was not measured because any changes in ICP with \( \text{PaCO}_2 \) manipulations would be extremely small in these normal brains and would easily be compensated for. However, under pathological circumstances, such as a stiff brain after head injury, an increase of 49% of blood volume in the arterioles could have a great impact on ICP.
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The fact that the mean vessel diameter in the experimental group increased to a value of 4.4% above baseline is not easily explained. Changes in sodium, potassium, and calcium concentrations and an increase in the lactate/pyruvate ratio have been reported during hyperventilation.1,3-5,14,16 Either of these changes may have been responsible for the slightly increased diameter, but no measurements were made in this study to support such a contention.

The implications of this study are twofold. First, the results support the hypothesis that (extracellular) pH is more important in so-called “CO₂ reactivity” than CO₂ itself. Second, and more important, these findings indicate that hyperventilation is effective in reducing cerebral blood volume for less than 24 hours and that it should be used only during actual ICP elevations. If used preventively, as is sometimes advocated,2,16 its effect may have worn off by the time ICP starts to rise for other reasons, and further decreases in PaCO₂ are difficult to obtain. Moreover, the reduction in buffer capacity with lower bicarbonate concentration renders the vessels more sensitive to changes in PaCO₂. This could lead to more pronounced and dangerous ICP elevations during transient rises in PaCO₂, such as during endotracheal suctioning of severely head-injured patients. In fact, preliminary data from a trial conducted at our service in Richmond, in which severely head-injured patients were randomly assigned to receive ventilation to a PaCO₂ of 24 or 35 mm Hg, indicate that the hyperventilated group fared less well. This paper offers a possible explanation for this finding.

References