Tolerance of Low Cerebral Intracellular pH in Rats During Hyperbaric Hypercapnia

Yan Xu, PhD; Yoram Cohen, PhD; Lawrence Litt, PhD, MD; Lee-Hong Chang, PhD; and Thomas L. James, PhD

Background and Purpose: Brain acidosis from cerebral ischemia is characterized by average intracellular pH levels of approximately 5.8–6.2, which appear in turn to worsen cellular injury. We report that the brain is not injured when hypercapnia is used to reduce intracellular pH to about 6.2 during adequate oxygenation. A hyperbaric chamber is needed to achieve intracellular pH values so low because inspired CO₂ tensions must be increased to approximately 1 atm.

Summary of Report: Using in vivo phosphorus-31 and proton nuclear magnetic resonance spectroscopy, we measured brain intracellular pH and lactate concentration of rats inside a nonmagnetic polycarbonate chamber at a barometric pressure of 1,500 mm Hg. Intubated rats were ventilated with a 50% O₂/50% CO₂ gas mixture for specific times. All six rats ventilated for 15 minutes with CO₂ tensions of approximately 750 mm Hg woke up without neurological impairment, despite a decrease in intracellular pH to about 6.2. Higher CO₂ tensions and longer exposures resulted in cardiovascular collapse and sudden death, followed by the postmortem appearance of brain lactate.

Conclusions: Brain intracellular pH values near 6.2 can be induced briefly in vivo in ventilated rats without injury under hyperbaric hypercapnic conditions. If attempts are made to lower brain pH in vivo even further by increasing PₐCO₂ beyond 750 mm Hg, mean arterial blood pressure and cerebral blood flow decrease to values incompatible with life. (Stroke 1991;22:1303-1308)

Although skeletal muscle and most organs tolerate 1–2 hours of ischemia before injury occurs, irreversible brain damage can be caused by 3–15 minutes of anoxia or ischemia, depending on the methods of resuscitation. During global ischemia, average intracellular pH (pHᵢ), as measured with phosphorus-31 nuclear magnetic resonance (³¹P NMR) spectroscopy, can decrease as low as approximately 6.2. One putative cause of rapid brain injury is cerebral lactic acidosis, particularly when blood glucose levels are elevated. Although recent studies have been interpreted as showing that injury results from acidosis per se (i.e., without lactate anions), other studies suggest that brain acidosis may close N-methyl-D-aspartate glutamate receptors and thus limit the damage produced by calcium entry. Clinically, respiratory acidosis is known to be better tolerated than metabolic acidosis.

Humans have recovered without injury from unintentional ventilation with gas mixtures having PₐCO₂ values above 300 mm Hg, including one case of accidental ventilation with 100% CO₂ for several minutes. We previously described recovery, normal behavior, and no histological injury in well-oxygenated rats exposed to 75 minutes of PₐCO₂ of approximately 350 mm Hg (pHᵢ of about 6.60) and 15 minutes of PₐCO₂ of approximately 500 mm Hg (pHᵢ of about 6.45). Because of the rightward shift of the hemoglobin dissociation curve, decreases in Pₒ₂ that are required to reach higher PₐCO₂ values result in hypoxemia (i.e., oxyhemoglobin saturation of <80%) at ambient pressure. We hypothesize that the use of hyperbaric pressures will permit both adequate oxygenation and the administration of CO₂ tensions higher than those used previously and that it will be possible to demonstrate lower tolerable brain pHᵢ values in vivo. As a corollary to the hypothesis, we sought to determine the lowest tolerable hypercapnic pHᵢ.

Materials and Methods

A nonmagnetic, 6-in.-diameter polycarbonate resin (Lexan, material 1500, General Electric Co., Fremont, Calif.) chamber with a 1-in.-thick wall was specially constructed for use in our horizontal 4.7-T...
magnet. Polished, transparent flanges had provisions for electrical, gas, and fluid feed-throughs. Approval for the animal protocols was obtained from the Committee on Animal Research at the University of California, San Francisco. After 3 minutes of anesthetic induction with spontaneous inhalation of 4% isoflurane, 16 male Sprague-Dawley rats weighing 360±36 g underwent nontraumatic transoral endotracheal intubation with a plastic 2-in., 16-gauge intravenous catheter. Maintenance anesthesia was established with 1% isoflurane and included neuromuscular blockade with 1 mg i.p. pancuronium. The rats were placed prone on a temperature-controlled copper cradle that fit into the hyperbaric chamber.

The ventilation system, which was homemade, originated with an anesthesia machine that was modified to select and deliver high-pressure O2/CO2/N2/isoflurane gas mixtures through nylon tubing to a blow-by, T-breathing circuit inside the hyperbaric chamber. The endotracheal tube corresponded to the long stem of the T and the high-pressure gas flow, which ran across the top of the T, emptied into the hyperbaric chamber after passing through a specially constructed, remote-controlled pneumatic valve. Regular, momentary closures of the pneumatic valve with a separate gas system and a remotely operated solenoid forced blow-by gases into the endotracheal tube. Thus, controlled ventilation was accomplished with a system that had all electrical components 5 m from the magnet. A separate, regulated, gas exit port provided controlled gas leakage from the chamber so as to keep it at constant pressure. The designs of the valves, gas circuits, manifolds, and the special ventilation system, which included many nuances (Y. Xu, Y. Cohen, L. Litt, J.W. Severinghaus, T.L. James, unpublished observations) permitted stability and fine control of 4–30-ml tidal volumes at chamber pressures of up to 4 atm and during rapid pressure changes. During each study tidal volumes of approximately 4 ml were kept constant as inspired concentrations were changed to keep hemodynamic changes minimal. This was accomplished by altering gas percentages in the blow-by mixture without changing the total blow-by flow. Although no invasive physiological monitoring was performed during hyperbaric NMR studies, ventilatory chest wall motion and foot pad color were constantly observed through the transparent window.

After obtaining control spectra at 0% CO2 and 1 atm, the inspired CO2 concentration was increased to 20% for 8 minutes. Isoflurane was then discontinued, and the inspired CO2 concentration was increased to 50% for 8 minutes. (CO2 becomes anesthetic at a partial pressure of approximately 250 mm Hg, which corresponds to an anesthetic potency equal to that of 1% halothane.8 Although CO2 solubility in tissues is close to that of N2O, so that it might be expected to have the same anesthetic potency, CO2 is approximately three times more potent.9) Chamber pressure was then raised acutely to an absolute pressure of 2 atm. Groups of rats were assigned to exposure to different hyperbaric periods and percentages of CO2.

After the prescribed exposure, the inspired CO2 concentration was lowered to 20% for 8 minutes and then to 0%, at which time isoflurane was administered again. After 20 minutes, barometric pressure was gradually restored to 1 atm.

Two parallel sets of hyperbaric studies were conducted: 1) outside the magnet, in rats having femoral arterial catheters connected to aneroid manometers in the chamber that could be read from outside through the transparent window; and 2) inside the magnet, in rats that had relative cerebral blood flow determined during hypercapnia from fluorine-19
Time course of average changes in (top) intracellular pH and (bottom) relative phosphorus-31 metabolite concentrations for six rats that survived administration of 50% CO2 at absolute barometric pressure of 2 atm. Each data point represents average during one spectral acquisition (approximately 7 minutes). Vertical bars on data points represent standard errors. Horizontal segments above abscissa indicate periods that are normobaric and normocapnic (a and g), normobaric with 20% inspired CO2 (b), normobaric with 50% inspired CO2 (c), hyperbaric with 50% inspired CO2 (d), hyperbaric with 20% inspired CO2 (e), or hyperbaric and normocapnic (f). Pi, inorganic phosphate; PCr, phosphocreatine; ATP, adenosine triphosphate.

$\text{(19F) NMR measurements of the washout of sevoflurane (Maruishi Pharmaceutical Co., Osaka, Japan), a vapor anesthetic with a molecular structure that has six equivalent fluorine nuclei producing an enhanced, sharp NMR peak. Thirty }^{19}\text{F NMR washout spectra (1 minute per spectrum) were obtained in each of three rats after 20 minutes of ventilation with 1% sevoflurane. For each rat, washout data were taken during normocapnia and two levels of hypercapnia (20% CO2 at 1 atm and 40% CO2 at 2 atm absolute barometric pressure).}$

Interleaved $^{31}\text{P}/^{1}\text{H NMR spectra were recorded continuously from the beginning of each hypercapnia experiment. A 13 mm x 11 mm elliptical NMR coil with a double-tuned circuit (}$^{31}\text{P}/^{1}\text{H, 81.005 MHz/200.109 MHz}$) was placed over the rat's head and used for interleaved spectral acquisitions. The spectral width was ±3,125 Hz. Each acquisition of 4,096 complex data points for $^{31}\text{P}$ was interleaved with one for $^{1}\text{H}$, with a time-sharing of 0.84 seconds for $^{31}\text{P}$ and 1.27 seconds for $^{1}\text{H}$. For both $^{31}\text{P}$ and $^{1}\text{H}$, accumulations of 200 acquisitions in quadrature phase-detection mode were used to generate individual spectra. In the $^{31}\text{P}$ spectra, the broad background from bone and phospholipids was reduced during acquisitions with selective presaturation and then removed from the display by the convolution-difference method. Using a method similar to that used in previous studies, we determined pH from the parts per million frequency separation (δ) of the phosphocreatine (PCr) and inorganic phosphate (P) resonances according to the following relation:

$$\text{pH}_{i} = 6.683 + \log((δ-3.153)/(5.73-δ)).$$
### Table 1. Mean Arterial Blood Pressure in Rats During Parallel Experiments

<table>
<thead>
<tr>
<th>Chamber pressure (atm, absolute)</th>
<th>Inspired CO₂ (%)</th>
<th>Inspired isoflurane (%)</th>
<th>Blood pressure (mean±SEM mm Hg, gauge pressure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>140±5</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>1</td>
<td>133±17</td>
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<tr>
<td>1</td>
<td>50</td>
<td>0</td>
<td>158±38</td>
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<tr>
<td>1.6</td>
<td>50</td>
<td>0</td>
<td>160±15</td>
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<tr>
<td>1.8</td>
<td>50</td>
<td>0</td>
<td>108±18</td>
</tr>
<tr>
<td>2.1</td>
<td>50</td>
<td>0</td>
<td>Almost 0</td>
</tr>
<tr>
<td>1.8</td>
<td>20</td>
<td>0</td>
<td>123±32</td>
</tr>
<tr>
<td>1.8</td>
<td>0</td>
<td>1</td>
<td>130±11</td>
</tr>
</tbody>
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### Results

Six of six rats recovered metabolically and behaviorally after 15-minute exposures to a \(\text{PCO}_2\) of 750±30 mm Hg and \(\text{pH}\) decrements to 6.20±0.05. Figure 1 shows three representative in vivo brain \(^{31}\)P NMR spectra of one animal. Figure 2, top, depicts the average \(\text{pH}\) values for the six rats as a function of time. Relative changes in concentrations of the phosphorous metabolites are shown in Figure 2, bottom. Repeated-measures analysis of variance found no significant difference among adenosine triphosphate (ATP) concentrations (\(p>0.3\)). Concentrations of other metabolites, however, changed significantly during hypercapnia (\(p<0.004\) for each). The \(\text{P}i\) increase and \(\text{PCr}\) decrease are expected from the creatine kinase reaction. All metabolite concentrations returned to control values at approximately the same time.

The absence of a lactate signal in \(^1\)H NMR spectra was assumed to indicate adequate tissue oxygenation (Figure 3). During hyperbaric hypercapnia of greater duration or severity, however, 10 of 10 rats died suddenly. Death was detected from sudden changes in the \(^{31}\)P NMR spectra. Parallel studies of relative cerebral blood flow using \(^{19}\)F NMR with sevoflurane and mean arterial blood pressure in rats with femoral
artery cannulas confirmed cardiovascular stability at hyperbaric hypercapnic conditions of PCO2 values below 750 mm Hg. However, cardiovascular collapse occurred suddenly at hyperbaric PCO2 values just above 750 mm Hg. Table 1 gives average mean arterial blood pressure data obtained from two rats outside the magnet. Figure 4 exhibits typical NMR Kety-Schmidt washout data representative of the effect of hyperbaric hypercapnia. The 19F NMR sevoflurane data were obtained using techniques similar to those employed previously by us12 and others,13 and the same qualitative behavior of the washout spectra was seen in two other rats.

**Discussion**

Our use of an accurate model of brain acid–base relations2 predicts that a pHj of 6.2 at a PCO2 of 750 mm Hg causes an intracellular bicarbonate concentration of approximately 29.3 meq/l, assuming only general chemical buffering. In situ microelectrode studies of cerebral ischemia in rats have demonstrated hydrogen ion sequestration in at least three compartments: astrocytes (in which pHj can decrease to about 5.3), neurons, and the interstitial space (in which pHj can decrease to about 6.2).14 Although previous NMR spectroscopy studies of ischemia have not clearly resolved discrete low-pHj compartments such as are found in microelectrode studies, some measurements have found that the Pj peak broadens and shifts toward a lower pHj, apparently indicating cell populations with a continuum of lower pHj values.15 It is not known if multiple brain pHj compartments occur during severe hypercapnia as they do during ischemia. Figure 1, however, cannot exclude the existence of a small second Pj peak at the noise level having a pHj of approximately 5.8.

We have shown in previous normobaric studies of rats that no more than 10% of our measured 31P and 1H NMR signals are extracerebral for the NMR coil size we have chosen.7 We must assume that similar relations2 predicts that a pHj of 6.2 at a PCO2 of 750 mm Hg causes an intracellular bicarbonate concentration of approximately 29.3 meq/l, assuming only general chemical buffering. In situ microelectrode studies of cerebral ischemia in rats have demonstrated hydrogen ion sequestration in at least three compartments: astrocytes (in which pHj can decrease to about 5.3), neurons, and the interstitial space (in which pHj can decrease to about 6.2).14 Although previous NMR spectroscopy studies of ischemia have not clearly resolved discrete low-pHj compartments such as are found in microelectrode studies, some measurements have found that the Pj peak broadens and shifts toward a lower pHj, apparently indicating cell populations with a continuum of lower pHj values.15 It is not known if multiple brain pHj compartments occur during severe hypercapnia as they do during ischemia. Figure 1, however, cannot exclude the existence of a small second Pj peak at the noise level having a pHj of approximately 5.8.

We have shown in previous normobaric studies of rats that no more than 10% of our measured 31P and 1H NMR signals are extracerebral for the NMR coil size we have chosen.7 We must assume that similar limits to extracerebral contamination hold during hyperbaric studies because it is difficult to perform invasive brain metabolite extract studies in our small rat-sized hyperbaric chamber. In vivo measurements of physiological variables such as arterial blood pressure and cerebral blood flow are also difficult when the chamber is in the magnet. The washout of intracranial sevoflurane was seen with 19F NMR spectroscopy to be significantly delayed, if not arrested, by severe hypercapnia. The objective of the sevoflurane studies (to demonstrate a relative decrease in cerebral perfusion during hypercapnia) was a qualitative one because quantification requires the knowledge of several physiological variables that are difficult to measure in our chamber. Also, because sevoflurane is preferentially soluble in scalp and muscle compared with brain, as much as 20% of the 19F NMR signal in our particular arrangement might be extracerebral, in contrast to the 31P and 1H NMR signals. It has been known for some time that accurate cerebral blood flow quantification is difficult with 19F NMR signals from tracer compounds12,16 and that careful spatial localization of 19F NMR signals is challenging, although it has been accomplished in nonhyperbaric environments after removal of the scalp and cranial bone and with depth pulsing,12,16

In conclusion, we find that CO2 and carbonic acid are not injurious to rats at PCO2 values near 750 mm Hg, despite the concomitant generation of pHj values near 6.2, which occurs during injurious cerebral ischemia.1 During adequate oxygenation, however, cerebral injury from extracerebral infusions occurs only after the extracellular pH has been reduced below approximately 5.3,17,18 In vivo tests of higher PCO2 values that might drive pHj closer to this value would require cardiovascular and circulatory support.

**Acknowledgments**

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**References**


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