Cerebral Oxygen Tension in Rats during Deliberate Hypotension with Sodium Nitroprusside, 2-chloroadenosine, or Deep Isoflurane Anesthesia

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Thirty-four male Sprague-Dawley rats were divided into four groups: control animals and those receiving sodium nitroprusside (SNP), 2-chloroadenosine, or a high, inspired concentration of isoflurane to produce deliberate hypotension to a mean arterial blood pressure of 50 mmHg. Ventilation was controlled (FiO2 = 0.3); control animals and those treated with sodium nitroprusside or 2-chloroadenosine breathed isoflurane 1.4 vol%, whereas isoflurane, 3.9 vol%, was required to produce hypotension by deep anesthesia alone. Multiple tissue oxygen tension values (PtO2) were measured at intervals of 10 μm over a distance of 2 mm by advancing an oxygen microelectrode through the parietal cerebral cortex of all animals. The frequency of low tissue PtO2 values (<10 mmHg) was increased with all forms of deliberate hypotension, but the magnitude of this change (a shift to the left in the frequency histogram) was significantly different among techniques. The shift toward lower PtO2 values during hypotension was least in animals receiving deep isoflurane anesthesia, intermediate in those receiving SNP, and greatest in those treated with 2-chloroadenosine. In rats, areas of the brain appear to be at risk for significant tissue hypoxia during hypotension produced by 2-chloroadenosine. (Key words: Anesthetics, volatile; isoflurane. Anesthetic techniques: adenosine; hypotension; nitroprusside. Blood pressure: hypotension, induced. Brain: oxygen tension.)

Several methods have been used to produce deliberate hypotension in humans and animals. Although sodium nitroprusside has been widely used for deliberate hypotension, adenosine compounds and isoflurane have gained increasing interest recently. Disadvantages associated with sodium nitroprusside include tachyphylaxis, rebound hypertension when the drug is discontinued, and the potential for cyanide toxicity. The value of any hypotensive technique is determined by its safety; despite a decreased perfusion pressure, organ function and tissue metabolism must be maintained, and newer hypotensive techniques should at least match the results (both clinical and experimental) that have been obtained with sodium nitroprusside treatment. Among other considerations, it is crucial that brain function and cellular integrity be well maintained and that cerebral hypoxia be avoided during deliberate hypotension. Several techniques have been used to evaluate the influences of deliberate hypotension on cerebral metabolism and energy state, including measurements of the cerebral metabolic rate for oxygen (CMRO2), cerebral high energy phosphate compounds and lactate production, and brain surface oxygen tension. However, the metabolic measurements are only indirect indicators of tissue oxygen tension. In the present study we used the microelectrode technique to measure directly the tissue oxygen tension (PtO2) in the parietal cerebral cortex of normotensive rats and those made hypotensive by administration of sodium nitroprusside, 2-chloroadenosine, or deep isoflurane anesthesia. The study was designed to answer the following questions: 1) Does deliberate hypotension alter the cerebral tissue oxygenation? and 2) Are there differences among the hypotensive techniques in terms of cerebral tissue oxygenation?

Materials and Methods

Thirty-four male Sprague-Dawley rats (315 ± 3 g) were divided into four groups: control animals and those receiving sodium nitroprusside, 2-chloroadenosine or a high inspired concentration of isoflurane. All animals were anesthetized initially with isoflurane, 1.6–2.0 vol% inspired, in order to prevent purposeful movements during tracheostomy and cannulation of the left femoral artery and vein (PE 50 catheters). The animals were then paralyzed with pancuronium bromide (1 mg·kg⁻¹, iv) and ventilated (FiO2 = 0.3) with a rodent ventilator (Harvard Apparatus Company, Inc., Millis, MA); PaCO2 was maintained at approximately 30 mmHg. Mean arterial pressure was recorded continuously using a standard pressure transducer (Statham Instruments, Inc., Hato Rey, Puerto Rico). Body temperature was maintained at 36–38°C by a heat lamp. The rats were placed in a stereotaxic head holder (David Kopf Instruments, Tujunga, CA); a 3 mm burr hole was placed 4 mm posterior and lateral to the bregma, and the dura was exposed. The inspired isoflurane concentration was maintained at 1.4 vol% for at least 20 min before the experiment was begun. Mean arterial pressure in treated rats was decreased over 3–5 min to approximately 50 mmHg by infusion of sodium nitroprusside or 2-chloroadenosine (10⁻² or 10⁻³ M, respectively) in isotonic saline; infusion volumes were 1–2 ml·h⁻¹. The total amount of sodium nitroprusside was 3.8 ± 0.1 mg·kg⁻¹; the total dose of 2-chloroadenosine was 0.81 ± 0.18 mg·kg⁻¹. In animals receiving isoflurane-induced hypotension, the inspired concentration was...
TABLE 1. Arterial Blood Values and Heart Rates in Normotensive and Hypotensive Animals

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>HI</th>
<th>SNP</th>
<th>CIADO</th>
<th>Significant Differences*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>104 ± 3</td>
<td>49 ± 1</td>
<td>50 ± 1</td>
<td>50 ± 1</td>
<td>C &gt; HI, SNP, CIADO</td>
</tr>
<tr>
<td>P&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; (mmHg)</td>
<td>128 ± 5</td>
<td>122 ± 2</td>
<td>120 ± 4</td>
<td>117 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>P&lt;sub&gt;CO&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; (mmHg)</td>
<td>29 ± 1</td>
<td>29 ± 1</td>
<td>28 ± 1</td>
<td>29 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>H&lt;sup&gt;+&lt;/sup&gt; (nEq・L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>36.63 ± 1.87</td>
<td>42.89 ± 1.10</td>
<td>41.45 ± 1.54</td>
<td>36.54 ± 1.60</td>
<td>NS</td>
</tr>
<tr>
<td>(pH)</td>
<td>(7.44)</td>
<td>(7.37)</td>
<td>(7.38)</td>
<td>(7.44)</td>
<td></td>
</tr>
<tr>
<td>Heart rate (min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>581 ± 11</td>
<td>349 ± 6</td>
<td>406 ± 11</td>
<td>237 ± 9</td>
<td>C, SNP &gt; HI &gt; ADO</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>47 ± 1</td>
<td>45 ± 1</td>
<td>41 ± 1</td>
<td>41 ± 1</td>
<td>C, HI &gt; SNP, CIADO</td>
</tr>
</tbody>
</table>

*P < 0.05, Duncan’s Multiple Range Test for comparisons among groups.

C = normotensive; HI = deep isoflurane anesthesia; SNP = sodium nitroprusside treatment; CIADO = 2-chloroadenosine treatment; NS = no significant differences.

briefly (3–5 min) increased to approximately 5 vol% to reduce arterial pressure initially, and then maintained at a concentration (3.9 ± 0.2 vol%), which held arterial pressure constant at 50 mmHg. Saline (1.5 ml・h<sup>-1</sup>) was infused in these animals and in those remaining normotensive so that fluid administration was identical in all groups.

A platinum oxygen microelectrode (tip diameter 1–2 μm) was used to measure P<sub>O</sub><sub>2</sub> values. The electrode was polarized with 0.7 V negative relative to a silver–silver chloride reference electrode. The resultant current was measured with a sensitive picocammeter (Keithley® Model 602; Keithley Instruments, Inc., Cleveland, OH). Electrodes were calibrated in brain by allowing the animal to breathe 100% nitrogen (zero oxygen value) at the end of each experiment, and then in saline equilibrated with air.

The zero oxygen value was obtained when the current stabilized at a minimal value; two points were adequate to calibrate the electrodes because these microcathodes give a linear response to oxygen over a range of values that exceeds the physiologic values for oxygen tension in tissue. This calibration method was verified by numerous preliminary tests (in oxygen concentrations of 0, 1, 2, 5, 12, and 21%), which confirmed that: 1) the method produced a minimum current for the zero oxygen value; 2) the calibration was linear over a range of oxygen values well in excess of those which occur in tissue; 3) results were reproducible for a given electrode; and 4) isoflurane did not interfere with the polarographic measurement of oxygen. The O<sub>2</sub> microelectrode was positioned initially at approximately 100 μm beneath the parietal cerebral cortex in order to minimize influences of oxygen diffusing in from the brain surface. After a 5-min period of stable hypotension, the electrode was advanced in 10 μm intervals through the cerebral cortex by an electronically controlled microelectrode holder (Burleigh Instruments, Inc., Fishers, NY). The electrode was positioned at each location for 9 s, and local P<sub>O</sub><sub>2</sub> was recorded at approxi-}

mately 200 sites (over a distance of 2 mm) in each rat. Total duration of hypotension was approximately 40 min. Arterial P<sub>O</sub><sub>2</sub>, P<sub>CO</sub><sub>2</sub> and pH values were measured with a standard blood gas analyzer (Radiometer® BMS Mark II; Radiometer America, Inc., Westlake, OH) before and after the P<sub>O</sub><sub>2</sub> determinations, and the average values of both determinations were used for statistical calculations. Hematocrit values were determined at the end of each experiment by the micromethod. Heart rate was determined from the pulsatile arterial pressure trace at 10-min intervals. Statistical analyses were performed using Duncan’s Multiple Range Test for comparisons among treatment groups and chi-square test for analysis of P<sub>O</sub><sub>2</sub> frequencies. The Kolmogorov-Smirnov test was used for comparisons of P<sub>O</sub><sub>2</sub> frequency histograms. Skewness and kurtosis of the histograms, parameters that describe and quantify the asymmetry of a distribution curve, were calculated by standard procedures. All data are presented as means ± SEM or as percentages of the total observed P<sub>O</sub><sub>2</sub> values. Significance was accepted for P < 0.05.

Results

Results for arterial blood gas values, hematocrit, and heart rates are reported in table 1. Mean arterial pressure was 104 ± 3 mmHg in normotensive animals and approximately 50 mmHg in hypotensive animals. Arterial pressure could be decreased easily with each of the hypotensive techniques. However, sodium-nitroprusside-treated animals needed increasing drug doses to maintain constant arterial pressure. In two out of 11 sodium-nitroprusside-treated rats, we were unable to maintain the desired blood pressure because of tachyphylaxis (those animals were not included in the data analysis). Arterial P<sub>O</sub><sub>2</sub>, P<sub>CO</sub><sub>2</sub>, and pH values were similar in all animals, although there was a tendency for decreased pH in animals receiving deep isoflurane anesthesia or sodium nitro-
prusside as compared with those normotensive. Heart rates were within the normal physiologic range except in those receiving 2-chloroadenosine, which decreased heart rate significantly. Hematocrit values tended to decrease in all hypotensive animals (perhaps due to hemodilution in response to decreased capillary hydrostatic pressure).

The $P_{tO_2}$ frequency histograms for the four treatment groups are presented in figure 1. All histograms were significantly different from a normal Gaussian distribution and from each other. The $P_{tO_2}$ histograms were shifted toward the lower range of observed values as illustrated by the positive values for skewness. Deep isoflurane anesthesia was associated with the least skewness ($S = 0.2$). Hypotension produced by isoflurane resulted in a slightly flattened histogram ($K = 2.4$) with two frequency peaks for oxygen tension, one in the least $P_{tO_2}$ range (below 5 mmHg) and the second around 50 mmHg. In 2-chloroadenosine-treated animals, almost 40% of all observed $P_{tO_2}$ values were between 0 and 5 mmHg, resulting in a marked left shift in the $P_{tO_2}$ frequency histogram ($S = 1.8$) and a kurtosis value of 6.1. Significant differences among the group median $P_{tO_2}$ values (average of the median for each animal within each treatment group) were observed only between rats treated with 2-chloroadenosine (least median value) and those receiving deep isoflurane anesthesia (greatest median value).

Cumulated frequencies for several $P_{tO_2}$ ranges are shown in table 2. Deliberate hypotension resulted in increased frequencies of $P_{tO_2}$ values below 15 mmHg with all hypotensive techniques. However, 2-chloroadenosine treatment resulted in the greatest number of low $P_{tO_2}$ values for all frequency intervals. For ranges between 0–4 mmHg, $P_{tO_2}$ frequencies were similar in animals receiving deep isoflurane anesthesia or sodium nitroprusside treatment. However, if the $P_{tO_2}$ ranges were increased to incorporate values of 5 mmHg or greater, then fewer low

### Table 2. Cumulated $P_{tO_2}$ Frequencies for the $P_{tO_2}$ Interval 0–15 mmHg

<table>
<thead>
<tr>
<th>$P_{tO_2}$ (mmHg)</th>
<th>C</th>
<th>HI</th>
<th>SNP</th>
<th>CIADO</th>
<th>Significant Differences*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>3.1</td>
<td>10.7</td>
<td>9.3</td>
<td>17.1</td>
<td>C &lt; SNP, HI &lt; ADO</td>
</tr>
<tr>
<td>0–2</td>
<td>4.6</td>
<td>12.4</td>
<td>12.4</td>
<td>21.8</td>
<td>C &lt; HI, SNP &lt; ADO</td>
</tr>
<tr>
<td>0–5</td>
<td>5.8</td>
<td>13.5</td>
<td>14.4</td>
<td>27.5</td>
<td>C &lt; HI, ADO</td>
</tr>
<tr>
<td>0–4</td>
<td>7.4</td>
<td>14.9</td>
<td>16.9</td>
<td>35.2</td>
<td>C &lt; HI, SNP &lt; ADO</td>
</tr>
<tr>
<td>0–5</td>
<td>9.0</td>
<td>16.0</td>
<td>19.6</td>
<td>38.9</td>
<td>C &lt; HI, ADO</td>
</tr>
<tr>
<td>0–10</td>
<td>17.9</td>
<td>22.1</td>
<td>31.4</td>
<td>51.5</td>
<td>C &lt; HI &lt; SNP &lt; ADO</td>
</tr>
<tr>
<td>0–15</td>
<td>31.5</td>
<td>27.6</td>
<td>45.5</td>
<td>62.2</td>
<td>C &lt; HI &lt; ADO</td>
</tr>
</tbody>
</table>

$C =$ normotensive; $HI =$ deep isoflurane anesthesia; $SNP =$ sodium nitroprusside treatment; $CIADO =$ 2-chloroadenosine treatment.

* $P < 0.5$, chi-square test.
PtO₂ values were observed in animals receiving deep isoflurane anesthesia as compared with those receiving sodium nitroprusside.

Discussion

In the present study, deliberate hypotension resulted in increased frequencies of low PtO₂ values with all hypotensive techniques. However, 2-chloroadenosine treatment was associated with far greater frequencies of low PtO₂ values as compared with those in each of the other groups, whereas animals receiving deep isoflurane anesthesia or sodium nitroprusside showed only moderately increased frequencies of low PtO₂ values as compared with those in normotensive animals.

Low PtO₂ values in the frequency histogram suggest that there may be areas that are hypoxic or that are at greater risk for tissue hypoxia. No single value that represents the threshold for unequivocal neuronal injury has been established, so that the definitive, "critical" PtO₂ value for brain tissue is not well defined. Certainly this value must be greater than the minimum PtO₂ value for mitochondria, which has been estimated in vitro to be approximately 0.05 to 0.1 mmHg. However, there is evidence to suggest that the likely critical intracellular PtO₂ value in vivo is well above these values, perhaps in the range of 5–8 mmHg. We, therefore, have focused our interests on the PtO₂ values of 10 mmHg or less, because it appears likely that the critical value is in this region rather than in the range greater than 15 mmHg. The duration of low PtO₂ values at a given site was not determined in these investigations, so we cannot state with certainty that cellular damage occurred in any of these animals. However, Manil and colleagues demonstrated that local cerebral PtO₂ values varied only slightly (±5%) over time, so that we would not expect major changes in the local PtO₂ values that we observed in our animals. Our results do allow relative comparisons of the risks for tissue hypoxia and cellular damage during deliberate hypotension, and they identify hypotensive techniques which may render the parietal cortex at the greatest risk for hypoxic injury. Indeed, an advantage of the PtO₂ method is that it identifies shifts in the PtO₂ frequency histogram (suggesting a decreased margin of safety for tissue oxygenation) before cellular hypoxia may have occurred.

Our normotensive control animals exhibited greater frequencies of PtO₂ values below 10 mmHg than were observed by Maekawa et al. in cats. These authors observed only about 1% of values below 10 mmHg. However, Leniger-Follert and Hossmann demonstrated frequencies of around 12% for PtO₂ values below 10 mmHg in cats, and Gyngax and Wiensperger found intracortical PtO₂ frequencies of 10–15% in the interval of 0–8 mmHg in cats also. We doubt that the PaCO₂ values of approximately 30 mmHg in our animals elicited cerebral vasocostriction and tissue hypoxia. In our previous investigations, awake rats had PaCO₂ values of approximately 30–55 mmHg and reported values for PaCO₂ in awake rats range from 22 to 40 mmHg, with several investigators reporting values of approximately 53 mmHg. Rather, we attribute the very low frequency of PtO₂ values below 10 mmHg in the report by Maekawa et al. to their experimental design and especially to their measurement technique. They studied a different species, they measured only brain surface PtO₂ (which could be influenced greatly by oxygen in the atmosphere and thus give artifactually increased local oxygen values), they used methoxyflurane anesthesia combined with practolol treatment, and they administered oxygen sufficient to produce a PaO₂ of approximately 160 mmHg. Others reported that surface electrodes, as compared with tissue electrodes, gave steady-state values that approximated mean venous oxygen tension, and the method did not reproduce the minimum values that occurred in tissue.

 Whereas Maekawa and associates did not observe changes in the frequency of PtO₂ values below 10 mmHg during the administration of sodium nitroprusside, we found these frequencies to be significantly increased in our sodium-nitroprusside-treated animals. It is likely that this finding represents the increased sensitivity of the intracerebral measurement of tissue oxygen tension, as opposed to the surface measurements recorded by Maekawa et al. We doubt that cyanide toxicity explains the increased frequency of very low PtO₂ values in the animals receiving sodium nitroprusside because cyanide toxicity would likely increase, not decrease, tissue oxygen tension. Further, our rats did not demonstrate the metabolic acidoses that accompanies cyanide toxicity. Data from canines suggest that cyanide toxicity is associated with base excess values that exceed −10 mEq·l⁻¹; whereas the base excess value was only −2.6 mEq·l⁻¹ in our rats treated with sodium nitroprusside. It is of interest that Maekawa and coworkers reported a reduced CMRO₂ in hypotensive animals receiving sodium nitroprusside, a finding that might indicate some degree of intracortical hypoxia. However, sodium-nitroprusside-induced hypotension in dogs and rats was without effect on CMRO₂, although the former investigators noted a tendency for CMRO₂ to decrease, and the latter lowered the MAP to 65 mmHg only. Further, CMRO₂ measures overall brain oxygen consumption and may not be a sensitive indicator of local changes in cortical oxygenation.

There is evidence from other investigations that iso-
flurane is a suitable agent for induced hypotension. Results in both humans and animals indicated that isoflurane reduced blood pressure by decreasing systemic vascular resistance primarily, whereas there were only minor changes in cardiac output. In dogs, the ratio of cerebral oxygen delivery to consumption was well maintained over a wide range of isoflurane concentrations, presumably due to the decrease in CMRO₂ that occurs at light levels of isoflurane anesthesia as compared with other anesthetics.

In the present study, even isoflurane-induced hypotension resulted in increased frequencies of low P₉₀ values. For intervals of 4 mmHg or less, these frequencies were similar when hypotension was induced by either sodium nitroprusside or deep isoflurane anesthesia (table 2). However, when the range was extended to include values from zero to 5, 10, or 15 mmHg (the more likely ranges for critical P₉₀), the frequency of low P₉₀ values was significantly less in animals receiving deep isoflurane anesthesia as compared with those receiving sodium nitroprusside. The occurrence of two peaks in the P₉₀ frequency histogram during deep isoflurane anesthesia (one peak in the range between 0–5 mmHg, the other around 50 mmHg, fig. 1) indicates a bimodal distribution and might reflect some inhomogeneity of cerebral blood flow, with some areas receiving very low flow and others receiving greater flows, perhaps indicating “patchy” tissue perfusion.

Deliberate hypotension induced with 2-chloroadenosine resulted in the greatest frequencies of low P₉₀ values among all treatment groups, and it caused an extensive left shift in the P₉₀ frequency histogram (fig. 1). 2-Chloroadenosine is metabolized less rapidly than adenosine itself, and therefore 2-chloroadenosine was used in the present investigation. Our pilot studies with adenosine, or combined adenosine and diprydiamol treatment, proved to be unsatisfactory because we were unable to administer sufficient concentrations of these drugs to achieve the desired level of hypotension due to their limited solubilities.

Fukunaga et al. emphasized the usefulness of adenine compounds for deliberate hypotension in rabbits. Adenosinetriphosphatase (ATP) or adenosine were shown to be effective hypotensive agents in humans also. With adenine compounds, hypotension could be achieved rapidly, tachyphylaxis did not occur, and no rebound hypotension was observed after termination of treatment. In humans, cardiac output was well maintained, and cerebral oxygenation was sufficient to prevent cerebral lactate acidosis. In baboons, hypotension induced by ATP did not result in morphologic evidence of ischemic cell damage. However, morphologic techniques (especially those employing light microscopy only) are likely to be insensitive indicators of subtle changes in aerobic metabolism.

There are disturbing reports associated with the use of adenine compounds for deliberate hypotension also. DeJong et al. reported severe cardiac arrhythmias in ATP-treated rats with several animals dying during the first minutes of treatment. These authors questioned the use of ATP for deliberate hypotension. In dogs, deliberate hypotension with adenosine or ATP resulted in increased cerebral lactate and decreased cerebral energy charge, results that also question the usefulness of adenine compounds for deliberate hypotension. Our results also indicate that adenosine may render at least some areas of the brain at risk for tissue hypoxia.

In summary, deliberate hypotension with each of these techniques resulted in increased frequencies of low cerebral P₉₀ values, suggesting that the margin of safety for brain tissue oxygenation is decreased with deliberate hypotension in rats. Among hypotensive techniques, the frequency of P₉₀ values in the apparent critical range was least during deep isoflurane anesthesia, slightly greater when sodium nitroprusside was used, and markedly increased in animals treated with 2-chloroadenosine. These results question the safety of adenine compounds for deliberate hypotension, at least as they relate to cerebral oxygenation in rats.

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