The effects of hypovolemic hypotension on high-energy phosphate metabolism of traumatized brain in rats

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To clarify the effect of hypovolemic hypotension on high-energy phosphate metabolism in head injury, sequential changes in in vivo phosphorus-31 magnetic resonance (31P MR) spectra were compared in 35 rats after impact injury with and without hypotension. Fourteen rats were subjected to hypotension alone (mean arterial blood pressure (MABP) of either 40 or 30 mm Hg for 60 minutes), seven to fluid-percussion impact injury (4 to 5 atm) alone, and 14 to impact injury and hypotension (MABP of 40 to 30 mm Hg). Impact injury alone caused a transient decrease in the phosphocreatine (PCr) level and an increase in the inorganic phosphate (Pi) value. While hypotension alone produced only small changes on 31P MR spectra, impact injury plus hypotension caused pronounced changes. Impact injury and an MABP of 40 mm Hg caused a 50% decrease in PCr concentration and an approximately twofold increase in Pi level, which were significantly greater than values in rats with impact injury alone. Impact injury and an MABP of 30 mm Hg also caused a significant decrease in adenosine triphosphate value, which was not observed in rats with impact injury alone or with an MABP of 30 mm Hg alone. Decreases in intracellular pH were greater in rats with impact injury and hypotension. After traumatic injury, the brain is extremely vulnerable to hypovolemic hypotension, as reflected in the loss of high-energy phosphates in brain.

KEY WORDS • head injury • hypotension • magnetic resonance spectroscopy • phosphorus-31 • rat

SHOCK is a frequent and important complication in severely head-injured patients, especially in the face of multiple injuries.29 Neurological outcome after head injury without and with hypotension has been compared in humans29,35 and in animals.32 Hypotension significantly increases the mortality rate.

Changes in cerebral circulation and metabolism after head injury have been studied both experimentally,4,9,28,37,38,51 and clinically,6,12,39,40 and autoregulation is impaired after traumatic head injury.22,21 Cerebral blood flow (CBF) decreases as the systemic blood pressure falls, and ischemia can cause additional damage to the injured brain. Brain metabolism, which may not be coupled with the change in CBF after trauma, probably is adversely affected by reduced cerebral perfusion pressure.

High-energy phosphate metabolism in the brain can be studied noninvasively and sequentially after various insults or treatments with in vivo phosphorus-31 magnetic resonance (31P MR) spectroscopy. Relative concentrations of phosphocreatine (PCr), adenosine triphosphate (ATP), and inorganic phosphate (Pi) and intracellular pH can be measured using a high-field magnet and a radiofrequency (RF) surface coil that is placed directly on the rat's head. The present study was designed to characterize the effect of hypovolemic hypotension on posttraumatic changes in phosphorus metabolites in head-injured rats.

Materials and Methods

Surgical Procedure

The experimental procedure was approved by the Animal Experimentation Committee of the University of California at San Francisco. Adult male Sprague-Dawley rats, each weighing 350 to 400 gm, were sedated with intraperitoneal chloral hydrate (0.3 mg/kg). Surgical sites were infiltrated with 0.5% Maracaine (bupivacaine), and a cannula (PE-60) was introduced into each femoral artery, one for the measurement of arterial blood pressure and analysis of blood gases, and the other for the withdrawal of blood to produce hypovolemic hypotension.* A third cannula, for the injection of blood that had been withdrawn, was inserted into the femoral vein.

* Statham P23-ID transducer, manufactured by Gould, Inc., Oxnard, California; pH/blood gas analyzer, Model 178, manufactured by Corning Medical and Scientific Co., Corning Glass Works, Medfield, Massachusetts.
TABLE 1
Comparison of physiological changes in the five experimental groups*

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>No. of Rats</th>
<th>Preinsult Data</th>
<th>MABP</th>
<th>MABP After Impact</th>
<th>MABP During Hypotension</th>
<th>MABP 10 Min After Hypotension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (MABP 40)</td>
<td>7</td>
<td>155 ± 19</td>
<td>35 ± 4</td>
<td>7.42 ± 0.02</td>
<td>114 ± 8</td>
<td>—</td>
</tr>
<tr>
<td>Group 2 (MABP 30)</td>
<td>7</td>
<td>149 ± 12</td>
<td>33 ± 4</td>
<td>7.42 ± 0.02</td>
<td>113 ± 5</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>Group 3 (impact)</td>
<td>7</td>
<td>147 ± 19</td>
<td>32 ± 4</td>
<td>7.44 ± 0.04</td>
<td>111 ± 6</td>
<td>153 ± 18</td>
</tr>
<tr>
<td>Group 4 (impact + MABP 40)</td>
<td>7</td>
<td>151 ± 14</td>
<td>33 ± 3</td>
<td>7.42 ± 0.02</td>
<td>113 ± 9</td>
<td>152 ± 14</td>
</tr>
<tr>
<td>Group 5 (impact + MABP 30)</td>
<td>7</td>
<td>154 ± 11</td>
<td>33 ± 4</td>
<td>7.43 ± 0.02</td>
<td>110 ± 7</td>
<td>158 ± 22</td>
</tr>
</tbody>
</table>

* MABP = mean arterial blood pressure, expressed in mm Hg. Values are means ± standard deviation.
† MABP obtained 60 minutes after impact.

A linear skin incision was made in the right temporal region, the temporal muscle was reflected, and a circular 4 mm-diameter craniectomy was made with a dental drill just above the zygoma. A polyethylene catheter (PE-350) filled with sterile water was placed against the intact dura mater and fixed securely to the skull with dental acrylic.

The rats were then intubated and ventilated† with a mixture of 30% oxygen, 70% nitrogen, and 1% isoflurane. The animals were placed in the prone position on a cradle and the body temperature was controlled at 37° to 38°C. A 7 × 11-mm oval two-turn balanced-matched surface coil was placed over the intact scalp and each rat was placed in a custom-made horizontal magnet (10.2-cm bore diameter) with a field strength of 5.6 tesla corresponding to a 95.9-MHz resonance frequency for 31P. The polyethylene catheter placed over the right temporal region was connected to the fluid-percussion device that was positioned about 1.0 m away from the rats.

Fig. 1. Changes in phosphocreatine (PCr), inorganic phosphate (Pi), β-adenosine triphosphate (β-ATP), and pH levels following hypotension. Open triangles indicate results in Group 1 rats, subjected to a mean arterial blood pressure (MABP) of 40 mm Hg for 60 minutes; closed triangles show results in Group 2, with an MABP of 30 mm Hg for 60 minutes.
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The rats were sacrificed after each experiment and the brains were removed for macroscopic histological examination.

\[ ^3P \text{ Magnetic Resonance Spectroscopy} \]

A single-pulse excitation-acquisition experiment was performed with an interpulse delay of 800 msec and a presaturation pulse of 600 msec to remove a broad component of immobile phosphorus from the bone and scalp.\(^1\)\(^,\)\(^14\) A spectrum was obtained by Fourier transformation of 224 averaged free-induction decays obtained in about 3 minutes. A control spectrum was obtained and spectra were recorded every 3 minutes for the first 15 minutes and then at 22, 30, 45, 60, 75, and 90 minutes after the insult.

Signal intensities of phosphate metabolites (PCr, \(\beta\)-ATP, Pi, and total phosphorus) were determined from the areas under Lorentzian-Gaussian curves using a line-fitting simulation program.\(\dagger\) The pH values were calculated from the chemical shift of the Pi peak relative to the PCr resonance (\(\delta_{\text{Pi}}\), expressed in parts per million) according to the equation:

\[
pH = 6.72 - \log[(\delta_{\text{Pi}} - 5.69)/(3.27 - \delta_{\text{Pi}})].
\]

\[ \text{Statistical Analysis} \]

Data were expressed as means \(\pm\) standard deviation (SD). A Wilcoxon signed-rank test was used to compare the PCr, \(\beta\)-ATP, Pi, and pH values with control values in each group. Between-group comparisons of the change in PCr, \(\beta\)-ATP, and Pi were made with one-way analysis of variance and with Student's t-test of independent samples using the Bonferroni correction. Between-group comparisons in pH were made with the Kruskal-Wallis test. A probability of 0.05 or less was considered to be significant.

\[ \text{Results} \]

\[ \text{Study Groups} \]

The 35 rats were divided randomly into five groups with seven rats in each group: Group 1: hypotension alone, mean arterial blood pressure (MABP) of 40 mm Hg; Group 2: hypotension alone, MABP of 30 mm Hg; Group 3: impact injury alone; Group 4: impact injury and hypotension, MABP of 40 mm Hg; and Group 5: impact injury plus hypotension, MABP of 30 mm Hg.

\[ \text{Physiological Changes} \]

Physiological changes are summarized in Table 1. Values for MABP, \(\text{PaO}_2\), \(\text{PaCO}_2\), and arterial pH did not differ among the groups and were stable for at least 10 minutes before each impact. The \(\text{PaO}_2\) was kept above 100 mm Hg and the \(\text{PaCO}_2\) was maintained between 30 to 35 mm Hg throughout the experiment.

Impact injury caused an immediate increase in MABP by approximately 40% and returned to control

\(\dagger\) NTCCAP program for the Nicolet 1180 computer obtained from General Electric Medical Systems Co., Fremont, California.
levels within 3 minutes. In Group 1, 2, 4, and 5 rats, hypotension was obtained by blood withdrawal in the next 5 minutes, and MABP was maintained at either 40 ± 3 or 30 ± 3 mm Hg for 60 minutes. With the reinfusion of blood, MABP recovered to near control levels within 5 minutes in all groups.

**31P Magnetic Resonance Spectroscopy**

**Hypotension Alone.** The time course of the changes in PCr, β-ATP, Pi, and pH in Groups 1 and 2 are shown in Fig. 1. The total phosphorus concentration did not change throughout the experiment. In Group 1 rats (MABP 40 mm Hg), there was a consistent drop of 0.2 pH units (p < 0.02). In Group 2 rats (MABP 30 mm Hg), PCr decreased from control values by 15% (p < 0.02) and Pi increased by 70% (p < 0.02). The pH value also decreased by approximately 0.25 units (p < 0.02), but β-ATP remained unchanged. After 30 minutes of normotension, the spectra returned to control levels. A typical spectral change in a Group 2 rat is shown in Fig. 2.

**Impact Injury Alone.** Impact injury caused transient changes in the spectra in Group 3 rats (Figs. 3 and 4). The PCr level decreased by 13% of control values (p < 0.05), the Pi concentration increased by 41% of control values (p < 0.05), and the pH content decreased by 0.1 unit (p < 0.02). Changes occurred within 15 minutes of impact injury and returned to near control levels within the next 30 minutes. The β-ATP level was not significantly changed.

**Impact Plus Hypotension.** Changes in the spectra were more pronounced in rats with impact injury and hypotension (Groups 4 and 5, Figs. 5 and 6). The PCr level decreased and the Pi content increased progressively for up to 60 minutes. In Group 4 (impact injury and MABP 40 mm Hg), a 21% decrease in PCr content and a 137% increase in Pi level were significantly greater than increases found for Group 3 rats (p < 0.05 and p < 0.02, respectively). The pH value decreased by 0.3 units (p < 0.02). In Group 5 rats (impact injury and MABP 30 mm Hg), the β-ATP content decreased significantly below control values (21%, p < 0.02), which was not observed in any other group. A 41% decrease in the PCr level and a 198% increase in the Pi content were also significantly greater than those seen in Group 3 rats (p < 0.01 and p < 0.005, respectively). Recovery after 30 minutes of normotension was significantly less than in rats with impact injury only (p < 0.05 and p < 0.005, respectively). The total phosphate concentrations visible on 31P MR spectra remained nearly constant. The 0.4-unit fall in the pH level was significantly greater than that in Group 3 rats (p < 0.005).

**Discussion**

Recently, 31P MR spectroscopy has been used widely in animal experiments and occasionally in humans to measure noninvasively the relative concentrations of high-energy phosphorus metabolites, intracellular pH, and unidirectional chemical fluxes. Most background signals from bone and scalp can be eliminated. In
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**Fig. 4.** A typical spectral change after impact injury in a Group 3 rat. A decrease in phosphocreatine level (downward arrow), an increase in inorganic phosphate content (upward arrow), and a drop in pH level were observed 15 minutes after impact injury. These changes returned to normal 30 minutes later. PPM = parts per million.

After impact injury alone, $^{31}$P MR spectra showed a transient decrease in PCr with an increase in Pi and a decrease in pH. These changes were observed within the first 15 minutes after impact injury, after which the spectra became essentially normal over the next 30 minutes. Nilsson and Nordstrom used a freeze-clamping technique to measure high-energy phosphates after acceleration concussion and reported similar results; PCr and ATP decreased progressively during the first 4 minutes after injury and returned to normal by 15 minutes posttrauma. It has been suggested that this transient loss of energy stores indicates a hypermetabolic state that follows concussive injury.

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is the characteristic response to trauma. Duckrow, et al.,11 supported this concept with the observation that fluid-percussion injury produced consistent transient oxidation of cytochrome aa₃, a finding similar to that seen with spreading cortical depression or seizures.

A transient increase in CBF associated with high metabolic activity has also been reported.4,9,10,28,37 However, CBF and metabolism may not remain coupled after impact injury. Using the same concussion model, Nilsson and coworkers37,38 found that CBF increased for only 2 to 4 minutes after injury and that the initially decreased PCr or ATP levels did not return to normal until 15 minutes after impact injury. Using the hydrogen clearance method in our rat model, we recently observed a 50% to 80% decrease in CBF, predominantly in the ipsilateral hemisphere, for more than 15 minutes after temporal lobe impact (unpublished results). The reduction in CBF was not associated with systemic hypotension. Cerebral ischemia could be a major factor contributing to the consumption of high-energy phosphates after impact injury.

Changes in regional metabolites have been studied using enzymatic-fluorometric techniques.26 Yang, et al.,52 observed a significant decrease of PCr in the hippocampus 1 hour after mild fluid-percussion impact injury in cats. There have been reports that there are substantial changes in PCr and ATP levels in brain regions within or adjacent to hemorrhagic tissue.15,49 In our present study, the MR coil was placed over both hemispheres and received signals from both. The ATP content did not change significantly after impact injury, but it is possible that focal changes were undetected or underestimated.

Impact injury and hypotension caused a significantly greater decrease in PCr and increase in Pi. An MABP of 40 mm Hg alone caused little alteration in the spectra, but resulted in significant changes in the spectra after impact injury. Impact injury followed by an MABP of 30 mm Hg caused a significant decrease in ATP levels as well as marked changes in concentrations of PCr and Pi. These findings clearly show that traumatized brain is more vulnerable to hypotension than is normal brain and supports findings that impact injury produces an impairment of pressure autoregulation.22 With the loss of autoregulation, CBF varies passively with cerebral perfusion pressure; that is, the difference between MABP and intracranial pressure. Intracranial pressure increased only transiently during the first several seconds after impact;24 therefore, systemic hypotension strongly influenced CBF and led to metabolic deterioration. Wei, et al.,51 found that pial vessel dilatation with hemorrhagic hypotension disappeared after percussion injury. Attenuation of the response to hypoxia23 or to hypocarbia23,45 also has been reported.
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After impact with 60 min hypotension

**CONTROL**

**AFTER 30 MIN RECOVERY**

**pH = 7.27**

**pH = 6.88**

FIG. 6. A typical spectral change in a Group 5 rat, with impact injury and an MABP of 30 mm Hg for 60 minutes. A decrease in the phosphocreatine level (large downward arrow) and an increase in the inorganic phosphate content (large upward arrow) were more pronounced than those in Group 3 rats (impact injury alone, see Fig. 4). The adenosine triphosphate level decreased significantly from the control value (small arrows). The pH value fell from 7.27 to 6.88. Spectral recovery was not complete after 30 minutes of normotension. PPM = parts per million.

Generation of superoxide and other free radicals may take part in these abnormal arteriolar responses. In rats with impact injury and an MABP of 30 mm Hg, the spectra did not recover completely after 30 minutes of normotension. Recovery of $^{31}$P MR spectra must be interpreted with caution, however. The spectra were essentially normal 24 hours after focal ischemia or impact injury and hypoxia, when MR images and neuropathology studies showed large lesions. The $^{31}$P MR spectra may not always reflect the severity of structural damage and may not detect even severe brain injuries several hours after insult. While the correlation between spectral and pathological changes after brain injury must be clarified, it was found that rats that had delayed or incomplete recovery of spectral characteristics after insult had a much worse neurological outcome and greater histopathological damage than did rats in which the $^{31}$P MR spectra returned to normal shortly after insult.

It has been reported that the severity of cerebral acidosis can be used to predict the degree of brain damage after trauma. Recently, severe lactic acidoisis itself has been recognized as a cause of secondary or delayed cellular damage, and an alkalinizing agent has been reported to improve the outcome following head trauma. In the present study, brain acidosis was clearly worsened in rats when impact injury was coupled with hypotension.

The implications of our results for patient care are clear. Hypotension reduces the stores of high-energy phosphates in traumatized brain. Metabolic impairment is more pronounced as hypotension becomes more severe and as the length of hypotension increases. The detriment of this combination of insults underscores the need for vigorous and continuous blood pressure resuscitation after head injury.

Acknowledgments

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