Cerebral energy metabolism following fluid-percussion brain injury in cats

ANDREAS W. UNTERBERG, M.D., BRUCE J. ANDERSEN, M.D., GEOFF D. CLARKE, PH.D.,
AND ANTHONY MARMAROU, PH.D.

Richard Roland Reynolds Neurosurgical Research Laboratories, Division of Neurosurgery, Department
of Surgery, and the Department of Radiology, Medical College of Virginia, Richmond, Virginia

Clinical and experimental evidence suggests that head injury can cause alterations of cerebral energy metabolism. However, the etiology of this metabolic perturbation is not known. The objective of this study was to determine the effect of fluid-percussion trauma on cerebral energy metabolism. Seven ventilated, chloralose-anesthetized cats were subjected to a 3.2-atm fluid-percussion brain injury. Before and for 8 hours after trauma, continuous phosphorus-31 magnetic resonance spectroscopy was obtained to noninvasively monitor tissue pH, phosphocreatine (PCr), and inorganic phosphate (Pi) levels. Measurement of cerebral blood flow (CBF) by the radioactive microsphere technique and calculation of oxygen and glucose consumption (CMRO₂ and CMRG₁) were also performed before trauma as well as 30 minutes and 1, 2, 4, and 8 hours after trauma. The data showed a moderate decrease in tissue pH from 7.04 to 6.89 at 30 minutes following trauma with return to control levels by 3 hours posttrauma. During the 8-hour observation period, CBF, CMRO₂, and CMRG₁ remained at control levels. Tissue PCr and Pi levels were also unchanged.

Fluid-percussion trauma at the 3.2-atm level in ventilated cats causes a moderate and transient decrease in tissue pH that returns to control levels after trauma. No other metabolic changes are seen later than 30 minutes posttrauma. This indicates that a mild metabolic disturbance occurs after trauma in the ventilated animal and quickly returns to normal.

Key Words • head injury model • metabolism • cerebral blood flow • magnetic resonance spectroscopy • tissue pH • lactic acidosis • cat

Preservation of sufficient energy metabolism is of utmost importance for the clinical course of head trauma, since ion transport, synaptic function, and membrane synthesis and repair all require provision of energy. The preservation, support, or re-establishment of energy supply therefore represents a prerequisite to improved outcome after head injury.

Clinical and experimental evidence suggests that severe head injury is followed by alterations in cerebral energy metabolism. However, it is unknown whether cerebral energy metabolism is perturbed by experimental fluid-percussion head injury itself. Clinically, disturbances in cerebral energy metabolism are indicated by the presence of lactic acidosis in the cerebrospinal fluid (CSF) after head injury, suggesting an increase in anaerobic glycolysis. Furthermore, experimental studies have shown that cerebral trauma in baboons caused a decrease in blood flow and in the cerebral metabolic rate of oxygen (CMRO₂) as well as an increase of tissue lactate, while concussion of rat brains also resulted in decreased levels of cerebral blood flow (CBF), CMRO₂, tissue phosphocreatine (PCr) concentration, and an increase in tissue lactate. In fluid-percussion brain injury of normally ventilated cats, the immediate response to trauma is a marked but transient increase in CBF with preservation of oxidative metabolic activity. Lack of disturbance in cerebral energy metabolism in this model is not totally consistent with the increased CSF lactate levels seen clinically, which has been interpreted as indicating cerebral metabolic alterations.

The goal of the present investigations was to characterize for the first time the response of cerebral energy metabolism to fluid-percussion injury in normally ventilated cats. Phosphorus-31 (31P) magnetic resonance (MR) spectroscopy was used because it offers the unique opportunity for noninvasive and continuous monitoring of tissue pH, PCr, and inorganic phosphate (Pi) concentrations in the brain. By combining 31P MR spectroscopy with measurement of CBF by the radioactive microsphere technique, as well as of glucose and oxygen consumption (CMRO₂ and CMRG₁), we were able to determine the actual tissue concentrations of...
Energy metabolism following brain trauma

high-energy phosphates and to characterize the turnover of substrates for energy production.

Materials and Methods

Surgical Preparation

Cats of either sex, each weighing between 2.5 and 3.5 kg, were used for these experiments. Anesthesia was induced by intravenous methohexital sodium (10 mg/kg) and maintained with intravenous α-chloralose (40 mg/kg initially followed by 20 mg/kg after 8 hours). The cats were then tracheostomized and placed on a ventilator adjusted to deliver a tidal volume of about 15 cc/kg at a rate of 10 to 12 breaths/min. Muscle paralysis was obtained with intravenous pancuronium bromide (0.5 to 1.0 mg/kg). After placement of an arterial catheter in the femoral artery for blood gas monitoring, ventilation was adjusted to maintain arterial pCO2 at 30 to 32 mm Hg and pO2 above 100 mm Hg by manipulating the tidal volume, ventilation rate, and supplemental O2. The arterial pH was kept at 7.35 to 7.40 by small injections of bicarbonate, given to correct base deficit when present. A second arterial catheter was introduced into the brachial artery for microsphere withdrawal and both arterial catheters (femoral and brachial) were connected to strain-gauge transducers for continuous monitoring of the systemic arterial blood pressure. A left thoracotomy was then performed, a pericardial window created, and polyethylene tubing (inner diameter 0.76 mm, outer diameter 1.22 mm) was sutured into the left atrium for the purpose of microsphere injection. All incisions were surgically sutured.

The animals were placed in a stereotaxic head-holder. The skull was exposed and the muscles of mastication were removed from the superior and left temporoparietal region. A lateral craniectomy was made (diameter 1 cm) for the attachment of the head-injury coupling device. A second craniectomy was made above the sagittal sinus 1.5 cm posterior to the bregma. Under magnification, a polyethylene catheter (inner diameter 0.58 mm, outer diameter 0.96 mm) was inserted into the sinus for withdrawal of cerebrovenous blood. Both the lateral and superior craniectomies were closed with Gelfoam and acrylic cement. Two MR spectroscopy surface coils (23 x 19 mm) were then cemented in place above the left lateral craniectomy and above the right marginal gyrus. Throughout the experiments, the body temperature of the animals was kept constant at 37°C by means of a thermostatic heating blanket.

Experimental Protocol

After surgical preparation, the animals were transferred to a Bruker Biospec 400/2.3 spectrometer,* and control 31P MR spectroscopy determinations of tissue pH, P, and Pi were made. Simultaneously, CBF was measured and the CMRO2 and CMRGl were calculated. The animals were then randomly assigned to either a control group (seven cats) or a trauma group (seven cats). The control animals remained in the spectrometer, and 31P MR spectra were obtained approximately every 8 minutes for 8 hours. The animals in the trauma group were removed from the spectrometer and subjected to a 3.2-atm lateral fluid-percussion injury. Immediately following trauma, the animals were returned to the spectrometer and 31P MR spectra were obtained approximately every 8 minutes for 8 hours, and CBF, CMRO2, and CMRGl were measured 30 minutes, 1, 2, 4, and 8 hours posttrauma. In the control group, CBF, CMRO2, and CMRGl determinations were not performed at 30 minutes. At 8 hours following trauma (or assignment to the control group), the animals were sacrificed by intravenous KCl injection and the brains were removed.

Magnetic Resonance Spectroscopy

The MR spectroscopy measurements were made in a spectrometer with a usable diameter of 340 mm and a B field of 2.35 tesla (31P resonant frequency of 40.71 MHz) operating in the pulse Fourier transform mode. Magnetic field homogeneity was optimized by shimming on the hydrogen signal from the water in the brain. Rectangular two-turn surface coils (23 x 19 mm) were used for both transmission and reception of the radiofrequency signals. The coils were shaped from copper wire 1 mm in diameter and were attached to the resonant circuitry via a 150- to 180-mm long miniature coaxial cable. Using a sweep generator and a 50-ohm impedance bridge, measured quality factors of the coils at 41.7 MHz ranged from 250 to 300. A pulse width of 22 μsec was empirically determined to produce a 180° nutation angle at the surface of the skull and approximately a 75° nutation angle at a depth of 4 mm. The spectral width was 6 kHz and 8192 data points were collected in each scan in a single scan acquisition time of 0.68 seconds. Scans were repeated every 0.9 seconds, and 512 scans were summed to achieve an adequate signal-to-noise ratio. Total time to obtain an averaged spectrum was 7.68 minutes. The relatively rapid pulsing improved the accuracy of the tissue pH measurements by reducing the signals from phosphomonoester and phosphodiester resonances near the Pi peak. Free induction decay signals were processed using a 10-Hz exponential filter before the Fourier transform.

---

* Animal ventilator manufactured by Harvard Apparatus Co., Millis, Massachusetts.
† Blood gas analyzer, Model 158, manufactured by Corning Medical, Medfield, Massachusetts.
‡ Statham pressure transducer manufactured by Gould Inc., Cleveland, Ohio.
∥ Sweep generator, Model 1080, manufactured by Wavetek Corp., San Diego, California.
The broad resonance from relatively immobile phosphates was removed by a baseline correction procedure supplied with the spectrometer. The intracellular pH was calculated from the chemical shift of the Pi peak using the form of the Henderson-Hasselbalch equation developed for cerebral intracellular pH using the PCr resonance as an internal reference. The area under the Pi, PCr, and the three adenosine phosphate peaks above an arbitrary baseline was determined in each spectrum by a computerized integration procedure provided by the spectrometer. The PCr:Pi ratio was calculated by dividing these specific areas.

Cerebral Blood Flow Measurement

Cerebral blood flow was measured by injection of radioactive microspheres. This technique allowed for five individual measurements of regional CBF. The protocol used in our laboratory has been previously described in detail. Briefly, for each determination of CBF, microspheres labeled with an individual radioisotope were injected into the circulation via the left atrium (0.9 to 1.8 × 10⁵ microspheres, 15 μ in diameter). Arterial reference samples were then taken from the brachial and femoral arterial lines. After the experiment, the brain was removed and fixed for 48 hours in aldehyde fixatives. After fixation, the brains were dissected into specific subsections, and the number of radioactive microspheres in each section was counted by a gamma counter. The relative number of microsphere counts per area corresponds to the CBF to that area at the time of microsphere injection.

Cerebral Metabolic Rates of Oxygen and Glucose

For determination of CMRO₂ and CMRG₁, arterial and cerebrovenous (sagittal sinus) blood samples were taken and the arterial-venous differences of oxygen and glucose were multiplied by the CBF at that time. Blood glucose was determined enzymatically, and oxygen content was determined by a Lex-O₂-Con analyzer that measures total oxygen content.

Fluid-Percussion Trauma

The fluid-percussion trauma was delivered using the device described by Sullivan, et al. Briefly, a variable-pressure pulse wave of fixed duration is produced by a calibrated pendulum and is transmitted through a saline-filled tube in contact with dura under the left temporoparietal craniectomy. An in-line strain-gauge transducer records the pulse and displays it on a storage oscilloscope, which allows the pressure pulse to be recorded and calibrated. The degree of injury corresponds to the amplitude of the recorded pressure wave and is reported in atmospheres (1 atm = 760 mm Hg). The degree of injury used (3.2 atm) is just below that degree (> 3.4 atm) that causes concomitant brain-stem injury.

Statistical Analysis

All data are expressed as means ± standard error of the means. Statistical comparisons were carried out using Student’s t-test for independent variables. A 95% confidence level was considered statistically significant.

Results

The results of this study are presented in Table 1. In the sham-operated control animals, blood pressure remained relatively constant throughout the entire observation period (135 ± 15 mm Hg). Fluid-percussion injury consistently resulted in a brief and sudden increase of blood pressure up to 274 ± 22 mm Hg. This hypertensive episode is commonly seen at this level of injury in fluid-percussion trauma, and lasted less than 10 minutes. During the following 60 minutes blood pressure was slightly hypotensive, 110 ± 10 mm Hg, returning to 120 ± 10 mm Hg by 90 minutes post-trauma. For the remainder of the 8-hour experiment, blood pressure remained constant at 135 ± 15 mm Hg until sacrifice of the animals.

Cerebral Blood Flow

Mean total CBF’s before and after fluid-percussion injury are shown in Fig. 1. Control values of CBF in sham-operated control animals were 29.6 ± 2.2 and 34.8 ± 3.8 ml/100 gm/min, respectively. In both control and trauma groups, CBF remained near control values at all measurement points except the final (8-hour) determination. At 8 hours, both control

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral blood flow and metabolic parameters in control and trauma groups*</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>Factor</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>control group</td>
</tr>
<tr>
<td>CBF</td>
</tr>
<tr>
<td>CMRO₂</td>
</tr>
<tr>
<td>CMRG₁</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>PCr:Pi</td>
</tr>
<tr>
<td>trauma group</td>
</tr>
<tr>
<td>CBF</td>
</tr>
<tr>
<td>CMRO₂</td>
</tr>
<tr>
<td>CMRG₁</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>PCr:Pi</td>
</tr>
</tbody>
</table>

* CBF = cerebral blood flow (ml/100 gm/min); CMRO₂ and CMRG₁ = oxygen and glucose consumption, respectively (μmoles/gm/min); PCr:Pi = phosphocreatine:inorganic phosphate ratio.

† Significant difference (p < 0.001) when compared with control values.
Energy metabolism following brain trauma

and trauma groups had elevated CBF at 42.1 ± 4.11 and 50.7 ± 0.35 ml/100 gm/min, respectively (p < 0.001 in both groups when compared with respective control values). This late increase in flow is attributed to hemodilution caused by the numerous blood samples being replaced with normal saline.

Cerebral Metabolic Rates of Oxygen and Glucose

The mean control CMRO₂ value in sham-operated control animals was 1.17 ± 0.14 μmoles/gm/min and remained unchanged throughout the experimental period (Fig. 2 left). In traumatized animals, CMRO₂ did not deviate statistically from control values between 30 minutes and 8 hours posttrauma (Fig. 2 left).

The time course of the CMRGl is given in Fig. 2 right. In sham-operated control animals, CMRGl was 0.32 ± 0.06 μmoles/gm/min and remained unchanged for the first 4 hours. At 8 hours it increased moderately to 0.43 ± 0.02 μmoles/gm/min (p < 0.05). In the trauma group, CMRGl remained at control values when measured during the entire 8-hour experiment.

Magnetic Resonance Spectroscopy

Figure 3 shows five ³¹P MR spectra obtained with the surface coil above the injury site (left temporoparietal region) before and at various time intervals after trauma. The time course of mean tissue pH values, as determined by the chemical shift of the Pi peak in the ³¹P MR spectra, is summarized in Fig. 4. The first spectra after trauma (15 minutes posttrauma) indicates a drop in pH from 7.04 ± 0.02 to 6.90 ± 0.02 (p < 0.05) and at 30 minutes posttrauma the pH dropped to 6.89 ± 0.05 (p < 0.001 compared with control values). After 30 minutes posttrauma the pH gradually began to return to normal and reached control values by 3 hours posttrauma.

A sensitive MR spectroscopy parameter used to indicate tissue energy depletion is the PCr:Pi ratio (Fig. 5). It is important to look at this ratio in order to describe the response of energy metabolism after trauma. The control value of the PCr:Pi ratio was 1.5 ± 0.33 in the trauma group. After trauma the PCr:Pi ratio was statistically unchanged and remained so for the entire experiment. From these data, there is no indication that tissue energy production was decreased between 30 minutes and 8 hours after trauma.

Neuropathology

Macroscopic neuropathological examination of the traumatized brains often revealed minor subarachnoid blood clots around the brain stem. Petechial hemorrh-
A. W. Unterberg, et al.

**Discussion**

This study demonstrates that a 3.2-atm fluid-percussion brain injury in the ventilated cat does not significantly affect energy metabolism between 30 minutes and 8 hours after trauma. All metabolic parameters studied remained at control values except for tissue pH which showed a slight but significant (p < 0.001) decrease and returned to normal within 3 hours after trauma.

In the present investigations, a global CBF of about 30 ml/100 gm/min was found under control conditions. Numerous previous studies with measurement by either the radioactive microsphere technique or the hydrogen clearance technique have reported normal CBF values in the cat to be in this range (27 to 35 ml/100 gm/min). The finding that CBF is not significantly altered, at least between 30 minutes and 8 hours posttrauma, is in agreement with previous findings in fluid-percussion brain injury. DeWitt, et al., reported that moderate levels of injury (2.68 ± 0.19 atm) resulted in a blood flow increase at 1 minute after injury from 30 to 60 ml/100 gm/min which returned to normal by 30 minutes posttrauma. Earlier hydrogen-clearance studies of CBF after high levels of fluid-percussion injury also indicated no significant changes in resting flow under normal ventilation between 1 and 3 hours after trauma. However, trauma abolished the responsiveness of CBF to increased CO₂ and to decreased O₂ (hypoxemia) and produced a generalized loss of CO₂ autoregulation for several hours after trauma. Only very high levels of injury (> 3.4 atm) concomitant with severe brain-stem dysfunction might be associated with a persistent global

---

**Fig. 3.** ³¹P magnetic resonance (MR) spectra of a cat brain under control conditions and at 30, 60, 240, and 480 minutes after fluid-percussion injury. The spectra were obtained with a coil (23 x 19 mm) placed above the injury site (left temporoparietal region). Sugar phosphates and phosphomonoesters (SP/PME), inorganic phosphate (Pi), phosphodiesters (PDE), phosphocreatine (PCr), and adenosine triphosphates (ATP) are indicated. Cerebral blood flow (CBF) and mean arterial blood pressure (MABP) are given to the right of each spectrum. The scale at the bottom refers to the chemical shift in parts per million, with respect to the frequency of the PCr signal. Note that at 30 minutes after trauma, pH (determined as the chemical shift of the Pi peak) is decreased. Also, at later times after trauma, the ³¹P MR spectra do not show alterations as compared to the control spectrum.

---

**Fig. 4.** Graph showing tissue pH as determined by the chemical shift of the Pi peak in the ³¹P magnetic resonance spectra obtained from the coil above the injury site (left temporoparietal) in control (sham-operated) and traumatized animals. Before trauma, pH was 7.04 ± 0.02 (mean ± standard error of the mean). The earliest posttraumatic pH (15 minutes after trauma) revealed a decrease to 6.90 ± 0.02 (p < 0.05). At 30 minutes posttrauma, pH decreased further to 6.89 ± 0.05 (p < 0.001), but then rose to 6.97 at 1 hour postinjury. Later, tissue pH showed no alterations in either the control or the experimental group.

---

Rhages smaller than 2 mm in diameter were observed within the brain stem pontine region in four animals. Two animals had supratentorial petechial hemorrhage in the nucleus caudalis and thalamus on the traumatized side.
Energy metabolism following brain trauma

In the fluid-percussion model of brain injury in cats, data concerning oxygen or glucose consumption have not been available prior to these experiments. The present investigations do not reveal significant alterations of CMRO₂ or CMRGl between 30 minutes and 8 hours posttrauma. These data are in the same range as control data for anesthetized cats reported previously. Nilsson and Nordström reported that experimental head injury in the rat resulted in an initial increase in CMRO₂ at 5 to 20 minutes following trauma, with return to normal values after that. Meyer, et al., found that severe concussive blows to the brain of baboons decreased CMRO₂ at 1 to 5 minutes posttrauma with recovery within 5 to 20 minutes. Neither study can be strictly compared with our data, since we measured oxygen and glucose consumption no earlier than 30 minutes following impact. Based on data following fluid-percussion injury in cats that showed a brief posttraumatic CBF increase and a consistent oxidation of cytochrome a₃, it is unlikely that CMRO₂ decreases in the acute posttraumatic period in the cat. In the light of unaltered CBF and oxygen and glucose consumption, the question arises whether normal CBF and energy substrate metabolism are sufficient to meet the energy demands of brain tissue which might be altered after trauma. This question can be answered by looking at indicators of energy stores of the tissue (PCr and Pi).

In the present investigations, PCr and Pi were assessed by ³¹P MR spectroscopy. One of the objectives of ³¹P MR spectroscopy is to quantify cerebral phosphorus metabolite concentrations. Under certain specific conditions, the integral of the peak of a specific metabolite reflects its actual tissue concentration. However, in the present experiment, absolute concentrations could not be measured directly because of uncertainties regarding the volume of the brain and its position relative to the inhomogeneous radiofrequency field and the tuning of the MR spectroscopy coil. To compensate for these uncertainties, the ratios of the MR peak areas is represented as being proportional to the ratios of metabolite concentrations. A sensitive ratio that possibly indicates cerebral energy depletion is the PCr:Pi ratio. Since a decrease in tissue PCr is most likely accompanied by an increase in Pi, the PCr:Pi ratio amplifies even subtle decreases of PCr. In the present investigations, the PCr:Pi ratio was not significantly changed at any point after trauma. Therefore, MR spectroscopy offers no indication that energy supply to the tissue is altered after trauma under the given conditions.

The findings of an unaltered PCr:Pi ratio after fluid-percussion injury is supported by biochemical analysis of high-energy phosphate concentrations (PCr, adenosine triphosphate, and adenosine diphosphate) and energy charge potential in traumatized brain tissue. Also, after concussive brain trauma in rats, as described by Nilsson and Nordström, moderate concussive insults did not alter tissue high-energy phosphate concentrations, and severe insults only transiently decreased the PCr content, which was most pronounced in the brain stem. At 15 minutes posttrauma, restoration of normal tissue PCr concentration was achieved. It should be mentioned that Ishige, et al., and Vink, et al., recently reported that fluid-percussion injury is accompanied by a decrease in PCr and an increase in Pi, resulting in a considerably decreased PCr:Pi ratio. These studies were performed in unventilated rats and are thus not directly comparable to our investigations. The respiratory distress that often occurs after brain trauma in general and in fluid-percussion injury in particular could explain their preliminary observations of alterations in tissue high-energy phosphates.

Concussive brain trauma may be the initiating event predisposing the patient to additional insult, but it is only one component of the clinical syndrome of head injury. To fully understand this complex problem, its component parts need to be isolated and characterized. The present investigation presents data to help answer the question as to whether brain trauma alone causes cerebral metabolic changes. As the results show, tissue acidosis occurs after trauma in the ventilated animal.
but is moderate and transient, and within 3 hours after trauma the tissue pH level has completely recovered. It is postulated that this transient acidosis is the result of increased lactate produced by glycolysis immediately following trauma. Despite this slight and transient acidosis, fluid-percussion brain trauma alone does not appear to mimic the degree of metabolic alterations seen clinically in many head-injured patients. These studies support the hypothesis that a secondary insult superimposed upon mechanical brain trauma compounds the metabolic disturbance caused by trauma alone, and may better explain the clinical data.

Conclusions

These investigations demonstrate that fluid-percussion brain trauma in the normally ventilated cat results in mild metabolic disturbance 30 minutes after trauma, as evidenced by a slight transient decrease in tissue pH. However, in the clinical situation of head trauma, CMRO₂ is frequently decreased; also, numerous studies have shown persistently elevated lactic acid levels in CSF, implying an increase in anaerobic glycolysis perhaps due to ischemia. Additionally, neuropathological investigations stress that ischemic alterations are found in approximately 90% of fatal head injuries. We found no evidence of ischemia or alterations of CMRO₂ and thus believe that fluid-percussion brain injury in ventilated animals does not sufficiently mimic the situation of many head-injured patients in regard to cerebral energy metabolism. This also leads to the assumption that other factors following trauma contribute to the development of the cerebral energy derangements frequently seen clinically.

Acknowledgments

The authors are grateful for the technical assistance of Dr. Hanns Gruemer, Dr. Kenneth Kraft, Nancy Nieling, Kimberly Battista, Hanno Rittner, and Lisa Nugent.

References

6. Graham DI, Adams JH, Doyle D: Ischaemic brain dam-


Manuscript received April 30, 1987.
Accepted in final form September 30, 1987.
This work was funded by Grants 5RO1NS19235-03 and 2RO1NS12587-12 from the National Institutes of Health, and by the Richard Roland Reynolds Neurosurgical Research Fund.

Address reprint requests to: Anthony Marmarou, Ph.D., Division of Neurosurgery, Medical College of Virginia, Box 508, MCV Station, Richmond, Virginia 23298-0001.