Influence of early posttraumatic hypothermia therapy on local cerebral blood flow and glucose metabolism after fluid-percussion brain injury

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Object. Using autoradiographic image averaging, the authors recently described prominent foci of marked glucose metabolism-greater-than-blood-flow uncoupling in the acutely traumatized rat brain. Because hypothermia is known to ameliorate injury in this and other injury models, the authors designed the present study to assess the effects of post-traumatic therapeutic hypothermia on the local cerebral metabolic rate of glucose (LCMRglu) and local cerebral blood flow (LCBF) following moderate parasagittal fluid-percussion head injury (FPI) in rats.

Methods. Either cranial hypothermia (30°C) or normothermia (37°C) was induced for 3 hours in matched groups of rats immediately after FPI; LCMRglu and LCBF were assessed 3 hours after concluding these temperature manipulations.

In rats subjected to FPI, regardless of whether normothermia or hypothermia ensued, LCBF was reduced relative to the sham-injury groups. In addition, when FPI was followed by hypothermia (FPI–30°C group), the subsequent LCBF was significantly lower (35–38% on average) than in FPI–37°C rats. Statistical mapping of LCBF difference imaging data revealed confluent cortical and subcortical zones of significantly reduced LCBF (largely ipsilateral to the prior injury) in FPI–30°C rats relative to the FPI–37°C group. Local glucose utilization was reduced in both hemispheres of FPI–37°C rats relative to the sham-injury group and was lower in the right (traumatized) hemisphere than in the left. However, LCMRglu values were largely unaffected by temperature manipulation in either the FPI or sham-injury groups. The LCMRglu/LCBF ratio was nearly doubled in FPI–30°C rats relative to the FPI–37°C group, in a diffuse and bihemispheric fashion. Linear regression analysis comparing LCMRglu and LCBF revealed that the FPI–37°C and FPI–30°C data sets were completely nonoverlapping, whereas the two sham-injury data sets were intermixed.

Conclusions. Despite its proven neuroprotective efficacy, early posttraumatic hypothermia (30°C for 3 hours) nonetheless induces a moderate decline in cerebral perfusion without the (anticipated) improvement in cerebral glucose utilization, so that a state of mild metabolism-greater-than-blood-flow dissociation is perpetuated.

KEY WORDS • head injury • hypothermia • hypoperfusion • autoradiography • image processing • brain temperature • neuroprotection

THE pathophysiological mechanisms of traumatic brain injury (TBI) are complex and known to involve both hemodynamics and metabolism. In both head-injured patients and animal models of brain injury, regional or global alterations in cerebral blood flow (CBF) have been noted,^{6,13,16,23,25,29,33,37,50} although initial CBF reductions are typically only mild to moderate in degree and lie above the thresholds associated with frank ischemic injury.²² In contrast with these CBF reductions, experimental studies have shown posttraumatic elevations of local cerebral glucose consumption.^{24,51,52}

We recently completed a study of experimental fluid-percussion brain injury (FPI) in rats to assess interrelationships between local glucose utilization and blood flow in matched series,¹⁸ taking advantage of powerful three-dimensional autoradiographic image averaging strategies

developed in our laboratory.^{53,54} That study, which was performed within the 1st hour after FPI occurred, revealed evidence of a marked, region-specific uncoupling of local glucose metabolism and blood flow, which was characterized by up to 2.5-fold elevations in the local cerebral metabolic rate of glucose (LCMRglu)/local CBF (LCBF) ratio in zones known to be pathologically lesioned in this model. These results suggested that trauma-induced, enhanced metabolic demand, when combined with mild posttraumatic oligemia, might produce a marked local bioenergetic stress that would contribute to the injury process.¹⁸

It is now well established that mild-to-moderate hypothermia may confer striking neuroprotection against both traumatic and ischemic brain injury.⁹ Indeed, a recently completed single-center randomized study demon-

strated improved clinical outcomes in hypothermia-treated head-injured patients,³¹ and a multicenter randomized trial of moderate hypothermia therapy (32°C lasting 48 hours) for acute head injury is now in progress.¹¹ In small animal models of head injury, we¹⁵ and others¹² have shown that moderate hypothermia reduces contusion volume and neuronal necrosis. The aforementioned studies provide the rationale for the current investigation, in which we wished to ascertain the manner in which a therapeutically effective hypothermia regimen following acute head injury affected subsequent LCMRglu-LCBF interrelationships.

Materials and Methods

Surgical Preparation

These studies were conducted using 40 male Sprague-Dawley rats with a mean weight of 336 ± 27 g. Protocols for these studies were approved by the University of Miami Animal Care and Use Committee. Following initial induction of anesthesia by intraperitoneal infusion of Equitensin (a cocktail of pentobarbital, propylene glycol, magnesium sulfate, and chloral hydrate), the animals were placed in a stereotactic frame. As previously described,¹⁵ a 4.8-mm craniotomy was produced over the right parietal cortex (3.8 mm posterior to the bregma and 2.5 mm lateral to the midline). A plastic injury tube was placed over the exposed dura and bonded to surrounding structures with dental acrylic. The injury tube was plugged with a Gelfoam sponge and the scalp was closed with sutures. The animals recovered in their cages overnight.

Fluid-Perfusion Brain Injury

On the next day, anesthesia was again induced in the rats and they were intubated and ventilated on a mixture of 70% nitrous oxide, 1.5% halothane, and a balance of oxygen. An FPI device was connected to the injury tube already in place. The device consisted of a saline-filled cylindrical Plexiglas reservoir with a rubber-coated piston at one end, a transducer housing for measuring pressure transients, and an injury screw adapted to the rat's skull. The descent of a pendulum transmitted a pressure transient ranging from 1.7 to 2.1 atm to the right dorsolateral parietal cortex. A thermistor probe was inserted into the left temporal muscle and the rat's cranial temperature was regulated at 36.5°C before and during the insult by using a warming lamp located above the head. Rectal temperature was also monitored and was maintained at 37°C throughout the study. Sham-injured rats, which underwent injury-tube placement and all procedures except for the injury itself, were also studied.

Posttraumatic Temperature Manipulations

Rats that underwent FPI or sham FPI were randomly allocated to two groups and subjected to either posttraumatic cranial hypothermia or normothermia. In the hypothermia group, cranial (temporal muscle) temperature was lowered to 29.5 to 30.5°C within 2 to 5 minutes after FPI or sham injury, and this level of cranial temperature was maintained for 3 hours. In the normothermia group, the temporal muscle temperature was regulated at 36.7 to 37°C for 3 hours. In both groups, rectal temperature was maintained throughout at 36.7 to 37.2°C. Cranial cooling was achieved using liquid nitrogen vapor, which was delivered by a fan assembly over the head; cranial warming was accomplished using a separate small lamp located over the rat's head.

Following the 3-hour period of hypothermic or normothermic cranial temperature manipulation, the cranial temperatures of all rats were held at 36.2 to 37°C for another 3 hours. Autoradiographic studies were performed after the procedure. The animals were maintained in a state of anesthesia with controlled ventilation throughout the entire study.

Autoradiographic Studies of LCBF and LCMRglu

In each group (FPI or sham FPI with posttraumatic hypothermia or normothermia), the rats were allocated to one of two subgroups,

in which either LCBF or LCMRglu was measured. To measure LCBF, ¹⁴C-iodoantipyrine ([IAP], 20 μ Ci; specific activity 40 μ Ci/mmol) dissolved in 1 ml of isotonic saline was injected 6 hours after FPI or sham injury. The radioactive tracer solution was administered over a period of 45 seconds by constant intravenous infusion via an infusion pump. Arterial blood samples were obtained at 2-second intervals from a short, freely flowing femoral arterial catheter.

To measure LCMRglu, the radioactive tracer ¹⁴C-2-deoxyglucose ([2-DG], 20 μ Ci; specific activity 40–45 μ Ci/mmol) dissolved in 0.1 ml saline was administered as an intravenous bolus 5.75 hours after FPI or sham injury. Arterial blood samples were obtained every 10 seconds during the 1st minute, at 1-minute intervals for 5 minutes, and at 2.5- to 5-minute intervals for the remainder of the 45-minute study period. Plasma samples were assayed for 2-DG by liquid scintillation counting and for plasma glucose by means of a glucose analyzer. Because the approximate center of 2-DG uptake into the brain occurs 15 to 20 minutes after its bolus administration, a point coinciding with the 6-hour time point of the LCBF study, valid comparisons between LCMRglu and LCBF data were permitted.

At the conclusion of the radioisotope studies, the rats were decapitated by a computer-activated device and their brains were quickly removed and frozen gradually over liquid nitrogen. The rat brains were subsequently embedded and sectioned subserially in a cryostat (20- μ m thickness at 100- μ m intervals), as previously described.⁵³ Along with calibrated ¹⁴C-methylmethacrylate standards, the brain sections were exposed to film for 10 days. The films were then digitized with an 8-bit charge-coupled device camera interfaced with an image-analysis system. Subsequent image processing was performed using a computer workstation (266 MHz with 128-MB random access memory) equipped with software developed in-house in C language. Operational equations of the IAP method⁴² and the 2-DG method modified for variable plasma glucose^{43,47} were used to compute the LCBF and LCMRglu, respectively. Normal values for the kinetic constants and the lumped constant of the 2-DG model were assumed⁴⁷ because LCBF post-FPI was in the supraschismic range (see *Results*).

Three-Dimensional Autoradiographic Image Averaging

Autoradiographic image reconstruction was based on novel image-alignment algorithms previously reported by our group.^{53,54} A linear affine transformation was first used to register sequential coronal sections of each individual brain. Each coronal section was aligned to its adjacent neighbor by applying a reverse transformation with the estimated translation and rotation parameters. Next, corresponding coronal sections of all replicate brains of each study subgroup were placed in register with one another at a common coronal reference level (bregma + 0.7 mm). At each coronal level, all brain sections were mapped into a common contour derived from one brain of the series, by a method similar to that used for image alignment. These procedures resulted in the generation of quantitative three-dimensional image datasets of the average LCBF or LCMRglu for each animal group. Next, an image data set was derived from the mean LCMRglu/LCBF ratio for each group, computed from individual image data sets by a previously described ratio double-averaging algorithm.^{4,18}

Autoradiographic region-of-interest (ROI) analysis was facilitated by superimposing digitized atlas templates derived from the atlas of Zilles⁵⁵ on the mapped individual data sets at each coronal level of interest. This allowed multiple cortical or subcortical ROIs identified on the atlas templates to be outlined by means of a polygon sampling tool, yielding mean and standard deviation (SD) values for each ROI. The coronal levels used in this analysis spanned levels anterior and posterior to the lesion epicenter, which was located at bregma -3.8 mm.¹⁸

Statistical Analysis

Values are reported as the mean $\pm$ SD. Repeated-measures analysis of variance (ANOVA) with post hoc Bonferroni testing was used to assess intergroup LCBF or LCMRglu differences. One-way ANOVAs with post hoc Tukey tests were used to compare structure mean values across study groups and paired t-tests were used for right-left hemisphere comparisons within groups.

TABLE 1

*Physiological variables in 40 rats subjected to FPI or sham FPI and treated with hypothermia or normothermia**

Group/Subgroup	No. of Rats	MABP (mm Hg)	pH	PCO ₂ (mm Hg)	PO ₂ (mm Hg)	Plasma Glucose (mg/dl)
FPI-30°C						
LCMRglu series	5	124 ± 24	7.445 ± 0.024	38.7 ± 1.1	132 ± 10	167 ± 30
LCBF series	6	122 ± 12	7.475 ± 0.043	38.5 ± 1.8	128 ± 20	
sham FPI-30°C						
LCMRglu series	4	131 ± 12	7.476 ± 0.027	37.0 ± 1.8	124 ± 13	181 ± 19
LCBF series	5	122 ± 14	7.458 ± 0.018	38.4 ± 1.6	122 ± 13	
FPI-37°C						
LCMRglu series	7	125 ± 12	7.449 ± 0.020	38.5 ± 1.5	125 ± 8	173 ± 47
LCBF series	5	125 ± 15	7.464 ± 0.022	39.4 ± 1.0	136 ± 17	
sham FPI-37°C						
LCMRglu series	4	119 ± 11	7.444 ± 0.019	38.9 ± 0.3	127 ± 13	174 ± 27
LCBF series	4	121 ± 14	7.483 ± 0.029	38.4 ± 1.6	126 ± 11	

* Values are expressed as the mean ± standard deviation. Abbreviation: MABP = mean arterial blood pressure.

To compare autoradiographic image datasets obtained under differing conditions, we applied the Mann-Whitney U test on a pixel-by-pixel basis. This powerful nonparametric procedure is especially useful in the case of small sample sizes.⁴⁵ The U statistic scores computed from accumulated data ranks for each pixel were converted to probability values, which, in turn, were assigned to the respective image-pixel locations. Coherent foci of pixels for which threshold statistical criteria were exceeded thus denoted regions of statistically significant intergroup difference (see *Results*).

Sources of Supplies and Equipment

The radioactive tracers IAP and 2-DG were purchased from New England Nuclear (Boston, MA). The IAP was administered using an infusion pump obtained from Harvard Apparatus, Inc. (S. Natick, MA). Yellow Springs Instrument Co., Inc. (Yellow Springs, OH) manufactured the glucose analyzer. Brain sections were exposed to

Hyperfilm Beta-max film provided by Eastman Kodak (Rochester, NY); the film was digitized using a charge-coupled device camera, obtained from Xillix Technologies Corp. (Vancouver, British Columbia, Canada), which was interfaced with the MCID M2 image analysis system manufactured by Imaging Research, Inc. (St. Catherine, Ontario, Canada). The Digital Alpha Station (based on the UNIX operating system) was purchased from Digital Equipment Corp. (Maynard, MA) and was used for image processing. The SAS System for Windows, version 6.12 (Cary, NC) was used to perform statistical analyses.

Results

Physiological Variables

The physiological variables are shown in Table 1. The mean arterial blood pressure and blood gas values were in normal ranges in all four animal groups included in this study. Mild hyperglycemia was present to an equal degree in all study groups. There were no significant intergroup differences for any physiological variable.

Local Cerebral Blood Flow

Average autoradiographic data sets for LCBF and LCMRglu were analyzed in detail at six representative forebrain levels (Table 2). Digitized atlas templates^{18,55} were fitted to the autoradiographic data at each level by disparity analysis⁵⁴ and were used as the basis for multiple ROI measurements from the 19 representative anatomical structures listed in Table 2.

Figure 1 presents LCBF data for the 19 anatomical structures of interest (Table 2) in the four animal groups in this series. Fluid-percussion injury, whether followed by normothermia or hypothermia, was associated on average with reduced LCBF compared with the corresponding sham-injury groups (Table 3). Right-hemisphere (ipsilateral to the trauma) LCBF values were lower than left-hemisphere values in both temperature groups (Table 3).

Unexpectedly, when FPI was followed by induced hypothermia (FPI-30°C group), subsequent LCBF was significantly lower than when FPI was followed by normothermia (FPI-37°C group). This was the case for 14 of 19 anatomical structures located in the right hemisphere, and for nine of 19 structures in the left hemisphere (Fig. 1). On average, right-hemisphere LCBF values in the FPI-30°C

TABLE 2

Anatomical structures used for measurements of LCBF and LCMRglu

Structure	Structure No.
bregma -0.8 mm	
paramedian frontal cortex	1
caudoputamen	2
lateral frontal cortex	3
bregma -1.8 mm	
paramedian frontoparietal cortex	4
caudoputamen	5
lateral frontoparietal cortex	6
bregma -3.8 mm	
paramedian parietal cortex	7
dorsolateral parietal cortex	8
hippocampus	9
thalamus	10
bregma -4.8 mm	
paramedian parietal cortex	11
dorsal & ventral hippocampus	12
thalamus	13
bregma -5.8 mm	
paramedian occipital cortex	14
dorsolateral occipital cortex	15
hippocampus	16
thalamus	17
bregma -7.3 mm	
occipital cortex	18
superior colliculus	19

FIG 1. Bar graphs showing a comparison of mean ($\pm$ SD) right- and left-hemisphere LCBF values for 19 hemispheric structures, listed in Table 2, in rats with FPI and sham injury that were exposed to posttraumatic normothermia (37°C) or hypothermia (30°C). *Significant difference between the 37°C and 30°C groups ($p < 0.05$; repeated-measures ANOVA followed by Bonferroni tests).

group were 38% lower than those in the FPI-37°C group and left-hemisphere values were 35% lower (Table 3). Representative average autoradiograms are shown in Fig. 2. The decrease in LCBF observed in FPI-30°C rats was particularly apparent on LCBF difference (FPI-37°C – FPI-30°C) images (Fig. 2C). A statistical analysis of intergroup difference data, performed using the Mann-Whitney U-test, revealed confluent cortical and subcortical zones of significantly reduced LCBF in FPI-30°C rats relative to the FPI-37°C group, particularly in the right hemisphere (ipsilateral to the injury) (Fig. 3).

Rats subjected to sham injury tended to have LCBF values in the normal range (Table 3). Nonetheless, intergroup comparisons of structure mean values by paired t-tests revealed that the sham-FPI-30°C group consistently had somewhat lower mean LCBF values than the sham-FPI-37°C group; this difference was statistically significant for the right hemisphere (ipsilateral to injury-tube placement; see Table 3) and thus may represent a milder manifestation of the same phenomenon noted in the FPI groups, in which hypothermia following perturbation to the brain reduced LCBF. Paired comparisons also revealed systematic average LCBF values that were greater in the right hemisphere than in the left for structures in both the sham-FPI-37°C and sham-FPI-30°C groups (Table 3).

Local Cerebral Glucose Utilization

Local cerebral glucose utilization values in the 19 anatomical structures of interest are plotted in Fig. 4 and the average values across structures are summarized in Table 3. On average, LCMRglu was reduced in both hemispheres of FPI-37°C rats relative to the corresponding sham-injury group; and the right hemisphere (ipsilateral to the earlier trauma) showed lower LCMRglu values than corresponding left-hemisphere structures (Table 3). In contrast with the pronounced influence of postinjury temperature alterations on LCBF, LCMRglu values were largely unaffected by the earlier temperature manipulation in either the FPI or the sham-injury groups (Fig. 4 and Table 3). Figure 5 shows representative average autoradiograms of LCMRglu in the four study groups, together with the respective difference images. In FPI rats the LCMRglu appeared to be mildly and diffusely increased in the FPI-30°C group relative to FPI-37°C animals (FPI-30°C – FPI-37°C difference image, Fig. 5C); however, ANOVA proved this effect to be significant only for two structures in each hemisphere (Fig. 4).

Metabolism/Blood Flow Ratio

In FPI rats, the decrease in LCBF associated with the

TABLE 3
Average values in 19 structures within the rat brain*

Variable	Rt Hemisphere†	Lt Hemisphere†	Rt-Lt Hemisphere Comparison (p value)‡
<i>LCBF (ml/g/min)</i>			
FPI-37°C	1.05 ± 0.24 (a, c)	1.27 ± 0.35 (e)	0.008
FPI-30°C	0.65 ± 0.17 (a, d)	0.83 ± 0.14 (e, f)	0.0000005
sham FPI-37°C	1.47 ± 0.36 (b, c)	1.39 ± 0.28	0.007
sham FPI-30°C	1.24 ± 0.21 (b, d)	1.20 ± 0.21 (f)	0.04
<i>LCMRglu (μmol/100 g/min)</i>			
FPI-37°C	31.3 ± 8.8 (g)	37.2 ± 7.7 (j)	0.0005
FPI-30°C	34.8 ± 7.6 (h)	43.5 ± 6.3	0.000002
sham FPI-37°C	48.6 ± 11.4 (g)	52.6 ± 12.6 (j)	0.0006
sham FPI-30°C	48.7 ± 8.5 (h)	49.6 ± 8.9	NS
<i>LCMRglu/LCBF ratio (μmol/100 ml)</i>			
FPI-37°C	32.6 ± 4.0 (k)	34.0 ± 4.3 (m, n)	NS
FPI-30°C	60.6 ± 8.8 (k, l)	58.6 ± 7.1 (m, o)	NS
sham FPI-37°C	37.5 ± 7.1	41.8 ± 8.4 (n)	0.0002
sham FPI-30°C	43.1 ± 5.8 (l)	46.7 ± 6.4 (o)	0.02

* Values are expressed as the average ± SD. Abbreviation: NS = not significant.

† Each letter (a, b, c, and so forth) designates two data points whose values differed significantly from one another ($p < 0.05$ by ANOVA followed by Tukey's test).

‡ Paired t-test on structure mean values.

antecedent hypothermia, combined with the slight tendency toward higher LCMRglu values in the hypothermic group, resulted in a near doubling of the LCMRglu/LCBF ratio in the FPI-30°C group compared with that in the

FPI-37°C group (Fig. 6 and Table 3). This effect was diffuse and bihemispheric (Fig. 6B). In sham-injured rats, however, temperature manipulation did not affect the LCMRglu/LCBF ratio, which was generally in the normal range in all four groups, albeit with an (unexplained) slight difference in which values on the right side were less than those on the left, possibly explicable on the basis of the LCBF effect in the sham-FPI animals alluded to earlier.

Figure 7 depicts the relationship between LCBF and LCMRglu for the structure mean values of the FPI and sham-injury groups. In rats that had suffered FPI, the confidence limits of the flow-metabolism relationship were entirely distinct for the FPI-37°C and FPI-30°C groups, reflecting the systematically lower LCBF values of the latter group (Fig. 7). By contrast, in the sham-injured animals, the sham-FPI-30°C and sham-FPI-37°C data overlapped completely (Fig. 7).

Discussion

In this study, we have shown in rats that were subjected to moderate parasagittal FPI followed by selective brain cooling (30°C for 3 hours) that LCBF at 6 hours postinjury is significantly reduced compared to animals with post-traumatic normothermia. This hypoperfusion chiefly involves ipsilateral cortical and subcortical sites (Figs. 1–3). By contrast, LCMRglu at this time is similar among rats with FPI, irrespective of the posttraumatic temperature manipulation (Figs. 4 and 5). This hypothermia-induced dissociation of LCBF and LCMRglu results in a near doubling of the LCMRglu/LCBF ratio in FPI rats that receive

FIG. 2. Average autoradiographic LCBF data obtained at the midparietal coronal level (bregma -4.8 mm) in the FPI series (A–C) and the sham-injury series (D–F). A and D: The LCBF in rats with 3 hours of normothermia (37°C, five and four animals, respectively). B and E: The LCBF in rats with 3 hours of hypothermia (30°C, six and five animals, respectively). C and F: The LCBF difference (37°C $-$ 30°C) images. Sham-injured brains without hypothermia (D) and those with hypothermia (E) have similar LCBF values (see difference image in F). The LCBF in brains with postinjury normothermia (A) appears comparable to the sham-injured series (D), whereas postinjury hypothermia leads to depressed LCBF (B), predominantly at cortical subcortical sites ipsilateral to injury (see difference image in C).

hypothermia therapy (Fig. 6 and Table 3). Indeed, the metabolism–blood flow relationship in the two groups is entirely nonoverlapping (Fig. 7).

The present results contrast with our previous data obtained in normothermic rats that were studied within the 1st hour after being subjected to a similar FPI.¹⁸ In that series, a moderate ipsilateral hemispheric hypoperfusion (mean extent 31%) coexisted with a moderately elevated glucose metabolic rate (mean 1.4-fold), resulting in a marked (twofold) ipsilateral metabolism-greater-than-flow dissociation. This early uncoupling was regionally selective, affecting predominantly the ipsilateral hippocampus and frontoparietal neocortex and, to a lesser extent, the contralateral hippocampus. Thus, despite the absence of frank ischemia, these affected regions were nonetheless subjected to an energy demand greatly disproportionate to their level of local perfusion.¹⁸ It is of interest that these sites of early metabolism-greater-than-blood-flow uncoupling corresponded closely to the predominant histopathological sites in this model (see later discussion). Other FPI studies have also included descriptions of CBF declines of 40 to 60% below control levels within 30 to 60 minutes postinjury.^{16,50} Early CBF reductions have also been reported in clinical studies of head injury.²⁹ Mechanisms of hypoperfusion in acute head injury include endothelial lesions, platelet accumulation with microvascular stasis, release of vasoactive substances with arteriolar vasoconstriction, cerebral edema and increased intracranial pressure, and autoregulatory disturbances (see the study by Dietrich, et al.,¹⁶ for a recent review).

Contrasting with our previous findings at 1 hour post-FPI, the present results, obtained 6 hours after FPI, reveal that in animals with posttraumatic normothermia, the earlier hypoperfusion has subsided, whereas in rats with posttraumatic hypothermia the degree of hypoperfusion (hemisphere mean CBF 0.65 ml/g/minute) is in fact worse than at 1 hour posttrauma. At 6 hours post-FPI, LCMRglu ipsilateral to the FPI has fallen to 50 to 55% of its 1-hour value in both the normothermic and hypothermic groups, and to 60 to 65% of its former value in the contralateral hemisphere. The marked metabolism-greater-than-flow dissociation present at 1 hour post-FPI has subsided by 6 hours in rats with posttraumatic normothermia, but is still present to a moderate degree in FPI rats treated with posttraumatic hypothermia (FPI/sham FPI, 1.3 to 1.4-fold, Table 3). These findings thus raise perplexing questions vis-à-vis the putative therapeutic benefit of posttraumatic hypothermia therapy. These issues are explored later in this paper.

Observations from other laboratories support our finding that posttraumatic hypothermia therapy may further decrease CBF. In the study conducted by Koizumi and associates²⁶ rats cooled to a cortical temperature of 32°C before and after sustaining contusion injury had CBF decreases within the contusion area that remained at hypoperfused levels for at least 4 hours, in contrast with the tendency toward CBF normalization in normothermic contused animals. Similar findings have emerged from clinical CBF studies in head-injured patients treated with moderate hypothermia.^{30,31} In these studies, the intravenous ¹³³Xe technique was used to measure CBF sequentially in severely injured patients randomly allocated to

FIG. 3. Statistical map of one minus probability value ($1 - p$) computed on a pixel-by-pixel basis by the Mann-Whitney U-test, comparing average LCBF in animals with brain injury followed by either normothermia (Fig. 2A) or hypothermia (Fig. 2B). The measured bregma level -4.8 mm, corresponding to the LCBF difference image in Fig. 2C. Relative to the normothermic series, postinjury hypothermia results in confluent zones of statistically significant LCBF reduction, mostly ipsilateral to the prior injury.

either normothermic or hypothermic (32–33°C brain temperature for 24 hours) treatment groups. During the cooling period, the CBF fell by approximately 19 to 26% relative to normothermic patients (approximately 5% per 1°C reduction in body temperature). On rewarming the patients, global CBF values increased to levels similar to the normothermic group,^{30,31} although cerebral perfusion pressure in previously hypothermic patients was approximately 13% below the level of the normothermic group ($p < 0.001$).³¹ Intracranial pressure declined with cooling to a greater extent (40%) than could be accounted for by the CBF decline alone. Interestingly, patients with initial Glasgow Coma Scale scores of 5 to 7 treated with hypothermia showed improved outcome at 3 and 6 months (but not at 12 months) relative to normothermic patients.³¹

On initial inspection, the results of our study are perhaps surprising in view of the fact that our previous FPI studies in the same model showed that hypothermia of this degree and duration confers at least partial histological and behavioral protection.^{7,8,15} The predominant histopathological characteristics in this model include focal contusions as well as necrotic neuronal changes at cortical and subcortical sites. In a 3-day survival series, posttraumatic hypothermia reduced the number of cortical necrotic neurons by approximately 50%, lowered contusion volume by approximately 75%, and diminished selective neuronal changes in the hippocampus and thalamus.¹⁵ Posttraumatic hypothermia has also been reported to re-

FIG 4. Bar graphs showing a comparison of the mean ($\pm$ SD) right- and left-hemisphere LCMRglu values for 19 hemispheric structures listed in Table 2, in rats with FPI and sham injury that were exposed to either posttraumatic normothermia (37°C) or hypothermia (30°C). *Significant difference between the 37°C and 30°C groups ($p < 0.05$; repeated-measures ANOVA followed by Bonferroni tests).

duce the number of damaged axons after cortical contusion.³²

The fact that postischemic hypothermia confers histopathological and behavioral protection would imply that the late metabolism-greater-than-flow uncoupling accentuated by hypothermic treatment is not injurious. By contrast, there is strong reason to infer that the early and profound degree of metabolism-greater-than-flow uncoupling arising in the 1st hour postinjury contributes substantively to the injury process.¹⁸ As mentioned earlier, there is a close correspondence between the anatomical sites of early metabolism-flow dissociation and zones of histopathological alterations.^{15,18} Focal elevations of glucose metabolism have been described by others following experimental trauma^{19,24,52} and can be suppressed by cytoprotective strategies such as excitatory amino acid antagonists²⁴ and hippocampal CA1 deafferentation,⁵¹ suggesting that glutamate-activated ion channels and energy-requiring ion-pumping mechanisms may be responsible. Increased glucose utilization occurring in the absence of frank ischemia is also a prominent early feature of severe head trauma in humans.⁵

Abundant evidence exists in the context of focal cerebral ischemia that metabolism-greater-than-flow uncoupling is also an important injury mechanism in the acute ischemic penumbra, where it appears to be mediated by

recurrent ischemic depolarizations that result in inappropriately elevated metabolic activity in the penumbra and a worsening of histopathological injury.^{1-4,21} Similar derangements of ionic homeostasis and episodes of depolarization have also been described in experimental head injury studies^{28,36} and, more recently, in human head trauma.³⁴ By contrast, the present results suggest that the moderate hypoperfusion and a milder degree of metabolism-greater-than-flow dissociation seen at 6 hours post-FPI, following the application of posttraumatic hypothermia, probably does not jeopardize cerebral recovery because hypothermia of this degree and duration is protective (see earlier discussion).

It should be emphasized here that the 2-DG method for quantitating local glucose utilization measures only the rate-limiting step in the glycolytic pathway—the conversion of glucose to glucose-6-phosphate—and, as such, is not able to distinguish aerobic from anaerobic glycolysis.^{46,47} Although one can safely assume that glucose metabolism measured under conditions of significantly reduced oxygen-glucose supply (for example, ischemia) is likely to be predominantly anaerobic,⁴⁹ this cannot necessarily be inferred when the extent of hypoperfusion lies above the frankly ischemic range,⁴ as was the case in rats with FPI followed by postischemic hypothermia in the present study.

FIG 5. Average autoradiographic LCMRglu data obtained at the midparietal coronal level (bregma -4.8 mm) in the FPI series (A–C) and the sham-injury series (D–F). A and D: The LCMRglu in rats with 3 hours of normothermia (37°C , seven and four animals, respectively). B and E: The LCMRglu in rats with 3 hours of hypothermia (30°C , five and four animals, respectively). C and F: The LCMRglu difference ($30^{\circ}\text{C} - 37^{\circ}\text{C}$) images. Glucose utilization appears virtually normal in sham-injured brains without (D) or with (E) prior hypothermia, but it is depressed at cortical and subcortical sites in brains with prior FPI, particularly in the ipsilateral hemisphere (A and B). Postinjury hypothermia (B) tends to improve LCMRglu slightly relative to postinjury normothermia (A) (see difference image [C]).

Although profound systemic hypothermia has long been recognized to decrease CBF, there have been surprisingly few studies on the effects of moderate degrees of selective brain cooling on cerebral circulation when systemic normothermia is maintained. In normal lightly anesthetized rats studied by laser-Doppler flowmetry during selective brain cooling to 31°C , Kuluz, et al.,²⁷ noted increases in cortical CBF that reversed after 30 minutes of subsequent rewarming. By contrast, the present results, taken together with those of our previous study,¹⁸ suggest that the acutely injured brain is incapable of sustained hyperemia in response to hypothermia. Increases in CBF, when they occur following FPI, are often delayed by many hours to days.^{25,33,40}

The factors mediating metabolism–blood flow relationships following traumatic head injury appear to be complex and multifactorial. Alterations in neurotransmitter/neuromodulator synthesis, release, and/or reuptake may be implicated, as well as alterations in endogenous antioxidants, the nitric oxide system,¹⁴ proinflammatory mediators, and other factors (for a comprehensive review, see McIntosh³⁵). Recent studies, for example, have implicated adenosine as one such factor. Brain interstitial adenosine levels increase greatly after FPI.²⁰ Adenosine acts via neuronal A1 receptors to inhibit adenylate cyclase activity and reduce neuronal activity,³⁹ and via A2 receptors on cerebral blood vessels to induce vasodilation.^{17,38} Clark and colleagues¹⁰ have shown, in adult patients with severe closed head injury, that increased adenosine concentrations in the cerebrospinal fluid are correlated with reduced cerebral arteriovenous oxygen content differences, suggesting that adenosine may lead to both cerebral vasodilation and/or a reduction in cerebral metabolism. In that

study, the elevated adenosine concentration in the cerebrospinal fluid was associated with an increased rate of mortality after TBI. This may be understood, perhaps, as an attempted protective mechanism arising in the course of severe injury, which, however, may exacerbate intracranial hypertension by luxury perfusion and increased cerebral blood volume.¹⁰ It would be of interest, in future

FIG 6. Average LCMRglu/LCBF ratios obtained by a double image-division algorithm^{4,18} in rats with FPI (A and B) and those with sham injury (C and D) exposed to antecedent normothermia (A and C) or hypothermia (B and D).

FIG. 7. Scatterplots demonstrating pooled right- and left-sided structure mean values for LCBF plotted against LCMRglu in the FPI (upper) and sham-injury (lower) groups. Data points represent the 19 structure mean values shown in Figs. 1 and 4. Solid circles: rats exposed to normothermia (37°C); open circles: rats exposed to hypothermia (30°C). In rats with FPI, the LCBF values are systematically lower in the FPI-30°C group than in the FPI-37°C group and the 99% confidence intervals of the respective linear-regression plots are completely nonoverlapping. By contrast, in rats with sham injury, the two datasets are intermixed.

studies, to use intracerebral microdialysis to explore the involvement of adenosine and other neuromodulators in posttraumatic hypothermia-induced CBF declines.

Both the present study and our previous investigation of metabolism-blood flow interrelationships in FPI¹⁸ were conducted under similar conditions with induced halothane/nitrous oxide anesthesia, ensuring the comparability of these two studies. Anesthetic agents may exert complex influences on both CBF and metabolism. Halothane is a cerebral vasodilator,⁴⁸ but it tends to decrease LCMRglu compared with the awake state.⁴⁴ In addition, halothane tends to reduce the spread of depression-like depolarizations in the setting of cerebral ischemia^{3,41} and, by extension, might have a similar effect on depolarizations induced by trauma.

In conclusion, we have shown that, despite its proven neuroprotective efficacy, early posttraumatic hypothermia (30°C for 3 hours) is nonetheless followed by an unexpected moderate decline in cerebral perfusion without the (anticipated) improvement in cerebral glucose utilization, so that a state of mild metabolism-greater-than-blood-flow dissociation is perpetuated. Because hypothermia is demonstrably neuroprotective, it is unlikely that this late hypothermia-associated uncoupling is detrimental to recovery. Further studies are needed, however, to explore the full implications of these findings.

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References

1. Alexis NE, Back T, Zhao W, et al: Neurobehavioral consequences of induced spreading depression following photothrombotic middle cerebral artery occlusion. **Brain Res** 706: 273–282, 1996
2. Back T, Ginsberg MD, Dietrich WD, et al: Induction of spreading depression in the ischemic hemisphere following experimental middle cerebral artery occlusion: effect on infarct morphology. **J Cereb Blood Flow Metab** 16:202–213, 1996
3. Back T, Nedergaard M, Ginsberg MD: The ischemic penumbra: pathophysiology and relevance of spreading depression-like phenomena, in Ginsberg MD, Bogousslavsky J (eds): **Cerebrovascular Disease: Pathophysiology, Diagnosis and Management**. Malden, MA: Blackwell Science, 1998, pp 276–286
4. Back T, Zhao W, Ginsberg MD: Three-dimensional image analysis of brain glucose metabolism-blood flow uncoupling and its electrophysiological correlates in the acute ischemic penumbra following middle cerebral artery occlusion. **J Cereb Blood Flow Metab** 15:566–577, 1995
5. Bergsneider M, Hovda DA, Shalmon E, et al: Cerebral hyperglycolysis following severe traumatic brain injury in humans: a positron emission tomography study. **J Neurosurg** 86: 241–251, 1997
6. Bouma GJ, Muizelaar JP, Choi SC, et al: Cerebral circulation and metabolism after severe traumatic brain injury: the elusive role of ischemia. **J Neurosurg** 75:685–693, 1991
7. Bramlett H, Green EJ, Dietrich WD, et al: Posttraumatic brain hypothermia provides protection from sensorimotor and cognitive behavioral deficits. **J Neurotrauma** 12:289–298, 1995
8. Bramlett HM, Dietrich WD, Green EJ, et al: Chronic histopathological consequences of fluid-percussion brain injury in rats: effects of posttraumatic hypothermia. **Acta Neuropathol** 93:190–199, 1997
9. Busto R, Ginsberg MD: The influence of altered brain temperature in cerebral ischemia. In Ginsberg MD, Bogousslavsky J (eds): **Cerebrovascular Disease: Pathophysiology, Diagnosis and Management**. Malden, MA: Blackwell Science, 1998, pp 287–307
10. Clark RSB, Carcillo JA, Kochanek PM, et al: Cerebrospinal fluid adenosine concentration and uncoupling of cerebral blood flow and oxidative metabolism after severe head injury in humans. **Neurosurgery** 41:1284–1293, 1997
11. Clifton GL: Systemic hypothermia in treatment of severe brain injury: a review and update. **J Neurotrauma** 12:923–927, 1995
12. Clifton GL, Jiang JY, Lyeth BG, et al: Marked protection by moderate hypothermia after experimental traumatic brain injury. **J Cereb Blood Flow Metab** 11:114–121, 1991
13. DeWitt DS, Jenkins LW, Wei EP, et al: Effects of fluid-percussion brain injury on regional cerebral blood flow and pial arteriolar diameter. **J Neurosurg** 64:787–794, 1986
14. DeWitt DS, Smith TG, Deyo DJ, et al: L-arginine and superoxide dismutase prevent or reverse cerebral hypoperfusion after fluid-percussion traumatic brain injury. **J Neurotrauma** 14: 223–233, 1997
15. Dietrich WD, Alonso O, Busto R, et al: Post-traumatic brain hypothermia reduces histopathological damage following concussive brain injury in the rat. **Acta Neuropathol** 87:250–258, 1994
16. Dietrich WD, Alonso O, Busto R, et al: Widespread hemodynamic depression and focal platelet accumulation after fluid percussion brain injury: a double-label autoradiographic study in rats. **J Cereb Blood Flow Metab** 16:481–489, 1996
17. Edvinsson L, MacKenzie ET, McCulloch J: **Cerebral Blood**

- Flow and Metabolism.** New York: Raven Press, 1993, pp 325–338
18. Ginsberg MD, Zhao W, Alonso OF, et al: Uncoupling of local cerebral glucose metabolism and blood flow after acute fluid-percussion injury in rats. **Am J Physiol** 272:H2859–H2868, 1997
19. Hayes RL, Katayama Y, Jenkins LW, et al: Regional rates of glucose utilization in the cat following concussive head injury. **J Neurotrauma** 5:121–137, 1988
20. Headrick JP, Bendall R, Faden AI, et al: Dissociation of adenosine levels from bioenergetic state in experimental brain trauma: potential role in secondary injury. **J Cereb Blood Flow Metab** 14:853–861, 1994
21. Hossmann KA: Perinfarct depolarizations. **Cerebrovasc Br Metab Rev** 8:195–208, 1996
22. Hossmann KA: Viability thresholds and the penumbra of focal ischemia. **Ann Neurol** 36:557–565, 1994
23. Jaggi JL, Obrist WD, Gennarelli TA, et al: Relationship of early cerebral blood flow and metabolism to outcome in acute head injury. **J Neurosurg** 72:176–182, 1990
24. Kawamata T, Katayama Y, Hovda DA, et al: Administration of excitatory amino acid antagonists via microdialysis attenuates the increase in glucose utilization seen following concussive brain injury. **J Cereb Blood Flow Metab** 12:12–24, 1992
25. Kelly DF, Martin NA, Kordestani R, et al: Cerebral blood flow as a predictor of outcome following traumatic brain injury. **J Neurosurg** 86:633–641, 1997
26. Koizumi H, Fujisawa H, Ito H, et al: Effect of mild hypothermia on cerebral blood flow-independent changes in cortical extracellular levels of amino acids following contusion trauma in the rat. **Brain Res** 747:304–312, 1997
27. Kuluz JW, Prado R, Chang J, et al: Selective brain cooling increases cortical cerebral blood flow in rats. **Am J Physiol** 265:H824–H827, 1993
28. Lee SM, Hovda DA, Becker DP: Core of degenerating neurons in the ipsilateral parietal cortex induces spreading depression following a lateral fluid percussion brain injury. **Soc Neurosci** 20 (Part 1):846, 1994 (Abstract)
29. Marion DW, Darby J, Yonas H: Acute regional cerebral blood flow changes caused by severe head injuries. **J Neurosurg** 74:407–414, 1991
30. Marion DW, Obrist WD, Carlier PM, et al: The use of moderate therapeutic hypothermia for patients with severe head injuries: a preliminary report. **J Neurosurg** 79:354–362, 1993
31. Marion DW, Penrod LE, Kelsey SF, et al: Treatment of traumatic brain injury with moderate hypothermia. **N Engl J Med** 336:540–546, 1997
32. Marion DW, White MJ: Treatment of experimental brain injury with moderate hypothermia and 21-aminosteroids. **J Neurotrauma** 13:139–147, 1996
33. Martin NA, Patwardhan RV, Alexander MJ, et al: Characterization of cerebral hemodynamic phases following severe head trauma: hypoperfusion, hyperemia, and vasospasm. **J Neurosurg** 87:9–19, 1997
34. Mayevsky A, Doron A, Manor T, et al: Repetitive cortical spreading depression cycle development in the human brain: a multiparametric monitoring approach. **J Cereb Blood Flow Metab** 15 (Suppl 1):S34, 1995 (Abstract)
35. McIntosh TK: Neurochemical sequelae of traumatic brain injury: therapeutic implications. **Cerebrovasc Br Metab Rev** 6:109–162, 1994 (Abstract)
36. Nilsson P, Hillered L, Olsson Y, et al: Regional changes in interstitial K⁺ and Ca²⁺ levels following cortical compression contusion trauma in rats. **J Cereb Blood Flow Metab** 13:183–192, 1993
37. Obrist ED, Langfitt TW, Jaggi JL, et al: Cerebral blood flow and metabolism in comatose patients with acute head injury. Relationship to intracranial hypertension. **J Neurosurg** 61:241–253, 1984
38. Phillis JW: Adenosine in the control of the cerebral circulation. **Cerebrovasc Brain Metab Rev** 1:26–54, 1989
39. Phillis JW, Kostopoulos GK, Limacher JJ: A potent depressant action of adenosine derivatives on cerebral cortical neurones. **Eur J Pharmacol** 30:125–129, 1975
40. Robertson CS, Contant CF, Narayan RK, et al: Cerebral blood flow, AVO₂, and neurologic outcome in head-injured patients. **J Neurotrauma** 9 (Suppl 1):S349–S358, 1992
41. Saito R, Graf R, Hübel K, et al: Reduction of infarct volume by halothane: effect on cerebral blood flow or perifocal spreading depression-like depolarizations. **J Cereb Blood Flow Metab** 17:857–864, 1997
42. Sakurada O, Kennedy C, Jehle J, et al: Measurement of local cerebral blood flow with iodo(¹⁴C)antipyrine. **Am J Physiol** 234:H59–H66, 1978
43. Savaki HE, Davidsen L, Smith C, et al: Measurement of free glucose turnover in brain. **J Neurochem** 35:495–502, 1980
44. Shapiro HM, Greenberg JH, Reivich M, et al: Local cerebral glucose uptake in awake and halothane-anesthetized primates. **Anesthesiology** 48:97–103, 1978
45. Siegel S: **Nonparametric Statistics for the Behavioral Sciences.** New York: McGraw-Hill, 1956
46. Sokoloff L: The radioactive deoxyglucose method. Theory, procedure, and applications for the measurement of local glucose utilization in the central nervous system, in Agranoff BW, Aprison MH (eds): **Advances in Neurochemistry.** New York: Plenum Press, 1982, pp 1–82
47. Sokoloff L, Reivich M, Kennedy C, et al: The (¹⁴C)deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. **J Neurochem** 28:897–916, 1977
48. Todd MM, Drummond JC: A comparison of the cerebrovascular and metabolic effects of halothane and isoflurane in the cat. **Anesthesiology** 60:276–282, 1984
49. Welsh FA, Greenberg JH, Jones SC, et al: Correlation between glucose utilization and metabolite levels during focal ischemia in cat brain. **Stroke** 11:79–84, 1980
50. Yamakami I, McIntosh TK: Alterations in regional cerebral blood flow following brain injury in the rat. **J Cereb Blood Flow Metab** 11:655–660, 1991
51. Yoshino A, Hovda DA, Katayama Y, et al: Hippocampal CA3 lesion prevents postconcussive metabolic dysfunction in CA1. **J Cereb Blood Flow Metab** 12:996–1006, 1992
52. Yoshino A, Hovda DA, Kawamata T, et al: Dynamic changes in local cerebral glucose utilization following cerebral concussion in rats: evidence of a hyper- and subsequent hypometabolic state. **Brain Res** 561:106–119, 1991
53. Zhao W, Ginsberg MD, Smith DW: Three-dimensional quantitative autoradiography by disparity analysis: theory and application to image averaging of local cerebral glucose utilization. **J Cereb Blood Flow Metab** 15:552–565, 1995
54. Zhao W, Young TY, Ginsberg MD: Registration and three-dimensional reconstruction of autoradiographic images by the disparity analysis method. **IEEE Trans Med Imag** 12:782–791, 1993
55. Zilles KJ: **The Cortex of the Rat.** New York: Springer-Verlag, 1985

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