Changes in Local Cerebral Blood Flow, Glucose Utilization, and Mitochondrial Function Following Traumatic Brain Injury in Rats

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Abstract

The pathophysiology of secondary brain damage following experimental traumatic brain injury was investigated by measuring local cerebral blood flow (ICBF), local cerebral glucose utilization (ICGU), and activity of succinate dehydrogenase (SDH), which is a mitochondrial enzyme of the tricarboxylic acid cycle, in the rat brain after moderate lateral fluid percussion injury. Measurements used autoradiography for ICBF and ICGU with [^{14}C]iodoantipyrine and [^{14}C]2-deoxyglucose, respectively. Regional SDH activity was determined using quantitative imaging of formazan produced from 2,3,5-triphenyl tetrazolium chloride by SDH. ICBF decreased at 1 hour after injury and was significantly lower than the preinjury level in almost all regions of both hemispheres at 6 and 24 hours, and remained low at 2 weeks. ICGU increased 1 hour after injury but was significantly decreased at 6 and 24 hours, and at 2 weeks in most regions of both hemispheres. The ipsilateral hemisphere showed a significant decrease in the activity of SDH in the cortices, hippocampus, thalamus, and caudate/putamen, most conspicuously 72 hours after injury, whereas no significant decrease was observed in the contralateral hemisphere at any time. Necrosis in the injured cortex and reduction of the number of neurons in the ipsilateral hippocampus were observed 2 weeks after injury. The present study showed that a decrease in ICBF and mitochondrial dysfunction occur with glucose hypermetabolism around 1 hour after lateral fluid percussion injury, and that ICBF, ICGU, and mitochondrial function all deteriorate after 6 hours. This suggests that ICBF and cellular metabolism may change dynamically during the several hours following traumatic brain injury, and afterwards neuronal damage may result in an irreversible change in the areas with depressed glucose hypermetabolism in the early period after injury in combination with mitochondrial dysfunction.

Key words: brain injury, cerebral blood flow, cerebral glucose utilization, lateral fluid percussion injury, mitochondria, succinate dehydrogenase

Introduction

Treatment for traumatic brain injury is mainly intended to prevent secondary brain damage and improve neuronal function. Better understanding of the underlying mechanisms of secondary damage will help develop more effective treatment and improve patient outcome. Clinical and experimental investigations have suggested that decreased local cerebral blood flow (ICBF) may be a major contributor to secondary neuronal damage after traumatic brain injury.^{2,5,6,31)} Various other contributing factors

have been proposed for secondary brain damage, including altered cerebral glucose metabolism,^{13,32)} release of neurotoxic excitatory amino acids with intracellular calcium accumulation,^{13,14)} oxygen free radicals,²⁶⁾ or other endogenous mediators, and delayed inflammatory response.¹⁸⁾ Furthermore, imbalance between ICBF and local cerebral glucose utilization (ICGU) in the injured brain may be a major determinant of outcome in humans as well as in animal experiments.^{1,7,15)} Previously, a rat model of acute subdural hematoma showed that decreased ICBF accompanied by hyperglycolysis occurring after lesion formation may contribute to neurotoxicity.¹⁵⁾ This phenomenon occurs only during the early period after brain injury,^{7,15)} but little is known about

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the mechanism of neuronal damage.

Mitochondrial dysfunction has been implicated as the cause of altered posttraumatic energy metabolism, which leads to mismatches of CBF and oxygen consumption and lactate production.^{3,28} Ischemic neuronal cell death may also be caused by mitochondrial membrane damage due to excessive Ca^{2+} adsorption into the mitochondria. Recently, 2,3,5-triphenyl tetrazolium chloride (TTC) staining has been used for measurement of the activity of succinate dehydrogenase (SDH), which is a mitochondrial enzyme of the tricarboxylic acid cycle for aerobic adenosine triphosphate (ATP) production, in models of focal cerebral ischemia¹⁶ and in rats subjected to lateral fluid percussion brain injury.^{20,28}

The rat fluid percussion injury model produces many features of head injury similar to the biochemical, physiological, pathological responses in humans.²² Our previous study characterized the location and extent of pathological changes shown by magnetic resonance (MR) imaging in vivo following moderate lateral fluid percussion injury in rats.²¹

The present study examined regional differences and chronological changes in mitochondrial function after moderate lateral fluid percussion injury in rats, to clarify the significance of circulatory disturbance and metabolic dysfunction in traumatic brain injury by comparing changes in SDH activity with changes in ICBF and ICGU.

Materials and Methods

I. Animal preparation

Male Wistar rats, weighing from 300 to 450 g, were anesthetized with ketamine hydrochloride (50 mg/kg, i.m.). During the surgical preparation and throughout the experiments, all wounds were infiltrated with 2.0% lidocaine hydrochloride. All experiments were performed according to the institutional guidelines for animal experiments. The right femoral artery and vein were cannulated with PE50 polyethylene tubing for arterial blood sampling and for continuous monitoring of arterial blood pressure in groups for measurement of ICBF and ICGU. Arterial blood gases were analyzed 5 minutes before and 5 minutes after injury using a blood gas analyzer (Instrumentation Lab., Lexington, Mass., U.S.A.). Each rat was fixed in a stereotactic frame, and a 4-mm-diameter burr hole was drilled over the right parietal cortex (3.5 mm posterior to the bregma and 3 mm lateral to the midline). A hard plastic tube connected to a reservoir was placed on the burr hole and fixed with Aron-alpha cement (Konishi Co. Ltd.,

Osaka). Rats were allowed to breathe spontaneously throughout all surgical procedures.

II. Fluid percussion injury

Our fluid percussion injury device consists of a cylindrical reservoir with a pressure transducer connected to a personal computer. The reservoir is fitted with a 10-cm-long hard plastic tube that is placed over the exposed dura overlying the parietal lobe. For these experiments, the entire system was filled with sterile isotonic saline at 37°C. Injuries were induced by dropping a metal pendulum from predetermined heights to strike the piston of the device, producing extracranial pressure pulses expressed in atmospheres (atm). During this procedure, body temperature was maintained between 36°C and 37°C with a heat lamp. This fluid percussion injury device was devised in our laboratory and was previously described.²²

III. Measurement of ICBF using autoradiography

Autoradiography was performed in five sham rats and in a total of 16 rats at 1 hour (5 rats), 6 hours (4 rats), 24 hours (4 rats), and 2 weeks (3 rats) after injury to measure ICBF according to the method of Sakurada et al.²³ ICBF was measured using [¹⁴C]iodoantipyrine (IAP) as a freely diffusible indicator. IAP in ethanol (DuPont/NEN, Boston, Mass., U.S.A.; 54.8 mCi/mmol) was dissolved in 1 ml of saline following elimination of ethanol. Prior to infusion of the radioisotope, arterial blood was sampled for blood gas analysis and mean blood pressure was measured. To induce similar conditions in each group, ketamine hydrochloride (50 mg/kg) was injected intramuscularly 2 hours before the measurement of ICBF in all groups. After intravenous infusion of isotonic saline containing 100 $\mu\text{Ci/kg}$ of [¹⁴C]IAP at a constant rate for 60 seconds through the femoral vein by an infusion pump, 20 μl samples of arterial blood were collected into heparinized tubes every 4 to 6 seconds from the femoral artery, after which 10 μl aliquots were transferred to scintillation vials to obtain the arterial concentration time curve of IAP. Ten ml of scintillation fluid was added to collected blood in scintillation vials. Radioactivity was measured by a liquid scintillation spectrometer and converted to disintegrations per minute. Animals were decapitated immediately after collecting the last blood sample. The skull was opened and the brain was rapidly removed and frozen at -80°C until sectioning. The frozen brains were cut into 20 μm coronal sections in a cryostat which was maintained at -22°C. Selected sections were mounted on glass coverslips, dried on a hot plate at 60°C, and autoradiographed with a set of

calibrated [^{14}C]methylmethacrylate plastic standards. The quantitative densitometric analysis was carried out with a computerized imaging-processing system (BAS 2000; Fujitsu, Tokyo). ICBF was calculated for the frontal, parietal, temporal, and occipital cortices, CA1 and CA2-3 sectors of the hippocampus, thalamus, corpus callosum, caudate/putamen, and cerebellar cortex using a National Institutes of Health (NIH) imaging analyzer.

IV. Measurement of ICGU using autoradiography

Autoradiography was performed in five sham rats and in a total of 16 rats at 1 hour (5 rats), 6 hours (4 rats), 24 hours (4 rats), and 2 weeks (3 rats) after injury to measure ICGU according to the method of Sokoloff et al.²⁷⁾ Most experimental procedures were the same as those for ICBF measurement. After arterial blood samples for blood gas analysis and plasma glucose measurement were collected, 100 $\mu\text{Ci/kg}$ of [^{14}C]2-deoxyglucose ([^{14}C]DG) (Amersham International, Little-Chalfont, Bucks., U.K.; 57.2 mCi/mmol) was intravenously injected. Timed arterial plasma samples were withdrawn during the following 45-minute period and immediately centrifuged to assess the time course of [^{14}C]DG concentration using a liquid scintillation spectrometer. Forty-five minutes after the [^{14}C]DG injection, the last blood sample was collected and the animals were decapitated. Autoradiograms were made in the same way as for measurement of ICBF. ICGU was calculated for the same regions as those of ICBF using an NIH imaging analyzer and the equations and constants determined by Sokoloff et al.²⁷⁾

V. Quantitative imaging of SDH activity

Animals subjected to injury were sacrificed by guillotine under anesthesia with ketamine hydrochloride 1 hour, and 6, 24, 48, and 72 hours, and 2 weeks ($n = 5$ each) after injury. Five sham control animals were also sacrificed for measurement of SDH activity. The brains were immediately removed and placed in a moist chamber maintained at 0°C , and four coronal sections (0, 3.5, 5.5, and 10 mm posterior to the bregma) corresponding to the levels of the frontal lobe, hippocampus, occipital lobe, and cerebellum, respectively, were prepared for quantitative imaging of SDH activity. Brain slices were incubated in 1% TTC in Dulbecco's tissue culture medium at 35°C for 6 minutes for quantitative imaging of SDH. The solution was circulated in a chamber with a water pump for homogeneous staining. Local changes in optical density of the coronal plane as a result of the accumulation of formazan produced from TTC by SDH were recorded using color standards at 1, 3,

and 6 minutes of immersion, using a CCD camera (XC-75/CE; SONY, Tokyo). This method of measurement was described elsewhere.¹⁶⁾ The local SDH activity was calculated from the initial rate of formazan production, expressed as a percentage of the SDH activity of cerebellum, and displayed using semicolor coding (NIH Image 1.57). Local SDH was measured for frontal, parietal, temporal, and occipital cortices, CA1 and CA2-3 sectors of the hippocampus, thalamus, corpus callosum, caudate/putamen, and cerebellum. Soon after measurement of SDH activity, brain slices were fixed with 10% buffered neutral formalin and prepared for histological examination.

VI. Histological evaluations

Brain slices used for measurement of SDH activity were prepared for microscopic examination by HE staining. The total number of apparently normal and abnormal neurons (dark, shrunken, and elongated, or light and swollen) in the hippocampal pyramidal layers in the rats sacrificed 14 days after injury and sham-operated rats was counted in a given region at a magnification of $\times 200$ in 10- μm -thick coronal sections in the CA1 and the transitional portion of CA2-3. Results were expressed as a percentage of the number of neurons on the contralateral side.

VII. Statistical analysis

All results are presented as mean $\pm$ SD. Physiological variables, reduction of the number of neuronal cells in the hippocampus, and regional differences in the changes of ICBF, ICGU, and SDH activity between sham and injury groups were compared using analysis of variance. P values less than 0.05 were considered statistically significant.

Results

I. Physiological variables, arterial blood glucose, and impact pressure

Physiological variables (mean $\pm$ SD) prior to infusion of radioisotope in groups for measurement of ICBF and ICGU are shown in Tables 1 and 2, respectively. There was no significant difference in the physiological variables or arterial blood glucose between the groups 5 minutes before the experiments. Rats showed stable blood pressure during the surgical procedures under anesthesia with ketamine hydrochloride and local infiltration with 2.0% lidocaine hydrochloride.

The impact pressure for the measurement of ICBF was 2.6 ± 0.3 atm for the 1 hour group ($n = 5$), 2.7 ± 0.2 atm for the 6 hours group ($n = 4$), 2.7 ± 0.5 atm for the 24 hours group ($n = 4$), and 2.7 ± 0.1

Table 1 Physiological values in experimental groups of rats for measurement of local cerebral blood flow

Variable	Sham (n = 5)	Time after injury			
		1 hr (n = 5)	6 hrs (n = 4)	24 hrs (n = 4)	2 wks (n = 3)
MABP (mmHg)	125 ± 6	120 ± 12	119 ± 9	122 ± 13	120 ± 8
PaO ₂ (mmHg)	108 ± 6	108 ± 9	109 ± 13	107 ± 9	111 ± 8
PaCO ₂ (mmHg)	40 ± 2	40 ± 3	40 ± 3	40 ± 2	39 ± 2
pH	7.39 ± 0.03	7.37 ± 0.02	7.38 ± 0.03	7.37 ± 0.01	7.38 ± 0.01

Values are mean ± SD. No significant difference in physiological variables between the groups ($p > 0.05$). MABP: mean arterial blood pressure, PaCO₂: arterial carbon dioxide tension, PaO₂: arterial oxygen tension.

Table 2 Physiological values in experimental groups of rats for measurement of local cerebral glucose utilization

Variable	Sham (n = 5)	Time after injury			
		1 hr (n = 5)	6 hrs (n = 4)	24 hrs (n = 4)	2 wks (n = 3)
MABP (mmHg)	118 ± 6	120 ± 11	125 ± 11	122 ± 13	124 ± 6
PaO ₂ (mmHg)	109 ± 14	103 ± 14	92 ± 11	98 ± 16	101 ± 6
PaCO ₂ (mmHg)	39 ± 3	41 ± 2	35 ± 3	38 ± 2	39 ± 2
pH	7.39 ± 0.03	7.38 ± 0.02	7.40 ± 0.03	7.38 ± 0.03	7.38 ± 0.01
Glucose (mg/dl)	124 ± 14	136 ± 24	132 ± 13	123 ± 13	125 ± 10

Values are mean ± SD. No significant difference in physiological variables between the groups ($p > 0.05$). MABP: mean arterial blood pressure, PaCO₂: arterial carbon dioxide tension, PaO₂: arterial oxygen tension.

Fig. 1 Changes in local cerebral blood flow (ICBF, closed and open circles), glucose metabolism (ICGU, closed and open triangles), and regional succinate dehydrogenase (SDH) activity (closed and open squares) in the parietal cortices ipsilateral (left) and contralateral (right) to the injury. These values are expressed as a percentage of sham control values. Symbols beside each value indicate significant decrease (asterisk) and increase (sharp) in ICBF, ICGU, and SDH, compared to sham values.

Table 3 Local cerebral blood flow in experimental groups following injury

Region	Sham	Time after injury			
		1 hr	6 hrs	24 hrs	2 wks
Ipsilateral hemisphere:					
Frontal cortex	114 ± 6	71 ± 7 ^{*c}	64 ± 6 ^{*c}	66 ± 4 ^{*c}	70 ± 5 ^{*c}
Parietal cortex	117 ± 4	48 ± 7 ^{*c}	29 ± 5 ^{*c}	37 ± 8 ^{*c}	18 ± 6 ^{*c}
Temporal cortex	116 ± 4	92 ± 28	77 ± 2 ^{*c}	91 ± 6 ^{*c}	76 ± 6 ^{*c}
Occipital cortex	111 ± 6	97 ± 20	74 ± 3 ^{*c}	78 ± 3 ^{*c}	75 ± 4 ^{*c}
Hippocampus					
CA1	106 ± 8	71 ± 4 ^{*c}	62 ± 2 ^{*c}	67 ± 5 ^{*c}	81 ± 3 ^{*b}
CA2-3	84 ± 3	72 ± 3 ^{*c}	58 ± 5 ^{*c}	69 ± 6 ^{*c}	62 ± 2 ^{*c}
Thalamus	113 ± 5	80 ± 10 ^{*c}	73 ± 1 ^{*c}	78 ± 4 ^{*c}	84 ± 3 ^{*c}
Corpus callosum	74 ± 4	48 ± 12 ^{*b}	54 ± 8 ^{*b}	58 ± 6 ^{*b}	65 ± 2 ^{*b}
Caudate/putamen	96 ± 9	82 ± 15	69 ± 2 ^{*c}	78 ± 4 ^{*b}	85 ± 4
Cerebellum	84 ± 7	78 ± 9	66 ± 1 ^{*c}	78 ± 4	78 ± 3
Contralateral hemisphere:					
Frontal cortex	113 ± 7	99 ± 22	76 ± 4 ^{*c}	72 ± 2 ^{*c}	74 ± 3 ^{*c}
Parietal cortex	117 ± 4	98 ± 25	67 ± 3 ^{*c}	74 ± 4 ^{*c}	77 ± 3 ^{*c}
Temporal cortex	116 ± 7	94 ± 27	78 ± 2 ^{*c}	90 ± 3 ^{*c}	70 ± 6 ^{*c}
Occipital cortex	109 ± 7	97 ± 23	75 ± 4 ^{*c}	76 ± 4 ^{*c}	75 ± 4 ^{*c}
Hippocampus					
CA1	107 ± 9	80 ± 10 ^{*b}	67 ± 2 ^{*c}	72 ± 5 ^{*c}	83 ± 3 ^{*b}
CA2-3	85 ± 2	71 ± 6 ^{*b}	57 ± 3 ^{*c}	68 ± 5 ^{*c}	63 ± 3 ^{*c}
Thalamus	115 ± 7	84 ± 11 ^{*b}	75 ± 4 ^{*c}	72 ± 3 ^{*c}	84 ± 4 ^{*c}
Corpus callosum	78 ± 7	47 ± 13 ^{*b}	55 ± 7 ^{*b}	61 ± 5 ^{*b}	64 ± 2 ^{*a}
Caudate/putamen	93 ± 7	81 ± 14	71 ± 3 ^{*c}	76 ± 5 ^{*b}	86 ± 3
Cerebellum	81 ± 4	78 ± 7	69 ± 3 ^{*b}	81 ± 3	78 ± 2

Values are mean ± SD (ml·100 g⁻¹·min⁻¹). ^{*}Significantly lower than values of sham group (^ap < 0.05, ^bp < 0.01, ^cp < 0.001).

atm for the 2 weeks group (n = 3), and was not significantly different between the experimental groups. The impact pressure for the measurement of ICGU was 2.7 ± 0.6 atm for the 1 hour group (n = 5), 2.6 ± 0.4 atm for the 6 hours group (n = 4), 2.6 ± 0.5 atm for the 24 hours group (n = 4), and 2.6 ± 0.2 atm for the 2 weeks group (n = 3), and was not significantly different between these experimental groups. The impact pressure for the measurement of SDH was 2.6 ± 0.7 atm for the 1 hour group (n = 5), 2.5 ± 0.2 atm for the 6 hours group (n = 5), 2.5 ± 0.9 atm for the 24 hours group (n = 5), 2.7 ± 0.7 atm for the 48 hours group (n = 5), 2.4 ± 0.4 atm for the 72 hours group (n = 5), and 2.7 ± 0.5 atm for the 2 weeks group (n = 5), and was not significantly different between the experimental groups. There was also no significant difference in the impact pressure between the experimental groups for the measurement of ICBF, ICGU, and SDH.

II. ICBF after fluid percussion injury

There was no hemispheric asymmetry of ICBF in the sham group following surgery (Table 3). In both hemispheres, CBF decreased in all regions 1 hour,

and 6 and 24 hours, and 2 weeks after injury. One hour after injury, CBF significantly decreased in the frontal and parietal cortices, hippocampus, thalamus, and corpus callosum compared to the corresponding areas of the sham group on the ipsilateral side, and also significantly decreased in the hippocampus (CA1 and CA2-3), thalamus, and corpus callosum on the contralateral side. There was no significant decrease of CBF in the temporal and occipital cortices, and in the caudate/putamen and cerebellum on both sides. Six hours after injury, CBF was lowest in most regions and ranged from 25% to 79% of the baseline values on the ipsilateral side. Twenty-four hours after injury, CBF was also significantly lowered in all regions except for the cerebellum. Two weeks after injury, CBF remained lower in these regions except for the caudate/putamen and cerebellum on both sides. In the parietal cortex near the injured site, CBF was significantly lower than that of the contralateral side at all times. Figure 1 shows changes in CBF values in the bilateral parietal cortices. There was a significant difference in CBF of the frontal cortex between the hemispheres at 1 hour, and 6 and 24 hours after in-

Table 4 Local cerebral glucose utilization in experimental groups following injury

Region	Sham	Time after injury			
		1 hr	6 hrs	24 hrs	2 wks
Ipsilateral hemisphere:					
Frontal cortex	94 ± 7	98 ± 14	70 ± 6 ^{*c}	69 ± 2 ^{*c}	61 ± 11 ^{*c}
Parietal cortex	96 ± 3	65 ± 19 ^{*b}	33 ± 5 ^{*c}	34 ± 8 ^{*c}	21 ± 4 ^{*c}
Temporal cortex	98 ± 3	104 ± 10	80 ± 3 ^{*c}	83 ± 2 ^{*c}	85 ± 2 ^{*c}
Occipital cortex	96 ± 3	115 ± 13 ^{#b}	79 ± 3 ^{*c}	78 ± 3 ^{*c}	77 ± 1 ^{*c}
Hippocampus					
CA1	85 ± 3	150 ± 13 ^{#c}	58 ± 3 ^{*c}	61 ± 3 ^{*c}	67 ± 3 ^{*c}
CA2-3	74 ± 3	86 ± 5 ^{#b}	50 ± 6 ^{*c}	56 ± 4 ^{*c}	60 ± 1 ^{*c}
Thalamus	91 ± 4	175 ± 11 ^{#c}	70 ± 2 ^{*c}	70 ± 5 ^{*c}	81 ± 1 ^{*b}
Corpus callosum	62 ± 4	73 ± 14	57 ± 3 ^{*a}	55 ± 4 ^{*a}	62 ± 5
Caudate/putamen	91 ± 3	141 ± 20 ^{#c}	65 ± 3 ^{*c}	65 ± 4 ^{*c}	75 ± 6 ^{*c}
Cerebellum	79 ± 6	88 ± 13	73 ± 3	74 ± 2	74 ± 2
Contralateral hemisphere:					
Frontal cortex	94 ± 7	159 ± 23 ^{#c}	79 ± 4 ^{*b}	79 ± 3 ^{*b}	80 ± 3 ^{*a}
Parietal cortex	94 ± 4	178 ± 15 ^{#c}	78 ± 2 ^{*c}	83 ± 2 ^{*c}	87 ± 3 ^{*a}
Temporal cortex	96 ± 5	181 ± 16 ^{#c}	76 ± 2 ^{*c}	82 ± 4 ^{*b}	77 ± 1 ^{*c}
Occipital cortex	94 ± 5	159 ± 22 ^{#c}	81 ± 2 ^{*b}	77 ± 3 ^{*c}	77 ± 2 ^{*c}
Hippocampus					
CA1	88 ± 5	176 ± 15 ^{#c}	50 ± 6 ^{*c}	57 ± 5 ^{*c}	65 ± 2 ^{*c}
CA2-3	75 ± 4	91 ± 11 ^{#a}	51 ± 4 ^{*c}	53 ± 2 ^{*c}	61 ± 4 ^{*b}
Thalamus	89 ± 5	172 ± 23 ^{#c}	70 ± 4 ^{*c}	72 ± 5 ^{*c}	78 ± 2 ^{*a}
Corpus callosum	64 ± 5	75 ± 12	63 ± 3	60 ± 5	62 ± 5
Caudate/putamen	83 ± 10	134 ± 15 ^{#c}	67 ± 3 ^{*a}	67 ± 2 ^{*a}	77 ± 4
Cerebellum	81 ± 8	87 ± 9	73 ± 3	73 ± 3	76 ± 2

Values are mean ± SD ($\mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$). [#]Significantly higher than values of sham group (^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$). ^{*}Significantly lower than values of sham group (^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$).

jury. In the temporal and occipital cortices, there was no significant difference between the hemispheres at any time.

III. ICGU after fluid percussion injury

There was no hemispheric asymmetry of ICGU in the sham group following surgery (Table 4). The values were lower than those in the conscious rat but were higher than those in the anesthetized rat measured by Sokoloff et al.²⁷⁾ ICGU increased significantly in most regions of both hemispheres 1 hour after injury, but decreased at 6 and 24 hours, and 2 weeks. However, ICGU of the ipsilateral frontal and temporal cortices did not increase significantly compared to sham values at 1 hour, and that of the parietal cortex near the injured site significantly decreased, whereas ICGU of the contralateral side increased markedly by 70% to 89% of the sham values ($p < 0.05$). In the CA1 of the hippocampus and thalamus, ICGU of the ipsilateral side increased markedly at 1 hour ($p < 0.05$) and afterwards decreased. An increase in ICGU of the CA2-3 was also significant, but only slight compared with that of CA1 (Fig. 2). At 6 and 24 hours after injury,

the ICGU of both hemispheres was significantly lower than that of the sham control except for the corpus callosum and cerebellum.

IV. Changes in local SDH activity after injury

Table 5 shows relative values for the activity of SDH in the sham and injured groups. SDH activity on the side ipsilateral to injury decreased in the frontal, parietal, and temporal cortices at 1 hour, and 6, 24, 48, and 72 hours following injury and was lowest at 72 hours (Fig. 3). SDH activity of the CA2-3 sectors in the hippocampus decreased with time after injury, and differed significantly from that in the sham group at 1 hour, and 48 and 72 hours (Fig. 2). SDH activity of the occipital cortex, CA1 of the hippocampus, thalamus, and caudate/putamen was significantly different from that in the sham group only 72 hours after injury. However, SDH activity had returned except for that of the parietal cortex near the injured site 2 weeks after injury. There was no significant decrease of SDH activity in all regions on the contralateral side at any time. Changes in SDH activity of the ipsilateral and contralateral parietal cortices and CA2-3 of the

Fig. 2 Changes in local cerebral blood flow (ICBF, closed and open circles), glucose metabolism (ICGU, closed and open triangles), and regional succinate dehydrogenase (SDH) activity (closed and open squares) in the CA2-3 of the hippocampi ipsilateral (left) and contralateral (right) to the injury. These values are expressed as a percentage of sham control values. Symbols beside each value indicate significant decrease (asterisk) and increase (sharp) in ICBF, ICGU, and SDH, compared to sham values.

Table 5 Local succinate dehydrogenase (SDH) activity in experimental groups following injury

Region	Sham	Time after injury					
		1 hr	6 hrs	24 hrs	48 hrs	72 hrs	2 wks
Ipsilateral hemisphere:							
Frontal cortex	107 ± 3	83 ± 7 ^{*c}	87 ± 12 ^{*b}	83 ± 16 ^{*a}	88 ± 9 ^{*b}	69 ± 11 ^{*c}	100 ± 16
Parietal cortex	99 ± 7	70 ± 5 ^{*c}	77 ± 8 ^{*a}	59 ± 17 ^{*a}	66 ± 10 ^{*c}	59 ± 13 ^{*c}	62 ± 9 ^{*c}
Temporal cortex	100 ± 9	83 ± 7 ^{*a}	80 ± 7 ^{*b}	77 ± 23	79 ± 8 ^{*b}	63 ± 9 ^{*c}	100 ± 7
Occipital cortex	91 ± 11	91 ± 10	90 ± 13	80 ± 13	89 ± 10	70 ± 10 ^{*a}	94 ± 14
Hippocampus							
CA1	134 ± 22	118 ± 17	119 ± 22	110 ± 15	115 ± 9	98 ± 16 ^{*a}	138 ± 15
CA2-3	142 ± 10	126 ± 12 ^{*a}	130 ± 13	126 ± 23	121 ± 12 ^{*a}	104 ± 11 ^{*c}	144 ± 20
Thalamus	119 ± 12	121 ± 7	112 ± 15	114 ± 18	104 ± 10	93 ± 8 ^{*b}	131 ± 11
Corpus callosum	59 ± 11	57 ± 15	52 ± 11	53 ± 13	56 ± 5	52 ± 6	61 ± 15
Caudate/putamen	105 ± 13	101 ± 5	107 ± 9	111 ± 13	95 ± 7	88 ± 9 ^{*a}	128 ± 9
Contralateral hemisphere:							
Frontal cortex	105 ± 10	95 ± 8	97 ± 13	99 ± 15	101 ± 11	97 ± 13	101 ± 12
Parietal cortex	96 ± 4	100 ± 8	95 ± 6	95 ± 15	97 ± 4	99 ± 8	92 ± 9
Temporal cortex	98 ± 3	105 ± 5	101 ± 10	109 ± 10	106 ± 7	97 ± 8	111 ± 6
Occipital cortex	95 ± 14	96 ± 12	95 ± 14	102 ± 12	95 ± 13	100 ± 12	100 ± 12
Hippocampus							
CA1	131 ± 25	121 ± 16	126 ± 13	124 ± 15	124 ± 15	123 ± 19	141 ± 13
CA2-3	143 ± 27	134 ± 9	134 ± 9	149 ± 18	136 ± 8	128 ± 9	153 ± 8
Thalamus	120 ± 16	127 ± 4	122 ± 12	123 ± 15	120 ± 5	111 ± 5	140 ± 26
Corpus callosum	61 ± 13	66 ± 18	54 ± 8	54 ± 10	53 ± 6	55 ± 7	61 ± 16
Caudate/putamen	113 ± 15	110 ± 4	121 ± 9	121 ± 13	113 ± 9	107 ± 9	131 ± 10

Values are mean ± SD (%SDH activity: each structure/cerebellum × 100) (n = 5 in all groups). *Significantly lower than values of sham group (^ap < 0.05, ^bp < 0.01, ^cp < 0.001).

Fig. 3 Quantitative imaging of succinate dehydrogenase (SDH) activity in sham control (A), and 1 hour (B), and 6 (C), 24 (D), 48 (E), and 72 hours (F), and 2 weeks (G) after injury. The regional SDH activity was displayed using semicolor coding (NIH Image 1.57). Higher activity of SDH is indicated by red, and lower activity by blue. The SDH activity gradually decreased until 72 hours after injury, but recovered except in the injured parietal cortex by 2 weeks. There was no significant change in SDH activity of all regions on the contralateral side. Arrowhead shows the injured site.

hippocampus after injury are shown in contrast to changes in ICBF and ICGU in Figs. 1 and 2, respectively.

V. Histological findings

Histological studies revealed petechial hemorrhage due to the impact in the injured cortex and at the border between the gray and the underlying white matter ipsilateral to the injury in 12 of the 25 rats examined within 72 hours after injury. Hemorrhage was not seen in any other region of the brain. Pyknosis and retraction of the cell body of the neurons around contusion were observed slightly in the injured cortex as early as 1 hour after injury, and these changes became more pronounced with vacuolated neuropils at 6 and 24 hours. By 72 hours after injury, the areas of injured and adjacent cortices had become slightly necrotic with more severe edema, and part of the area was separated from the

surrounding tissue shown by its pale staining. By 2 weeks after injury, the lesion had become an area of more prominent necrosis with a few remnant pyknotic neurons (Fig. 4A, B). No histological changes were observed in the hippocampus until 24 hours after the injury. However, pyknosis and retraction of the cell body of the pyramidal neurons were observed in the ipsilateral hippocampus 48 hours after the injury, and the number of neurons in the CA1 and CA2-3 regions ipsilateral to injury decreased by 2 weeks after injury compared to that of the contralateral side (Fig. 4C, D). Reduction of neurons was $86 \pm 14\%$ for CA1 and $83 \pm 12\%$ for CA2-3. Reduction of neurons in the CA2-3 was significant, compared to the sham group ($99 \pm 7\%$ for CA1 and $100 \pm 11\%$ for CA2-3) ($p < 0.05$). There were no marked changes in other regions including the thalamus, basal ganglia, and remote cortices from the impact site within 2 weeks.

Fig. 4 Photomicrographs showing the left contralateral (A) and right injured (B) cortices, and contralateral (C) and ipsilateral (D) CA2 of the hippocampus 2 weeks after injury. The area of cortical contusion became necrotic with pyknotic neurons (arrow), and reduction of the number of neurons in the CA2 of the hippocampus is seen with dark or pyknotic neurons on the side ipsilateral to the injury. HE stain, A and B, $\times 100$; C and D, $\times 200$.

Discussion

Numerous studies of changes in ICBF and glucose metabolism after experimental brain injury have been reported.^{4-6,8,10,14,15,19,22,24,25,30-32} Methods of brain injury have included fluid percussion injury and compression injury. However, the degree of injury and the time course of measurements are different in each study. In addition, only a few studies have associated changes in ICBF with those in ICGU after brain injury.^{7,15} In the present study, we measured both ICBF and ICGU from 1 hour to 2 weeks after the injury and also followed mitochondrial oxidative function at intervals from 1 hour to 2 weeks. Thus, we could characterize the chronological changes in both CBF and cellular metabolism after traumatic brain injury in the rat using a moderate fluid percussion injury device. Moderate lateral fluid percussion injury in the rat produces a combination of

focal cortical contusion and diffuse pathophysiological and subcortical morphological changes.^{9,21}

ICBF values of sham control rats were slightly lower in most regions than those in the conscious rat measured by Sakurada et al.²³ This is probably due to anesthesia with ketamine 2 hours before the measurement. ICBF decreases after lateral fluid percussion injury in rats and remains significantly lower than the preinjury values in both injured and contralateral hemispheres at 15 minutes,^{30,31} 30 minutes,⁶ and 1 hour.^{30,31} ICBF returns to the preinjury level in both hemispheres except for the injured parietal cortex at 2 hours after fluid percussion injury in the rat.³¹ ICBF decreased only in the ipsilateral hemisphere for 3 hours after injury in piglets.²⁵ Thus, most studies showed that ICBF decreases immediately and remains low in the ipsilateral hemisphere for 1 hour to 3 hours after injury. However, little is known about changes in ICBF

and ICGU after 3 hours. Furthermore, there is no study on simultaneous measurement of the long-term changes in ICBF and ICGU after fluid percussion injury in rats, though uncoupling of ICBF and ICGU was found during only the first hour after lateral fluid percussion injury.⁷⁾ In the present study, ICBF significantly decreased in the hippocampus, thalamus, and corpus callosum bilaterally and in the frontal and parietal cortices near the injured site 1 hour after injury. Furthermore, ICBF was significantly lower than the preinjury level in almost all regions at 6 and 24 hours after injury and remained low at 2 weeks in the bilateral hemispheres, though we did not measure ICBF at time intervals after 24 hours to 2 weeks, because ICBF remained low at 2 weeks. Our study indicates that ICBF remains low not only in the injured cortex but also in extensive areas of both hemispheres for a long period (up to 2 weeks) after unilateral fluid percussion of moderate injury level.

Cerebral glucose metabolism increases considerably in the hemispheres for a short period after fluid percussion injury,^{7,10)} concussive brain injury,^{14,24,32)} and induction of acute subdural hematoma¹⁵⁾ and then decreases. An increase in ICGU in the early period after injury is probably related to release of excitatory amino acids after experimental brain injury in rats.^{14,15,32)} ICGU increased immediately following midline fluid percussion injury in rats, suggesting that excitatory amino acid-activated ion channels are involved in the posttraumatic increase in ICGU, reflecting the energy demand of cells required to drive pumping mechanisms against an ionic perturbation seen immediately following injury.¹⁴⁾ This hypothesis is supported by the blocking of increase in glucose utilization by administration of excitatory amino acid antagonists.^{12,14)} Examination of three groups with the different injury levels (mild, moderate/severe, and severe) to assess whether the severity of injury correlates with the duration of prolonged metabolic depression found that ICGU in all brain regions ipsilateral to the injury increased over control values for all three injury groups after 30 minutes, and afterwards metabolic depression first seen at 6 hours after the injury remained significantly in approximately 50% of the structures on the injured side for 15 days in the moderate or severe lateral fluid percussion injury groups.¹⁰⁾ Increasing the injury severity resulted in a more prolonged metabolic derangement. Our results obtained with moderate fluid percussion injury are similar during the early and chronic periods. Another finding in the present study is that the response of ICGU on the contralateral side to injury was very similar to that of the injured hemisphere except in the frontal and

parietal cortices near the injured site, though ICGU in the contralateral hemisphere of rats was found to recover within 24 hours after cerebral concussion or contusional injury, whereas that in the injured hemisphere remained depressive.^{19,32)} This difference of response in the contralateral hemisphere is probably attributable to the degree of injury, suggested by the positive correlation between injury severity and prolongation of metabolic dysfunction.¹⁰⁾ We used a lumped constant for the normal rat²⁷⁾ to calculate ICGU in the present study as in others,^{7,10,12,14,15,19,24,32)} though it may change in the injured brain after fluid percussion injury. Factors which can affect the validity of this calculation include alterations in the blood-brain barrier permeability, severe hyperglycemia, and severe reduction of ICBF. With no evidence of blood-brain barrier disruption or ischemic conditions following fluid percussion injury, the use of Sokoloff's method can provide valid and reliable values of ICGU.^{14,32)} There is probably less change in the lumped constant in the rat brain after fluid percussion injury unlike cerebral ischemia, because no ICBF decreased beyond the ischemic threshold except for the injured parietal cortex in the present study. There may be some overestimation of the value of ICGU only in the injured site.

The relationship between ICBF and ICGU is coupled in the normal brain and even under abnormal circumstances such as suppression of brain function by the administration of drugs, epileptic seizures, or electrical stimulation of the cerebral cortex. Our data suggest that the relationship between ICBF and ICGU is coupled in the cerebral regions of the entire hemispheres for several hours following traumatic brain injury and then over 2 weeks, but that the relationship is uncoupled in the early period.⁷⁾ A coupled decrease in ICBF and ICGU in most regions of both cerebral hemispheres 6 hours after injury in the present study reflects a general influence on the brain caused by moderate lateral fluid percussion injury. Furthermore, the present study showed that both ICBF and ICGU remained depressed for 2 weeks in most regions of both hemispheres including the entire cerebral cortex, hippocampus, and corpus callosum in the chronic period, except for remote structures such as the cerebellum. These results suggest that unilateral traumatic brain injury causes prolonged metabolic dysfunction coupled to decreased CBF in both local and outlying areas. We used ketamine hydrochloride for relatively light anesthesia for measurement of ICBF and ICGU. Although ketamine may have caused some effect on excitatory amino acid receptors, this does not invalidate the results of the study, because there were

no data indicating any marked effect on the values of ICBF and ICGU in the sham control group.

There is a significant correlation between cortical lesion volume measured by TTC staining and severity of brain injury verified histologically.²⁰⁾ A small difference in SDH activity which was not detectable after enzymatic staining reaction had reached a plateau can be detected by measuring the initial rate of formazan production.¹⁶⁾ We examined regional changes in SDH activity in the early period when dynamic changes in ICBF and ICGU occur. Our method of using quantitative imaging of SDH was sensitive to regional disturbance in SDH activity from 1 hour after traumatic brain injury, in which time there was no clear manifestation of histological changes in regions other than the impact site. Furthermore, our results showed that the greatest decrease in SDH activity occurred 72 hours after injury, and that SDH activity recovered except in the injured parietal cortex 2 weeks after injury. SDH activity significantly decreased in the cortex soon after injury, and later in the hippocampus and other regions on the ipsilateral side to the moderate injury, and no significant change was seen in the contralateral hemisphere. SDH activity seems to show more gradual deterioration, compared with the decreases in ICBF and ICGU, and recovers earlier. Investigation of isolated mitochondria from the injured rat forebrain with glutamate plus malate as substrates showed that mitochondrial respiratory dysfunction occurred as early as 1 hour after injury, with a maximum effect around 12 to 24 hours, and persisted for at least 14 days following traumatic brain injury.²⁸⁾ Traumatic brain injury may have perturbed cellular Ca^{2+} homeostasis with excitatory amino-acid release and resulted in excessive Ca^{2+} adsorption to the mitochondrial membrane. Oxidative stress due to production of reactive oxygen species via various pathways after injury may damage mitochondrial structures in combination with Ca^{2+} .^{17,26,28)} Treatment with either an antioxidant or N-type calcium channel blocker, or both, restores mitochondrial function.^{17,29)} SDH activity of the cerebellum was used as a standard value in each brain to calculate the regional activity of SDH, because this structure was almost free from influence from injury in terms of changes in ICBF and ICGU. Although blood gas was not analyzed in these groups for measurement of SDH activity, our previous studies and data for measurement of ICBF and ICGU in the present study demonstrated no significant changes in blood gas after similar levels of injury. The result of changes in SDH activity measured by the method used in the present study is closely in agreement with that of a cytochrome ox-

dase histochemistry study¹¹⁾ in a concussion model using rats, which showed that as early as 1 day following fluid percussion injury, cerebral cortices and hippocampus ipsilateral to the side of injury entered a state of depression of cerebral oxidative metabolism lasting for up to 10 days.

Cellular oxidative metabolism decreased mostly in the ipsilateral hemisphere 1 hour after injury, though ICGU increased. By contrast, a decrease in regional SDH activity at the ipsilateral cortices correlated with a decrease in ICBF in the parietal and frontal lobes. Low oxygen delivery due to decreased ICBF probably increases anaerobic glycolysis, which requires much more glucose for a given energy production. In addition, postinjury impairment of the mitochondrial tricarboxylic acid cycle for aerobic ATP production may promote glucose metabolism in a compensatory fashion. The increase in ICGU seen within 1 hour to 2 hours after injury is likely to reflect energy demand. Posttraumatic transient increase in ICGU may be important as an energy supplement for ionic homeostasis. However, glucose metabolites may be utilized less effectively in the ipsilateral side to injury because mitochondrial ATP production is attenuated, even if glucose hypermetabolism reflects the energy demand of cells exposed to ionic perturbation.¹⁴⁾ Although posttraumatic derangement such as glucose hypermetabolism and a concomitant decrease in ICBF which leads to regional decreased oxidative metabolism may contribute to neurotoxicity especially in the ipsilateral hemisphere,¹⁵⁾ failed glucose hypermetabolism may be a more critical factor in neuronal cell damage. In the areas where hyperglycolysis cannot be accomplished, neuronal cell damage could worsen because of energy depletion.¹⁴⁾

Our previous experimental study using MR imaging showed that cerebral edema around the contusion appeared 4 hours after moderate lateral fluid percussion injury in rats and was most extensive between 6 and 24 hours, and also that the number of neurons in the hippocampus ipsilateral to injury decreased by 2 weeks after injury compared to those in the contralateral side and sham control.²¹⁾ Histological changes observed after injury were most severe at the injured cortex and cortex adjacent to the impact, where both SDH activity and ICBF deteriorated most conspicuously without hyperglycolysis as early as 1 hour after injury in the present study. A less conspicuous but gradual decrease in SDH activity was seen in the CA2-3 of the hippocampus where significant reduction of neuronal cells was observed on the ipsilateral side 2 weeks after injury. The method we used is more sensitive to regional changes in the SDH activity than TTC staining.²⁰⁾

This area of the hippocampus also showed less hyperglycolysis as well as a decrease in ICBF and SDH activity 1 hour after injury. Thus, the histological changes seem to correlate with the deterioration of SDH activity, absent or reduced hyperglycolysis, and decreased ICBF in the early period after injury. Other factors such as perifocal edema may contribute to histopathological changes in the CA1 of the hippocampus, in which ICGU markedly increased 1 hour after injury.²¹⁾

Our present study suggests that simultaneous development of decreased ICBF, mitochondrial dysfunction, and depressed glucose hypermetabolism under hyperglycolytic conditions in the early period after injury may produce unfavorable effects on the cells in the areas that correspond to the penumbra. Subsequently, ICGU as well as ICBF and SDH activity decreases by 6 hours after injury, as observed in our study and others.^{10,32)} This condition reflects the entire metabolic dysfunction of neuronal cells, some of which may suffer an irreversible change. A therapeutic window for preventing secondary injury may exist within 6 hours after traumatic brain injury.

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Commentary

These detailed and well executed experiments with a moderate fluid-percussion injury to the rat brain illustrate several points. They show nicely the extent of change even after a local injury, with moderate changes in blood flow and glucose utilization persisting for up to two weeks even contralaterally. Regions much more remote than could be affected by diaschisis showed significant changes at an early stage. The changes in SDH, which correlates more with mitochondrial dysfunction and later histological changes, were essentially only ipsilateral.

The alterations in glucose uptake, with considerable and widespread increases in the early hours after injury, were interesting. The possibility of increased utilization due to anaerobic glycolysis following reduced blood flow and oxygen delivery is mentioned, but it is pointed out elsewhere that the fall in blood flow, though statistically significant, was mostly not to ischemic levels. Disruption of mitochondrial function and metabolism via the TCA cycle is perhaps more likely. It would be interesting to see direct measurements of oxygen metabolism in future studies.

Changes occurring between the early measurements and six hours may, as discussed, indicate the development of irreversible processes. The possibility of a therapeutic window is raised, but the possible duration of this would need further study with the same experimental model between one and six hours after injury to determine more exactly when a possible irreversible stage is reached.

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The authors should be congratulated on an excellent study of the pathophysiology of secondary brain damage following experimental fluid percussion brain injury in rats. It has been generally postulated that secondary brain damage after traumatic brain injury and cerebral ischemia are important to determine the extent of final brain damage and outcome.

Various contributing factors have also been proposed for secondary brain damage including excessive release of excitatory amino acids which act on NMDA receptor-control ion channels to allow the influx of calcium and sodium ions, uncoupling of cerebral blood flow and metabolism, activation of lipoxigenase pathway, etc.

The authors measured both local cerebral blood flow (ICBF) and local cerebral glucose utilization (ICGU) from 1 hour to 2 weeks after traumatic brain injury and also followed the chronological change of mitochondrial oxidative function by quantitative measurement of regional succinate dehydrogenase activity. The present study showed that a decrease in ICBF and mitochondrial dysfunction occur with glucose hypermetabolism around 1 hour after brain injury, and that ICBF, ICGU, and mitochondrial function deteriorate after 6 hours. The authors suggest that final neuronal damage might occur especially in the areas with depressed glucose hypermetabolism under mitochondrial dysfunction in the early period. In other words, the cerebral metabolic disturbance is a key factor to determine final neuronal damage after traumatic brain injury. Thus, the conclusions in this paper are highly dependent on the data of the calculated ICGU. The authors tried to measure ICGU in the pathological condition by Sokoloff's 2-deoxyglucose (2-DG) method. However, Sokoloff limits use of the 2-DG method to measurement of ICGU under normal

physiological conditions because the lumped constant (LC) of the operational equation of the 2-DG method may change under pathological conditions.²⁾ LC dramatically increases in decreased brain tissue glucose concentration. Therefore, measurements of tissue glucose concentrations under individual conditions are essential for quantitative measurement of ICGU.^{1,2)} If the present study had measured brain tissue glucose concentration by either direct chemical assay or [¹⁴C]methylglucose method, the data would be more valid and concrete.

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