Study of Regional Cerebral Blood Flow in Experimental Head Injury: Changes Following Cerebral Contusion and During Spreading Depression

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Abstract

Changes in regional cerebral blood flow (rCBF) following fluid-percussion brain injury (cerebral contusion) were studied in rats using the autoradiographic method. The direct current potential was monitored to identify spreading depression (SD). The rCBF was measured during SD and 2, 4, and 24 hours after injury. rCBF was almost nil in the contused area and decreased considerably in the cortices of the injured side for 4 hours after insult, then recovered by 24 hours. Focal relative rCBF increase occurred in the parietal cortex during SD, and was probably hyperperfusion due to SD. However, the rCBF did not increase over the sham-operated control. The injury probably caused hypoperfusion within 4 hours of insult and abolished the vascular response to SD.

Key words: rat, head injury, cerebral blood flow, spreading depression

Introduction

Study of cerebral blood flow (CBF) and metabolism in experimental brain injury models has increased since Sullivan et al.27) developed the quantitative model using fluid percussion. This model essentially represents brain concussion or diffuse injury as defined by Gennarelli.s) Nakamura et al.10) modified the original fluid-percussion method to obtain a local brain contusion model in rat. Study of changes in local cerebral glucose utilization (LCGU) in this model showed that LCGU in the injured cerebral cortex was markedly increased in some animals 2 hours after the insult. Sunami et al.28) proved that spreading depression (SD) caused this accelerated glucose metabolism. SD usually occurred repeatedly in the injured cerebral cortex for 1-2 hours after the insult. The frequency of SD correlated with the degree of injury, but not all contused animals developed SD.19)

Here, we report our study of changes in regional CBF (rCBF) following brain contusion causing SD in the rat.

Materials and Methods

This study used 35 male Wistar rats, weighing 250-350 gm. The femoral artery and vein were cannulated with polyethylene catheters under light halothane-nitrous oxide anesthesia. Brain contusion was induced in 27 animals by Nakamura's method10) as reported elsewhere. A biphasic pressure wave of 20 msec from the fluid-percussion device induced a focal contusion in the unilateral cerebral hemisphere. The peak pressure was 0.5 kg/cm². The burr hole was then closed with bone cement. A hole was drilled in the outer table of the parietal skull. Direct current (DC)-potential electrodes were fixed on the skull on both sides using cyanoacrylate. The DC-potential electrode consisted of a Ag-AgCl wire embedded with agar in a polyethylene cap 4 mm in diameter.29) The reference plate type Ag-AgCl electrode was placed under the back skin. The animals were then loosely fixed and awoken. All procedures were finished within 20 minutes of percussion.

The DC potential was monitored with a DC amplifier (Model MEZ-8201 differential preamplifier; Nihon Koden Kogyo Co., Inc., Tokyo). Thirteen of the 27 animals demonstrated DC-potential negative shift within 2 hours after percussion. These 13 rats were divided into four groups, and the
rCBF was measured with Sakurada's 14C-iodoantipyrine autoradiographic method. Fifty μCi of 14C-iodoantipyrine was administered intravenously over 1 minute. Arterial blood samples were taken regularly for tracer concentration analysis. The animal was decapitated exactly 1 minute after measurement initiation. The brain was removed as rapidly as possible, frozen, sliced, and used for quantitative autoradiography. In the SD group (n = 4), the rCBF was measured during the recovery phase of the first or the second DC-potential negative shift (Fig. 1). In the 2 hours group (n = 3), the rCBF was measured 2 hours after percussion. The tracer was injected while the DC potential was stable. Similarly, the rCBF was measured 4 and 24 hours after percussion in the 4 (n = 3) and 24 hours groups (n = 3).

Eight animals received sham operation. rCBF measurements were made 2 (n = 4), 4 (n = 2), and 24 hours (n = 2) after sham procedures. The mean values from these animals were taken as controls.

**Results**

The groups demonstrated no significant differences in physiological variables (Table 1).

DC-potential negative shifts began about 30 minutes after percussion and finished at 3.5 hours in the most persistent case. The frequency of DC-potential negative shifts varied with individuals, from only a few before rCBF measurement to intervals of 6 or 7 minutes. rCBF measurements in the SD group were made at a mean of 76 minutes.

The contusion showed little accumulation of 14C-iodoantipyrine, showing that the contused area received little blood flow for at least 24 hours after insult. The rCBF markedly decreased in the cerebral cortices and subcortical structures of the injured side until 4 hours after insult, especially in the cerebral cortices of the SD group, where rCBF decreased to about 40% of the control. The rCBF also decreased in some structures of the non-injured side. The rCBF generally recovered to normal values at 24 hours except in the visual cortex (Figs. 2 and 3).

The SD group autoradiograms showed a band-like focus of increased tracer concentration relative to surrounding tissue in the parietal cortex of the injured side near the DC-potential electrode (Fig. 4). The rCBF of this area increased compared with other ipsilateral cortices, and was not significantly different from the comparable sites in the controls and the non-injured side (Fig. 2).

**Discussion**

Most previous CBF studies in head injury models investigated immediate changes. In the rat, concussion decreased the CBF to 30% of normal within a few minutes, and CBF normalized in 20–40 minutes. In the cat, concussion did not change the CBF value. In this study, rCBF was measured at intervals for 24 hours. The rCBF decreased for 4 hours after insult and then usually recovered by 24 hours. The contusion received little blood flow throughout the experiment. Crockard et al. reported that CBF decreased for at least 1 hour after insult in the rat penetrating brain contusion model. Hekmatpanah and Hekmatpanah investigated the contused rat brain histologically. Microvascular obstruction due to intravascular clots and extravascular pressure from destroyed and swollen tissue, petechial hemorrhage, and dissecting extraluminal clots extended far

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**Table 1 Physiological variables**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>PaO₂ (mmHg)</th>
<th>PaCO₂ (mmHg)</th>
<th>Hematocrit (%)</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>97.5 ± 3.8</td>
<td>37.1 ± 1.1</td>
<td>49.0 ± 0.9</td>
<td>126.0 ± 1.2</td>
</tr>
<tr>
<td>SD*</td>
<td>4</td>
<td>87.9 ± 3.3</td>
<td>41.3 ± 1.6</td>
<td>49.1 ± 0.6</td>
<td>118.3 ± 2.9</td>
</tr>
<tr>
<td>2 hours</td>
<td>3</td>
<td>107.8 ± 3.9</td>
<td>35.2 ± 0.2</td>
<td>49.0 ± 0.9</td>
<td>121.3 ± 1.1</td>
</tr>
<tr>
<td>4 hours</td>
<td>3</td>
<td>99.4 ± 3.0</td>
<td>33.0 ± 2.7</td>
<td>47.8 ± 1.3</td>
<td>120.4 ± 1.9</td>
</tr>
<tr>
<td>24 hours</td>
<td>3</td>
<td>95.6 ± 1.7</td>
<td>40.4 ± 0.5</td>
<td>48.8 ± 1.4</td>
<td>126.5 ± 2.4</td>
</tr>
</tbody>
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Data are means ± standard errors. *76 ± 7.5 minutes after injury. PaO₂: arterial oxygen tension, PaCO₂: arterial carbon dioxide tension.
beyond the apparent contused area for 1–3 hours. In contrast, Wei et al. found vasodilatation but no intravascular platelet aggregation in the cat concussion model. Decreased rCBF in the brain contusion models is therefore caused by disturbance of microcirculation around the contusion. Ekelund et al. reported intra and extracranial arterial spasm in the rat head trauma model, possibly due to subarachnoid hemorrhage and mechanical stretching. Our rat contusion model also demonstrated subarachnoid hemorrhage, possibly with such arterial reactions.

Nakamura et al. used a peak pressure of 0.4 kg/cm² in their LCGU study. Our study used a peak pressure of 0.5 kg/cm², so strictly the models are not identical. However, comparison of the results is interesting. Nakamura et al. reported that LCGU in the injured cerebral cortex generally decreased to 70–80% of the control 1 hour after insult, normalized between 2 and 4 hours, and then again decreased to 70% at 24 hours. Some animals demonstrated considerably increased LCGU in the cerebral cortices and hippocampal gyrus of the injured side. Sunami et al. showed that this was caused by SD based on the transiently depressed electroencephalographic activity, DC-potential negative shifts, and ¹⁴C-deoxyglucose autoradiograms. The LCGU of the cortex increased to 150–200% of the control. The peak pressure used was also 0.5 kg/cm². Induction of SD in normal rat cerebral cortex causes the extracellular potassium concentration to increase from 3 to about 60 mM. This recovers in about 1 minute after SD propagation. The increased clearance of extracellular potassium by the cellular Na-K pump requires

Fig. 2 Changes in rCBF in the visual (A), parietal (B), sensorimotor (C), and frontal cortices (D) of the injured (○) and the contralateral sides (●). rCBF decreased remarkably in the injured side following insult for 4 hours. After 24 hours, rCBF recovered except in the visual cortex. rCBF also decreased slightly in the non-injured side. rCBF in the relative hyperperfused area detected in the parietal cortex of the SD group (△) did not exceed the control. Bars represent standard errors. C on the abscissa means control. Numerals in parentheses indicate number of animals. Asterisks show statistically significant difference from the control (*p < 0.05, **p < 0.02) by Student’s t-test.
adenosine triphosphate and increases the rCBF to more than double the control value. The rCBF increase begins during the recovery phase of the DC-potential negative shift and lasts for 2–3 minutes. This manifests as a band of increased tracer concentration in the autoradiogram.

In this study, the SD group showed local increases in CBF compared with surrounding areas, suggesting increased rCBF accompanying SD propagation. In contrast to the 150–200% LCGU increase, the rCBF increased only slightly over the control or the contralateral side. This difference is possibly due to the reduced response of cerebral blood vessels in the contused brain. Cerebral concussion impairs autoregulation and the response to hypoxia and hypercapnia in cat cerebral blood vessels for a few hours. Damage to cerebral blood vessels will be greater in contused than concussed brain. Therefore, cerebral blood vessels in contused brain will not respond to the abrupt increase in metabolic demand.

Fig. 3  Changes in rCBF in the hippocampus (A), dentate gyrus (B), thalamus (C), and caudate nucleus (D) of the injured (○) and contralateral sides (●). rCBF decreased in the hippocampus of both sides for 4 hours after insult. rCBF of the injured side decreased in the dentate gyrus, thalamus, and caudate nucleus for 4 hours. The rCBF of the contralateral dentate gyrus also decreased after 4 hours. Bars represent standard errors. C on the abscissa means control. Numerals in parentheses indicate number of animals. Asterisks show statistically significant difference from the control (*p < 0.05, **p < 0.02) by Student’s t-test.

Fig. 4  Autoradiograms 2 hours after injury (left) and during SD (right). A band-like focal increase in rCBF is visible in the parietal cortex (arrow).
due to SD. SD in normal brain is considered a reversible and transient phenomenon. However, SD may cause permanent damage if the energy supply is restricted. We have found that repeated SD under hypoxia caused neural necrosis in the rat cerebral cortex (unpublished data). Nedergaard and Astrup found that neuronal death was related to SD in the rat middle cerebral artery occlusion model. Harris et al. reported that SD propagation caused irreversible disorder of the extracellular ion balance in hypoglycemia. Therefore, SD is probably harmful in the brain when the energy supply is restricted. However, SD is not easily induced in the cerebral cortex with impaired energy metabolism. Also, SD is harder to induce in human than rat brain, because the ratio of glia cells to neurons is higher in human than rat brain. Therefore, these experimental results cannot be directly linked to clinical situations, but requires further investigation. However, it is reasonable that impaired CBF due to low blood pressure or high intracranial pressure, or accelerated metabolism due to epilepsy will induce secondary brain damage in contused brain with reduced CBF and impaired vascular response.

The rCBF was generally reduced until 4 hours after the insult, then generally recovered by 24 hours in the sensorimotor and frontal cortices and deep regions. In contrast, Nakamura et al. reported that LCGU in the injured cerebral cortex was lowest at 70% of the control after 24 hours. This suggests that luxury perfusion occurred in the cerebral cortex 24 hours after injury. Obrist et al. measured the CBF and oxygen consumption in head-injured patients. Luxury perfusion occurred in 41 of 75 cases within 3 days after insult. Pappius et al. reported reduced LCGU developed after thermally induced rat cerebral cortex lesion, greatest in the cortical areas of the lesioned hemisphere 3 days after insult. Increased CBF occurred in all cortical areas. Shohami et al. reported that prostaglandin E2 (PGE2) was synthesized six and four times faster than the control in the contused and contralateral hemispheres, respectively, after 18 hours in the rat contusion model. PGE2 is known to dilate cerebral vessels. Possibly, increased PGE2 dilated the cerebral vessels spared from direct injury and increased the rCBF. Obrist et al. found that hyperemia and high intracranial pressure are correlated. Correlation between PGE2 and brain edema was also reported. However, edema formation following focal brain injury may also be due to leukotrienes. Increased serotonin is also suggested as the cause of reduced LCGU in the thermally lesioned cortex. Further investigation is needed to identify the causes of CBF and metabolic changes following brain injury, especially the reduced LCGU and relative increased CBF after 24 hours.

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