Spreading Depression Following Experimental Head Injury in the Rat

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Abstract

The direct current (DC) potential and electroencephalographic (EEG) changes were continuously monitored following fluid percussion head injury (brain contusion) in 10 conscious rats. Local cerebral glucose utilization (LCGU) was measured by the autoradiographic [14C]deoxyglucose method. Measurement of LCGU was started at the lowest point of the first or second DC potential negative shift when it occurred, and 2 hours after contusion if no DC potential changes were observed. The DC potential did not change in four rats (Group A), whereas DC potential negative shifts together with marked suppression of EEG activity occurred at 54 ± 6.9 minutes after injury in six rats (Group B). In Group A, LCGU was decreased nonsignificantly in both the right and left cortices. In Group B, however, LCGU in the lesioned cortex rose to 160-190% of the level observed in the contralateral cortex (p < 0.05). The autoradiographic pattern in Group B was identical to that seen in spreading depression. These findings can contribute to the effort to better understand the pathophysiology of head injury.

Key words: head injuries, cerebral metabolism, spreading depression, deoxyglucose, brain contusion

Introduction

Following brain concussion, a brief increase in glucose metabolism has been observed in some parts of the cerebral hemispheres and the brainstem. After severe head injury, the cerebral oxygen metabolic rate is believed to be consistently depressed, but local cerebral metabolic changes have not been clearly identified. Using the [14C]deoxyglucose method, Nakamura et al. found hypermetabolic changes in the cortex and hippocampus of rats 2 hours after mechanical brain injury. They suggested spreading depression (SD) as a possible mechanism, but the delayed occurrence of SD following head injury has not yet been verified. Measurement of the direct current (DC) potential along with electroencephalographic (EEG) recording are essential to document the occurrence of SD. We studied local cerebral glucose utilization (LCGU) in rats following brain contusion, using the [14C]deoxyglucose method (Sokoloff et al., 1977) combined with EEG and DC potential recordings. We then compared these quantitative data with the well-known EEG features of SD.

Materials and Methods

Ten male Wistar rats weighing 250-350 gm were used. In these experiments, the guidelines of the National Institute of Health for the care and use of laboratory animals were followed.

I. Experimental brain contusion

Brain contusion was produced over the lower left parietal region by mechanical impact. A fluid percussion device was designed to produce a uniform and localized unilateral contusion in one cerebral hemisphere. A biphasic pressure wave with a duration of 20 msec was delivered to the brain by impact against a vinyl chloride membrane attached to a fluid reservoir. This system produces a cortical contusion under a 4-mm burr hole through impact of a 0.5-atm positive peak pressure and a -0.1-atm negative peak pressure. Transient arterial hypertension, with apnea lasting about 20 seconds, always occurs immediately after the impact.
II. DC potential monitoring

An Ag-AgCl electrode connected to a DC amplifier (Model MEZ-8201 differential preamplifier; Nihon Koden Kogyo Co. Inc., Tokyo) was used to monitor the DC potential. A silver wire 0.5 mm in diameter was electrolytically coated with silver chloride with a 1.5-V battery in a dark chamber. This Ag-AgCl electrode was embedded in agar in a plastic cap 4 mm in diameter. A reference electrode of Ag-AgCl plate, 6 mm in diameter, was placed in the subcutaneous tissues of the back of the neck. It was confirmed in advance that a negative shift of 5-7 mV was detectable at the time of sacrifice.

III. Experimental protocol

Polyethylene catheters were inserted into the femoral artery and vein under light halothane-nitrous oxide anesthesia. A burr hole 4 mm in diameter was drilled in the lower parietal region; great care was taken to avoid damaging the dura mater. The fluid percussion device was fixed to the burr hole with cyanoacrylate, and 10 rats were given contusions as described above. The device was removed and the burr hole was closed with bone cement. Electrodes for measurement of DC potential and EEG monitoring were firmly attached with cyanoacrylate at the vertex of the skull on both sides. Only the outer table of the rat skull was drilled so as to prevent injury to the cortex and to obtain a sufficient DC potential gain. All animals were revived after the procedure and were restrained by means of a loose-fitting plaster cast placed around the lower abdomen. About 15 minutes elapsed before measurement of the DC potential and EEG recording were started. Quantitative [14C]deoxyglucose measurement was started at the lowest point of the first or second negative shift in the DC potential. If no negative shift in the DC potential occurred, [14C]deoxyglucose was measured after 120 minutes of observation.

IV. Measurement of physiological variables

The mean arterial blood pressure, plasma glucose concentration, and arterial pH, PaO2, PaCO2, and hematocrit were monitored immediately before the beginning of each 2-deoxyglucose experiment. Rectal temperature was maintained at 37°C with a heating lamp.

V. Measurement of LCGU

The procedure for measuring LCGU was described by Sokoloff et al.37) Briefly, a pulse of 100 μCi/kg of 2-deoxy-D-[14C]glucose was administered via a femoral venous catheter. Arterial blood samples were rapidly drawn immediately after the pulse, for 45 minutes at timed intervals. The red cells were immediately separated by centrifugation and the plasma samples were stored on ice until analysis for glucose and [14C]deoxyglucose concentrations. At the end of the 45-minute sampling period, arterial circulation was stopped by an intravenous bolus injection of 2 ml of saturated potassium chloride solution, and the brain was removed as rapidly as possible, frozen in isopentane, chilled to −30°C, sectioned, and subjected to quantitative autoradiography. LCGU was calculated from the time courses of the plasma [14C]deoxyglucose and glucose concentrations and the tissue 14C concentrations, by means of the operational equation for the [14C]deoxyglucose method.

VI. Statistical analysis

All data are expressed as the mean ± SEM. The data were statistically analyzed by Student’s two-tailed t-test. The results were considered significant at a confidence level of over 95%.

Results

I. DC potential

The animals were divided into two groups based on DC potential changes. Group A consisted of the four rats that showed no change in the DC potential, while Group B comprised the six animals in which negative shifts in the DC potential were seen (Fig. 1). The negative shifts in the DC potential appeared 37–88 minutes after the injury (54 ± 6.9 minutes), and the EEG activity was markedly depressed during the negative shifts. The frequency of negative shifts varied, the highest being every 6–7 minutes (Fig. 2).

II. LCGU

Autoradiographic and DC potential changes are shown together in Fig. 1. There were no significant differences in the uptake of [14C]deoxyglucose between the right and left cortices in the absence of negative shifts (Group A). In Group B, in which negative shifts in the DC potential were elicited, [14C]deoxyglucose uptake was clearly increased in the lesioned cortex relative to that in the contralateral cortex, while uptake in subcortical structures was decreased on the lesioned side.

LCGU values are shown in Table 1. In Group B, LCGU in the lesioned cortices reached 160–190% of the value obtained contralaterally (p < 0.05). In Group A, there were no significant differences between the right and left hemispheres and the values were generally lower than those of the lesioned cortices in Group B.
The subcortical LCGU is also shown in Table 1. In Group B, LCGU tended to be more markedly decreased on the lesioned side than on the contralateral side but the difference was not significant. In Group A, hemispheric differences in LCGU were also insignificant.

### III. Physiological variables

As shown in Table 2, there were no significant physiological differences between Groups A and B. No seizures, either behavioral or EEG, were noted in any animal.

### Discussion

Numerous experimental models of mechanical brain injury have been developed.1-3,12,39] The fluid percussion model has several advantages, as pointed out by Sullivan et al.39] We developed a modified fluid

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**Table 1** LCGU values (μmol/100 gm/min) in conscious rats

<table>
<thead>
<tr>
<th>Structure</th>
<th>Group A (n=4)</th>
<th>Group B (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td><strong>Cortical structures:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>visual cortex</td>
<td>47.7±7.3</td>
<td>59.8±11.0</td>
</tr>
<tr>
<td>parietal cortex</td>
<td>47.8±6.1</td>
<td>56.4±10.4</td>
</tr>
<tr>
<td>sensorimotor cortex</td>
<td>62.6±11.3</td>
<td>62.0±11.9</td>
</tr>
<tr>
<td>frontal cortex</td>
<td>60.7±6.6</td>
<td>68.6±9.8</td>
</tr>
<tr>
<td>cingulate gyrus</td>
<td>65.0±8.3</td>
<td>61.7±5.6</td>
</tr>
<tr>
<td><strong>Subcortical structures:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thalamus</td>
<td>56.1±4.4</td>
<td>58.7±6.5</td>
</tr>
<tr>
<td>medial geniculate</td>
<td>52.6±7.8</td>
<td>67.3±6.3</td>
</tr>
<tr>
<td>hypothalamus</td>
<td>43.8±6.5</td>
<td>42.5±5.8</td>
</tr>
<tr>
<td>caudate nucleus</td>
<td>69.1±5.0</td>
<td>73.5±6.1</td>
</tr>
<tr>
<td>hippocampus</td>
<td>47.0±7.2</td>
<td>49.6±5.9</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p<0.05 (intragroup, left vs. right side), **p<0.01 (intragroup, left vs. right side), p<0.05 (Group B vs. Group A, same side).
percussion technique\textsuperscript{28}) to produce contusion in the lateral aspect of the brain rather than in the vertex,\textsuperscript{5,30} which allows comparison of differences between the right and left hemispheres.

In this study, the DC potential and EEG recordings, as well as the glucose metabolism data, were all characteristic of SD. Since the first observation of SD by Ledo in 1944,\textsuperscript{21} a negative shift in the DC potential and EEG suppression have been considered the most reliable indicators of SD.\textsuperscript{22,23} In addition, Shinohara et al.\textsuperscript{35} noted an increase in LCGU in the hemisphere in which the DC potential negative shifts occurred, the pattern of increase (i.e., throughout the cortex) was identical to that of SD. Although a wave of EEG suppression spreading over the cortex at a speed of 3–5 mm/min\textsuperscript{21}) was not demonstrated in the present study, it is highly likely that SD occurred in the Group B rats. Earlier, using an ion-sensitive microelectrode technique, we observed a marked increase in the extracellular potassium concentration ([K\textsuperscript{+}]\textsubscript{o}) concurrent with the DC potential negative shift.\textsuperscript{18}

We did not anticipate the occurrence of multiple DC potential negative shifts. However, they were observed as often as 9–10 times/hour (Fig. 2), the frequency varying from rat to rat. We did not measure the DC potential just after impact; the first negative shift was observed 54 ± 6.9 minutes after contusion. SD may be a late phenomenon, as it appeared about 1–2 hours after brain contusion. When SD is induced by mechanical or other traumatic means, it always appears immediately after the stimulation.\textsuperscript{22,23} It is known that an increase in the [K\textsuperscript{+}]\textsubscript{o} is important both in the genesis of SD\textsuperscript{10,11} and as a consequence of SD.\textsuperscript{10,11,23} [K\textsuperscript{+}]\textsubscript{o} must exceed a certain threshold in order to induce SD.\textsuperscript{10,11} Takahashi et al.\textsuperscript{40} and Hubschmann and Nathanson\textsuperscript{13} noted a transient increase in the [K\textsuperscript{+}]\textsubscript{o} either immediately or a few minutes after experimental head injury. It is not clear how [K\textsuperscript{+}]\textsubscript{o} changes are brought about after 1 hour following head injury, but Nedergaard and Hansen\textsuperscript{59} reported that, in a middle cerebral artery occlusion model, [K\textsuperscript{+}]\textsubscript{o} of 40–60 mmol/l persisted for 2 hours at the center of the lesion and that the concentration slowly returned to the baseline level in the tissue near the ischemic area. Briefly transient and recurrent increases in [K\textsuperscript{+}]\textsubscript{o} or SD occurred farther away from the lesion. Our head injury model may therefore provide useful information concerning the occurrence of SD after head injury. That is, in both our model and the middle cerebral artery occlusion model, the [K\textsuperscript{+}]\textsubscript{o} is elevated due to damage of such components as vessels, neurons, and glial cells at the main point of mechanical impact.

There are problems with the [\textsuperscript{14}C]deoxyglucose method of quantifying LCGU under pathological conditions. The lumped and rate constants in Sokoloff’s equation\textsuperscript{43} may be altered and the LCGU data are probably distorted at and around the impact site. However, because the physiological findings in this study were not indicative of any severe pathology, such as marked ischemia or hypoxia, we conclude that the lumped constant and glucose content do not vary greatly, especially in areas remote from the contusion. Therefore, it is likely that the enhanced uptake of [\textsuperscript{14}C]deoxyglucose reflected an increase in LCGU.

Subcortical LCGU has been shown to decrease in SD,\textsuperscript{35} although in our study the decrease in Group B was not statistically significant. Shinohara et al.\textsuperscript{35} attributed this decrease to functional decortication due to SD. Comparison of LCGU in Groups A and B showed a statistically significant difference at the caudate nucleus, but the reason for this is unclear. It is noteworthy, however, that the SD on the lesioned side in Group B affected the subcortical structures. This in turn, may have some influence on the contralateral side.

SD is accompanied by marked changes in the extracellular microenvironment,\textsuperscript{4,10,14,27} glucose consumption,\textsuperscript{4,15} cortical blood flow,\textsuperscript{9} and energy metabolites,\textsuperscript{24} and these alterations last for several minutes. The increased LCGU in the cortex during SD is thought to be the consequence of increased [K\textsuperscript{+}]\textsubscript{o} and activation of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase.\textsuperscript{4,36} The cortical glycogen concentration and protein synthesis remain inhibited for a longer period.\textsuperscript{16,17} After SD, cortical blood flow is reduced by 30% on the affected side for at least 90 minutes.\textsuperscript{19,20} All these phenomena would result in deleterious extracellular

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**Table 2 Mean values of physiological variables**

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mg/dl)</th>
<th>Hematocrit (%)</th>
<th>MABP (mmHg)</th>
<th>PaO\textsubscript{2} (mmHg)</th>
<th>PaCO\textsubscript{2} (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=4)</td>
<td>204±33</td>
<td>48.8±1.9</td>
<td>123±5.7</td>
<td>86.1±1.8</td>
<td>35.9±2.3</td>
</tr>
<tr>
<td>B (n=6)</td>
<td>151±14</td>
<td>47.8±2.0</td>
<td>115±6.0</td>
<td>88.3±2.5</td>
<td>40.4±2.5</td>
</tr>
</tbody>
</table>

Values are means ± SEM. MABP: mean arterial blood pressure.
changes. The delayed occurrence of SD after brain contusion has not been previously reported, and it is still unknown how SD affects the contused brain. In the event of contusion, there is likely a marginal zone in which the cells are on the verge of death. Whether they will die or recover depends on the conditions in the extracellular environment. If SD occurs in a contused brain in which the environment has already been compromised by secondary changes, such as increased intracranial pressure and extracellular edema, further deterioration is likely, and the brain damage will spread to the marginal zone.

Although our results do not provide data regarding a possible mechanism for the occurrence of SD, they do provide information that may be useful in elucidating the pathophysiology of head injury. Although induction of SD is a much simpler matter in rats than in humans, the potential application of SD under certain circumstances in humans has been addressed. It will be very interesting to discover its role in human brain contusion.

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