Interrelationship of Brain Edema, Motor Deficits, and Memory Impairment in Rats Exposed to Focal Ischemia

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We investigated the relations of brain edema, ion shifts, motor performance, and memory impairment using a focal ischemia model in rats. Cortical infarction was produced by ligation of the middle cerebral artery and the ipsilateral common carotid artery combined with temporary occlusion of the contralateral common carotid artery for 1 hour. Water content and sodium, potassium, and calcium concentrations were measured until Day 14 after the ischemic insult. Significant edema formation was observed; it peaked on Day 3 \( (p<0.001) \) and then declined. The tissue sodium concentration changed in a manner similar to that of water content, but the tissue potassium concentration changed in an opposite fashion. Massive accumulation of calcium was detected as early as Day 1 after ischemia \( (\text{almost four times the normal level}) \). The increased calcium concentration was sustained even up to Day 14. Motor performance examinations performed on Day 3, including inclined plane, balance beam, and prehensile tests, demonstrated significantly reduced \( (p<0.001) \) motor ability that did not recover even by Day 7. Passive avoidance learning was carried out on Day 2, followed by a memory retention test on Day 3. Significant memory dysfunction was observed in ischemic compared with sham-operated rats \( (p<0.001) \). A high correlation coefficient \((r=0.91, p<0.01, n=13)\) was obtained between water content and calcium concentration on Day 3. Both the total motor score and the degree of disturbance of the passive avoidance reaction also correlated well with water content. \( \text{(Stroke} 1989;20:513–518) \)

The clinical outcome of cerebral infarction depends heavily on the formation of brain edema. Therefore, the mechanism of brain edema has been intensely studied by a number of investigators using ischemia models in various animal species. In general, the brain edema caused by ischemic damage has been classified as cytotoxic or vasogenic edema. The time courses of these classes of edema evaluated by the movement of water and electrolytes have been well investigated using focally or globally ischemic animals. The relation between ischemic brain edema and neurologic dysfunction has also been studied using electrophysiological techniques, such as electroencephalography (EEG) and DC and somatosensory evoked potentials. However, it is still not clear how ischemic brain edema affects neurologic functions such as motor capability and memory.

For this type of study, it is desirable to obtain both biochemical and behavioral data from the same animal. The advantages of using a rat model are compelling because it is possible to use large numbers of an inbred strain. Thus, we used the rat cortical infarction model developed by Chen et al, which has a low mortality rate, less deterioration of systemic conditions, and allows the relatively long-term observation of behavioral changes following the ischemic insult.

Materials and Methods

We used 84 adult male Sprague-Dawley rats (SPF, Zivic-Miller Laboratories, Inc., Zelienople, Pennsylvania) weighing 250–300 g housed in cages with a 12-hour light/dark cycle and a temperature of 24 °C. The rats had unlimited access to food pellets and water throughout the experiment. Anesthesia was induced and maintained with 1.0–2.5% halothane via a close-fitting mask. The bilateral common carotid arteries (CCAs) were gently exposed, and the right CCA was ligated at two places with 4-0 silk suture. To expose the right middle cerebral artery (MCA), the temporal muscles were cut and retracted; a small temporal craniotomy was then performed with a microdrill. With
the aid of a dissecting microscope, the dura was opened with a 26-gauge needle and the MCA was ligated with 10-0 suture. The bone defect was covered with a small piece of absorbable gelatin sponge. Immediately after MCA ligation, the left CCA was occluded with an aneurysm clip. After surgery, the rat was returned to its cage. One hour after clipping the left CCA, the awake rat was hand-held and the clip was released. Sham-operated control rats were subjected to the same procedure, without MCA ligation and CCA clipping.

Systolic arterial blood pressure (SABP) was measured using a noninvasive plethysmographic tail-cuff method (Model 29, IITC Inc., Woodland Hills, California) just before left CCA clipping. Body weight was measured before and 3 and 7 days after surgery.

To evaluate ischemic brain edema, we measured water content and tissue concentrations of sodium, potassium, and calcium using standard methods. The rats were decapitated and the excised cerebral hemisphere was placed in a preweighed crucible, and the wet weight (WW) was measured with a chemical balance (Model AE 100, Mettler, Hightstown, New Jersey). The tissue was then dried in an oven at 105°C until it reached a constant weight (DW). The water content was calculated as (WW-DW)/WW.

to determine the tissue electrolyte concentrations, the dried tissue was digested in concentrated nitric acid and diluted appropriately with distilled-deionized water. The concentrations of sodium, potassium, and calcium were measured at 589.6, 766.5, and 422.7 nm, respectively, with an atomic absorption spectrophotometer (Model Z-8000, Hitachi, Mountain View, California).

We examined motor performance with an inclined plane test, a balance beam test, and a prehensile test originally devised by Combs and D’Alecy15 to assess the motor weakness of rats subjected to global ischemic insults. We modified this method to fit our focal ischemia model. In the inclined plane test, a 60×30 cm board covered with a thin rubber pad was fixed at an angle of 60° from the horizon. The lower edge of the board was 50 cm above a thick sponge pad. The rat was placed at the top of the board in the head-up position, and the time that the rat spent on the board (up to 30 seconds) was measured. In the balance beam test, a wooden rod 70 cm long and 3.2 cm in diameter was positioned horizontally 60 cm above a thick sponge pad. The rat was placed at the center of the rod, and the time that the rat stayed on the rod (up to 30 seconds) was measured. In the prehensile test, a nylon rope 70 cm long and 4 mm in diameter was stretched horizontally 60 cm above a thick sponge pad. After hanging the forepaws of the rat on the rope, the animal was released. The time that the rat hung onto the rope (up to 30 seconds) was measured. The score was 0, <1 second; 1, 1-10 seconds; 2, 11-20 seconds; 3, 21-30 seconds; and 4, >30 seconds. The same weight was given to each test. Therefore, the total motor score ranged from 0 to 12 points. The motor deficit was quantified by the total scores obtained from the three tests.13 Two trials were given to each rat in each test, and the better score was recorded. To avoid fatigue, the trials were interspersed with a few minutes’ rest.

The passive avoidance test is one of the learning and memory retention tests originally described by Kurtz and Pearl16 and is widely used to detect memory dysfunction.17,18 It was performed in a dim and silent room. The apparatus consisted of a 40×40-cm illuminated compartment and a 10×10-cm dark compartment with a roof and electroconductive grids on the floor. The two compartments were connected by a 6×6-cm opening that could be closed by a transparent shutter. The rat was placed

![Figure 1. Time courses of mean ± SEM sodium, potassium, water, and calcium contents in rat brain. Difference between ischemic (—) ipsilateral (●) and sham-operated (—) ipsilateral hemispheres: *p<0.05, **p<0.01, ***p<0.001. ○, contralateral hemisphere.](http://stroke.ahajournals.org/Downloaded from)
at a fixed position in the large compartment and was allowed to explore both compartments for 3 minutes. The total time spent in the small compartment (all four paws past a boundary line between the compartments) was measured using a stopwatch. After 3 minutes, when the rat was in the small compartment, the shutter was closed and weak footshocks (0.3 mA, 30 seconds) were given. In this way, the rat received an averse stimulus (learning) and a passive avoidance reaction was established. Twenty-four hours after learning, memory retention was tested in the same manner as the exploration.

To record EEG, the halothane-anesthetized rat was immobilized with 0.8 mg/kg i.p. pancuronium bromide. After transoral intubation, artificial ventilation was carried out with a rodent respirator (Model 683, Harvard Apparatus, South Natick, Massachusetts). Bilateral bone screws were placed at the upper borders of the temporal muscle (at the midpoint of the coronal and lambdoid sutures) and a third screw was placed on the interocular midline as a reference electrode. Halothane anesthesia was then discontinued, and monopolar EEGs were videotaped through a Sony PCM-701 digital audio processor (Tokyo, Japan) and analyzed with a fast Fourier transform signal processing program developed by Tektronix Inc. (Beaverton, Oregon).

Three days after surgery, the rats were anesthetized with 50 mg/kg i.p. pentobarbital sodium and reperfused with normal saline via the ascending aorta. The animals were then decapitated, and the heads were kept in a 10% neutral buffered formalin solution for 3 days. Then the brain was removed carefully and embedded in paraffin. Coronal sections (6-7 μm) were stained with hematoxylin and eosin and were examined with light microscopy.

The day of surgery was defined as Day 0. Water content and ion concentrations were measured in ischemic rats on Days 1 (n=8), 3 (n=10), 7 (n=6), and 14 (n=6); normal brain was obtained by decapitation of unoperated rats (n=13). Motor deficit was quantified in ischemic rats on Days 3 (n=14) and 7 (n=9). The passive avoidance test was performed in ischemic rats on Days 2 and 3 (n=12) (i.e., exploration on Day 2 and memory testing on Day 3). EEG was recorded on Day 0 before the ischemic insult and just before the release of the left CCA occlusion and on Day 3 and/or 7 from the same rat (n=3-6). Sham-operated rats (n=5-12) were prepared for each ischemic group.

The results are expressed as mean±SD for physiologic parameters and as mean±SEM for water content, ion concentrations, motor deficit, and results of the passive avoidance test. The differences in physiologic parameters, water content, or ion concentration between the sham-operated and ischemic groups were evaluated using Student’s unpaired t test. The motor deficit and the results of the passive avoidance test were evaluated using Wilcoxon’s test. The difference was considered significant when p<0.05.

Results

SABP under halothane anesthesia just before clamping of the left CCA was 136±12 mm Hg (n=16) for the sham-operated group and 132±11 mm Hg (n=16) for the ischemic group; there was no significant difference. Before surgery, there was no significant difference in body weight between the sham-operated (262±9 g) and ischemic groups (270±18 g). However, in the ischemic group body weight during the first 3 days after surgery increased 7±12 g, significantly less than in the sham-operated group (26±9 g, p<0.001). On Day 7, this significant difference in body weight disappeared. Compared with another rat MCA occlusion model in which body weight progressively decreased until Day 7...

**FIGURE 2.** Total motor score for sham-operated (hatched columns) and ischemic (filled columns) rats on Days 3 and 7. Differences between groups were evaluated by Wilcoxon’s test.

**FIGURE 3.** Memory impairment in rats demonstrated by passive avoidance test. Ordinate, mean±SEM time spent in dark compartment; open columns, exploration tests conducted before footshocks; shaded columns, memory retention tests performed 24 hours later. *p<0.001, different from sham-operated control group.
after surgery, our focal cortical infarction model was less invasive and postoperative feeding was easier. Unless motor performance tests were conducted, no sign of motor deficit was recognized by casual observation throughout the experiment (except on Day 1, when spontaneous movement was relatively less).

Normal cerebral hemisphere water content was 79.17±0.03%, and the normal concentrations of sodium and potassium were 259±6 and 464±9 μmol/g dry wt, respectively; normal calcium concentration was 5.9±0.2 μmol/g dry wt. Figure 1 shows the time courses of water content and electrolyte concentrations of sham-operated and ischemic rats. In ischemic rats, water content increased and peaked on Day 3, then started to decrease. The time course for sodium concentration paralleled that for water content, but that for potassium concentration was opposite. The changes in potassium concentration were smaller than those of sodium. Among these ions, calcium had the greatest percent changes; its peak concentration was almost four times the normal value, and the concentration was elevated even at Day 14 (Figure 1).

In the sham-operated group, the total motor scores on Days 3 and 7 were 11.1±0.5 and 11.0±0.2, respectively; the total motor score for the ischemic group was 6.9±0.5 on Day 3 and 7.3±0.8 on Day 7. There were significant differences between groups for Days 3 and 7 (p<0.001; Figure 2). The extent to which the systemic condition of a rat affects its motor performance was studied, with body weight gain as a parameter reflecting systemic condition. The correlation between body weight gain and total motor score for ischemic rats during the first 3 days was not significant (r=-0.36; n=13).

During exploration, the time spent within the dark compartment by sham-operated and ischemic rats did not differ significantly. In the memory retention test, the time spent in the dark compartment was 8±23 seconds for the sham-operated group, while that of the ischemic group was 124±14

**FIGURE 4.** Photographs of coronal sections of rat brain 3 days after focal ischemia (hematoxylin and eosin stain). Arrows, well-defined cortical infarct.

**FIGURE 5.** Photograph of neuronal necrosis of pyramidal cells in CA1 region of hippocampus of rat 3 days after focal ischemia (hematoxylin and eosin stain, ×400). Necrotic cells were identified by eosinophilic cytoplasm and nuclear pyknosis as well as by abnormal shape (arrows).
seconds ($p<0.001$; Figure 3). When the rats were tested on Day 7 after exploration on Day 6, memory was still impaired (data not shown).

A well-defined massive infarct was found in the ipsilateral cortex (Figure 4). There were various stages of necrotic cells in the infarcted zone, with infiltration of neutrophils. Typical coagulation necrosis (shrinkage and eosinophilic changes of cytoplasm, nuclear pyknosis) was observed, especially on the borders of the infarct. Additionally, scattered necrotic neurons were found in the contralateral cortex. Several pyramidal cells in CA1 of the dorsal hippocampus underwent necrosis (Figure 5).

EEG amplitude of the ipsilateral hemisphere was decreased to its lowest value (64.2% of preischemia value) just before release of the left CCA occlusion. EEG amplitude then recovered to 77.4% of its preischemia value by Day 3. The ipsilateral:contralateral ratio of EEG amplitude decreased to 0.58 just before release of the left CCA occlusion but gradually recovered to 0.88 and 1.00 by Days 3 and 7, respectively.

**Discussion**

In conditions of brain ischemia, the characteristic features of ionic derangement, which may lead to the pathogenesis of edema and electrophysiological dysfunction, are accumulations of calcium and sodium ions as well as a depletion of potassium ions. Our results were in good agreement with reported data.

On Day 3, water content increase correlated well with calcium concentration increase ($r=0.91, p<0.01; n=13$) and with sodium concentration increase ($r=0.85, p<0.01; n=13$). Calcium ion accumulation has been hypothesized as one of the "autodestructive events" leading to functional and structural breakdown of the cell membrane. The large increase of calcium concentration by Day 3, almost four times normal, may support this notion. It has been suggested that increased intracellular calcium concentration may be at least partially responsible for the attenuation of ionic conductance. It is of particular interest that the accumulated calcium ions were not cleared even 14 days after focal ischemia. Calcium may be retained in the necrotic tissue.

The motor performance test we used revealed that motor deficits were closely correlated with brain edema on Day 3 (total motor score vs. water content: $r=-0.79, p<0.01; n=10$; Figure 6); the correlation between body weight gain and motor deficit was not significant. Thus, we propose that motor dysfunction may be caused by ischemic brain edema rather than by poor systemic conditions. According to Hall and Lindholm, the cortical motor area of rats is the anterior dorsal cortex, particularly those areas of the hindlimb and forepaw, which extends slightly laterally. As shown in Figure 4, these areas are near or on the dorsal border of the infarcted area indicated by histopathologic staining. This may explain the significant correlation between motor deficit and the extent of infarction expressed by water content. When motor deficit recovery was compared with water content, the degree of recovery was relatively smaller than that of brain edema. Motor deficits might be produced by irreversible cell damage that took place up to Day 3.

The passive avoidance test we used revealed that focal ischemia impaired memory. It has been generally considered that memory acquisition and its retention are anatomically related to the hippocampus, temporal lobe, and cholinergic system. At first, we could not explain why learning/memory was impaired by focal cortical infarction. We considered the possibility of hippocampal damage because its highly selective vulnerability is well known. Thus, we conducted the histopathologic study to determine whether the subcortical structure was intact. That study indicated the possibility of hippocampal damage. Another possibility is learning impairment caused by consciousness disturbance since passive avoidance learning was performed on Day 2 when brain edema was most prominent. The correlation between memory disturbance (shown by an increase of time spent in the dark compartment in the retention test) and water content of the ischemic hemisphere was significant ($r=0.77, p<0.01; n=13$) on Day 3, indicating that there may be a significant relation between brain edema and memory disturbance.

In summary, we have established protocols to study ischemic brain damage by which both neurologic deficits and biochemical changes can be evaluated in the same rat. Our method consists of the motor performance tests, the passive avoidance test, and concurrent measurement of brain edema as characterized by water content and sodium, potassium, and calcium concentrations. This method is being used successfully in our laboratory to evaluate the protective effects of possible anti-ischemic drugs against brain ischemia.
References


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