Effects of intraparenchymal hemorrhage on extracellular cortical potassium in experimental head trauma

OTAKAR R. HUBSCHMANN, M.D., AND DAVID KORNHAUSER, M.D.
Section of Neurosurgery, Veterans Administration Medical Center, East Orange, and University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey

A model of intraparenchymal hemorrhage was created in 11 cats. The development of the parenchymal hemorrhage was accompanied by a massive cellular depolarization and K⁺ release in anatomically intact areas in the vicinity of the hematoma. The K⁺ clearance was rapid and in most instances total. This initial K⁺ release was not ischemic in origin but was the result of mechanical and chemical factors of the extravasated blood on cellular membranes. In contrast, an increased water content of the white matter did not affect the cellular function or levels of K⁺ in the extracellular space of the cerebral cortex in the recording area. The experimental results suggest that K⁺ release takes place at the onset of trauma in subcritically injured cellular areas in the vicinity of a hemorrhage. The cellular elements undergo massive depolarization which is accompanied by a number of chemical and biochemical changes that lead to cellular swelling. Cellular depolarization and K⁺ release appear to be the initial response of the central nervous system to trauma. The extent of this response may strongly influence the final degree of cellular damage and, thus, the neurological deficit in patients with head trauma.

KEY WORDS: potassium ion, intraparenchymal hemorrhage, head injury, ischemia, cellular swelling, cellular depolarization

The mortality and morbidity of patients suffering from head injuries remain high despite recent additions to the diagnostic and therapeutic armamentaria. It is generally agreed that two processes principally determine the degree of neurological deficit: 1) the extent of “primary” mechanical trauma to neurons; and 2) the extent of “secondary” ischemic damage that develops sometime after the trauma.

Conceptually, two types of cellular response to mechanical injury are recognized. The first leads to the rupture of cellular membrane and a total anatomical destruction of the cell. Since there is no useful regeneration of neurons in the central nervous system, this damage is anatomically permanent. The second type of injury leads to a temporary cellular dysfunction without anatomical destruction of the cell. Cells with functional disturbance are capable of functional recovery, but these cells are particularly susceptible to secondary injuries. It is not clear, however, what is the initial response of this subcritically injured group of cells to trauma, or what is the role of ischemia in determining the degree of their dysfunction. It is presumed, however, that at the cellular level the onset of trauma is accompanied by a multitude of biochemical events that may 1) be ischemic in nature, 2) lead to ischemia directly, or 3) destabilize the cellular membranes to the point that the ischemic threshold is lowered and ischemia may develop much more readily.

One factor that may play an important role in this cycle is the K⁺ released from injured cells and accumulated in the extracellular space (ECS).1,6,7,10 Such elevations in K⁺ are known to adversely affect axonal transmission,8 synaptic function,5,7 metabolism of glial cells,5,7 and resistance of microvessels.6,11 Thus, K⁺ released at the onset of trauma could be responsible for the temporary neurological deficit caused by the axonal and synaptic block. It could also participate in the development of cerebral edema and secondary ischemia due to its ability to induce a cellular swelling and increased vascular resistance.5,11

We have attempted to determine whether release of K⁺ from anatomically intact cells in the vicinity of an intraparenchymal hemorrhage accompanies the onset of trauma, whether prolonged K⁺ accumulation takes place in the ECS in that region, and whether this K⁺ release is secondary to ischemia.
Materials and Methods

A total of 11 cats were used in this study. The animals were anesthetized with sodium pentobarbital (35 mg/kg) and maintained on regular intravenous doses using the animals' reaction to pain, pupillary size, and electrocorticogram (ECOG) pattern as indicators of the level of anesthesia. A tracheostomy was performed, the femoral vein and artery were cannulated, and blood pressure monitored. Blood gases were maintained within normal limits, keeping the pCO₂ between 27 and 32 mm Hg by allowing animals to breathe unassisted or on a respirator subsequent to a paralyzing dose of gallamine triethiodide (Flaxedil, 3 mg/kg). The cerebral cortex was exposed through a left frontal craniectomy and the dura was opened under magnification using Zeiss OPMI-1 operating microscope. The recordings were made from the posterior sigmoid gyrus. The cortical surface was kept moist with constant irrigation with mock cerebrospinal fluid, and cortical and rectal temperatures were maintained between 36° and 38°C using a heating lamp and pad connected to a temperature monitor and controller.* The electrical activity of the cortical cells was monitored with surface Ag-AgCl electrode referenced against cervical muscles in a monopolar fashion. Potassium ion-selective microelectrodes were manufactured using a modification of the technique described by Walker.† Borosilicate glass capillary tubing (1.5 mm in outside diameter) was cleaned and then pulled with a Narashige puller. Pipettes with tips between 5 and 10 μm were dipped into a fresh solution of 1% Siliclad in water until a small column of the solution appeared in the tip (200 to 500 μm). They were then placed tip up in an oven set at 200°C for at least 1 hour.

Ion-selective electrodes were filled by introducing the tip into the potassium ion exchanger‡ until the terminal portion of the tip contained a small column of the solution (10 μm). The electrodes were then back-filled with 100 mM KCl.

Reference electrodes with similar tip diameters were filled with 150 mM NaCl. An ion-selective electrode and reference electrode were glued together with a quick-setting epoxy so that their tips were within 50 μm of one another. The ion-selective electrode was inserted into a WPI Ag-AgCl electrode holder§ while an Ag-AgCl wire was inserted into the reference electrode. The activity from the electrode pair was fed directly into matched high-input F-223 WPI impedance probes (10¹⁵) and differentially amplified with a common mode rejection ratio of 10,000:1.

The potassium activity and reference potential were displayed on a polygraph and oscilloscope along with monopolar ECOG referenced against the neck musculature. The electrode pair was calibrated in solutions containing varying amounts of KCl (between 3 and 30 mM) and 150 mM NaCl just prior to use and immediately following a reading.

An experimental hematoma was produced in eight animals by 1 cc of autologous blood obtained from the femoral vein being injected into the frontal subcortical white matter at a distance from the recording site, using stereotaxic coordinates. Three cats were used as controls. In these animals, both hemispheres were exposed. Injection of 1 cc of saline into the left hemisphere served as a model of a diffuse white matter edema, while injection of the same amount of agar into the right hemisphere simulated the local mass effect of the hematoma. Following termination of the experiments, the animals were sacrificed with an overdose of pentobarbital (Nembutal). The K⁺ release that followed the cardiac standstill was used both as a model of global ischemia and confirmation that the K⁺ electrodes were functional. The animals were then perfused with 10% buffered formalin and the brains removed. The brains were then stored in 10% buffered formalin solutions for 4 weeks, after which they were cut serially in sections 1 cm thick. The location of the hematoma, ventricular configuration and size, presence of herniation, and presence of macroscopic changes in the brain stem were evaluated. The recording areas and representative slices from the vicinity of the hematoma and the brain stem at the midcricoidal level were obtained at 50 μm thickness, stained with cresyl violet, and evaluated under ×40 magnification.

Results

Intraparenchymal Hemorrhage

Following injections of blood, a local increase in the size of the anterior portion of the hemisphere was visible and, in one animal, a rupture of the cortex occurred. The onset of changes in cortical K⁺ and the cortical field potential were immediate and paralleled each other as mirror images. The K⁺ rise was abrupt, reaching a peak of between 18 and 28 mM (Fig. 1). This increase was concomitant with maximal cortical depolarization characterized by the development of a negative potential between −15 and 20 mV. The K⁺ clearance lasted between 100 and 210 seconds. The preinjection levels (3.15 ± 0.25 mM) were reached in four animals; in the remaining four animals, levels exceeding previously established baselines by 0.5 to 1.5 mM were recorded. In the one animal with the ruptured cortex, the levels remained elevated at 15 mM for the duration of the experiment. Sacrifice of the animals with an overdose of Nembutal was accompanied by a terminal depolarization and K⁺ release, which served both as a control

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* Temperature monitor and controller (BAT-8, TCAT-1) manufactured by Bailey Instruments, Saddle Brook, New Jersey.
† Pipettes manufactured by Clay-Adams, Inc., 141 East 25th Street, New York, New York.
‡ Potassium ion exchanger, Model 477317, manufactured by Corning Glass Works, Corning, New York.
§ WPI electrode holder manufactured by W-P Instruments, 60 Fitch Street, New Haven, Connecticut.  ```
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model of global ischemia and a confirmation that the K⁺ electrode was functional.

On injection of blood, the ECoG record showed immediate and profound depression in voltage and disappearance of the spindle pattern (Fig. 1), which did not change throughout the experiment. There was no improvement seen in the ECoG patterns with return of the K⁺ level to normal. The terminal ischemia was accompanied by immediate disappearance of all ECoG activity, occasionally accompanied by a short burst of seizure discharge. The K⁺ levels did not start to rise for a variable time period, from a few seconds to as late as 3 minutes. The maximum K⁺ levels achieved at 20 minutes ranged from 85 to 98 mM. The rise of K⁺ was gradual, and opposite in pattern to that seen following the injection (Fig. 2).

Agar Injection

The patterns of K⁺ release and uptake after agar injection were similar to those of hematoma, but both the peak levels (9 to 12 mM) and the clearance times (40 to 80 seconds) were substantially shortened. The ECoG showed changes similar to those of intracerebral hematoma, but were less pronounced. In some instances, a transient decrease of extracellular K⁺ levels followed the return to baseline (Fig. 1).

Injection of Saline

There was no release of K⁺ on injection of saline, and no effect was observed on the ECoG (Fig. 1). The field potential recordings have shown some changes in the slow potentials in both negative and positive directions.

Pathological Findings

The serial brain cuts demonstrated the presence of an intraparenchymal hemorrhage or an agar mass. In all animals, there was some subarachnoid blood present at the injection site, but no blood was present at the recording site. The lesion varied from 0.5 cm to 1.0 cm in diameter, which probably accounted for the variability of both the peak levels and clearance times. The main portion of the hematoma was located in the subcortical white matter (Fig. 3). There was no involvement of deep midline structures or thalamus, or penetration into the ventricles. The ventricular size and configuration were equal on both sides. Serial cuts of the brain stem revealed no macroscopic changes. The cellular structures of the recording area, examined under x 40 magnification, showed no evidence of gross cellular destruction in any of the experiments, with the exception of the one animal in which rupture of the cortex took place. Similar changes were seen in agar-injected animals, while the saline injection resulted in only small increase in the white matter volume.

Discussion

The advantages and disadvantages of the K⁺ ion-specific electrode in measuring ionic fluxes in experi-
mental animals have previously been reported. The main advantage of this technique is that it measures directly the changes in the extracellular ionic milieu, and indirectly the status of the cellular membranes. Using this electrode, one can assess the function of metabolic pumps, especially Na⁺/K⁺, and make a gross estimate about the blood flow through the area beneath the electrode. The main disadvantages are the difficulty in manufacturing the electrode and its susceptibility to mechanical damage. Nevertheless, despite its drawbacks, it is currently the only device that can provide information about the functional status of the cerebral cortex and the relationship between cerebral perfusion and function of metabolic pumps simultaneously.

These experiments showed a massive K⁺ release at the onset of trauma in the subcritically injured cortical cells. The extracellular K⁺ levels, reaching up to 28 mM, indicated a serious functional disruption of membrane integrity. This disruption resulted from cellular depolarization rather than cellular destruction, as evidenced by the absence of an anatomical lesion in the recording area. Furthermore, the K⁺ clearance was rapid, and in most instances total, which was different from the experiment where anatomical rupture of cortex took place and the K⁺ levels remained elevated. The significance of mildly elevated K⁺ at new steady states after the clearance was completed is not known, and it may be a result of technical rather than physiological factors, although a mild degree of Na⁺/K⁺ pump dysfunction cannot be excluded using present technology.

The rapidity of the K⁺ clearance indicates, however, that no significant failure of metabolic pumps took place in the anatomically intact areas of the cortex surrounding the hematoma. It further appears that the initial membrane depolarization was not ischemic, nor was it followed immediately by ischemia. This conclusion was based on several lines of evidence.

During events that cause a direct cellular depolarization, the extracellular K⁺ levels reach their maximum immediately after the insult (Fig. 1). This pattern has been observed in our model of subarachnoid hemorrhage (SAH), and was also reported by Takahashi, et al., although in their model the immediate elevations were not recorded due to a delay in the placement of the K⁺ electrode, and only the downhill K⁺ slope was seen. When this pattern occurs, only a mild degree of ECoG suppression is seen. Ischemia, on the other hand, produces the opposite pattern (Fig. 2). The extracellular K⁺ levels start to rise gradually after a variable delay, ranging from several seconds to as long as 3 minutes. The ECoG record shows a rapid total suppression of electrical activity immediately after the onset of ischemia, although occasionally a seizure-like burst of electrical activity may be seen shortly after the onset.

Although the blood flow cannot be assessed quantitatively using the electrode, the K⁺ release indicating ischemic threshold can be readily detected. Astrup, et al. have correlated the changes in the blood flow and the extracellular K⁺ measured by this electrode in ischemia, and found that no elevation in extracellular K⁺ is seen unless the blood flow falls below 8 ml/100 gm/min. According to those authors, the development of ischemic changes can be predicted equally well by measurement of either blood flow or extracellular K⁺ levels. Furthermore, there is an inverse relationship between the blood flow and extracellular K⁺ levels; that is, no ischemic release of K⁺ takes place unless the blood flow falls below the threshold level and, conversely, no clearance of K⁺ from the extracellular space takes place unless the blood flow is maintained above the threshold level. Therefore, while some changes in the blood flow in the area (particularly hyperemia) could have taken place undetected, the rapid K⁺ clearance indicates that significant ischemia did not take place.

The much greater release of K⁺ in hematomas compared to agar loculi indicates that extravasated blood has a specifically toxic effect on cellular structure. This is consistent with the effects of SAH on the cellular elements reported previously. The absence of K⁺ release in saline-injected animals indicates that increased water content of white matter (often used as a model of white matter edema) in itself does not affect the membrane function of the cortical cells in the vicinity.

Our experiments and those of others suggest that an initial K⁺ release accompanies all instances of trauma that are characterized by the presence of SAH. The cellular depolarization and brief K⁺ accumulation result in a transient block of neuronal transmission caused by the elevated extracellular K⁺ levels in the subcritically injured cells in the vicinity. In addition, as demonstrated previously, the release of K⁺ is also accompanied by Ca⁺⁺ depletion in the ECS and most likely also by a local accumulation of neurotransmitters. The changes in Ca⁺⁺ concentration, in addition to direct suppression of synaptic transmission, may also increase cellular permeability and decrease the activity of metabolic energy-dependent processes. Thus, the combination

![Fig. 3. The subcortical location of the experimental hemorrhage. The recordings were made from the adjacent gyrus.](image-url)
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of elevated K\(^+\) and decreased Ca\(^{++}\) may lead to cellular swelling, induced primarily by the intracellular uptake of chloride and water.\(^5\) The K\(^+\) release in the early stage of trauma may be responsible for the early neurological deficit caused by the blockage of electrical transmission. It also appears that the initial K\(^+\) release and Ca\(^{++}\) depletion are the actual causative factors of the cellular dysfunction, rather than the subsequent cellular edema, which is the end result of this process. Reversal or containment of this process may lead to a significant decrease in the number of cells that are destroyed secondarily, and open a new avenue to better treatment of head-injured patients.

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Address reprint requests to: Otakar R. Hubschmann, M.D., Neurosurgery Section (112), Veterans Administration Medical Center, East Orange, New Jersey 07019.