Secondary ischemia impairing the restoration of ion homeostasis following traumatic brain injury

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Object. It is well established that posttraumatic secondary ischemia contributes to poor outcome. Ion dysfunction leading to cytotoxic edema is a primary force in the formation of ischemic brain edema and is a principal component of traumatic brain swelling. Because cell swelling is the result of net ion and water movement, it is crucial to have a thorough understanding of these transient phenomena. The purpose of this study was to characterize the effects of secondary ischemia following traumatic brain injury (TBI) on the ability to restore ion homeostasis.

Methods. Twenty-four Sprague-Dawley rats were divided into four groups of six animals each. The rats underwent transient forebrain ischemia via bilateral carotid artery occlusion combined with hypotension: 15 minutes of forebrain ischemia (Group 1); 60 minutes of forebrain ischemia (Group 2); impact acceleration/TBI (Group 3); and impact acceleration/TBI followed by 15 minutes of ischemia (Group 4).

Ischemia resulted in a rapid accumulation of $[K^+]$: 41.94 ± 13.65 and 66.33 ± 6.63 mM, respectively, in Groups 1 and 2, with a concomitant decrease of $[Na^+]$: 64 ± 18 mM and 72 ± 11 mM in Groups 1 and 2. Traumatic brain injury resulted in a less severe although identical trend in ion dysfunction ($[K^+]$: 30.42 ± 11.67 mM and $[Na^+]$: 63 ± 33 mM). Secondary ischemia resulted in prolonged and sustained ion dysfunction with a concomitant elevation of intracranial pressure (ICP).

Conclusions. Analysis of these results indicates that ischemia and TBI are sublethal in isolation; however, when TBI is associated with secondary ischemia, ion dysfunction is sustained and is associated with elevated ICP.

KEY WORDS • traumatic brain injury • ischemia • ion-selective electrode • potassium • sodium • rat

Despite advances in the understanding and treatment of intracranial hypertension, the outcomes in patients with severe diffuse brain swelling or intractable intracranial hypertension remain poor. Based on a review of Traumatic Coma Data Bank records, the mortality rate in patients who suffered a diffuse head injury was 34%; a good outcome or moderate disability was documented in only 16.4%. Similarly, in patients with elevated ICP persisting after TBI, death or a poor outcome is almost universally observed.1,2 The deleterious effects of secondary insults are well defined, and several investigators have identified the relationship between secondary insults and poor patient outcome.3-9,20,22,32,36 Rose, et al.,20 reported that approximately one third of the deaths in head-injured patients are caused by complications due to secondary insults. In addition, they found that at least 75% of these insults were avoidable. In caring for severely head injured patients, the prevention and treatment of secondary ischemia is as important as the treatment of the primary injury.

Abbreviations used in this paper: ATP = adenosine triphosphate; BBB = blood–brain barrier; CA = carotid artery; CAO = CA occlusion; CBF = cerebral blood flow; EEG = electroencephalography; ICP = intracranial pressure; MABP = mean arterial blood pressure; SD = standard deviation; TBI = traumatic brain injury.

Ischemia and TBI trigger a cascade of events involving membrane depolarization, neurotransmitter release, and hypermetabolism and energy crisis, which lead to a host of pathological sequelae including ion dysfunction.2,17,24,44,50 Membrane depolarization resulting from the initial injury and the activation of ligand-gated channels via excitatory amino acids exacerbate ion dysfunction.2,9,22,24,50 The cellular response to ionic perturbations, in an attempt to restore ion homeostasis, results in increased energy use and augmented metabolic demand. Ion dysfunction and metabolic crisis are exacerbated in the presence of reduced ATP, resulting from the inability of mitochondria to produce sufficient amounts or the ischemic reduction of metabolic substrate.2,3

The ion flux, which accompanies brain injury, results in the net movement of ions out of the extracellular space into cells. The movement of ions is followed isosmotically by water, which causes cell swelling and cytotoxic edema. Ion dysfunction is the primary driving force in the formation of ischemic brain edema.2,3,30 Moreover, in a recent study the authors identified cytotoxic edema as a principal component of traumatic brain swelling.8 Blood edema and swelling are often uncertain and menacing sequelae associated with brain injury. These pathological processes contribute to raised ICP, which is associated with a poor prognosis and is a frequent cause of death in head-injured patients.19 Be-
cause cell swelling is the result of net ion and water movement, it is crucial to have a thorough understanding of the time course and magnitude of these transient phenomena. The purpose of this study was to characterize the effects of secondary ischemia following TBI on the ability to restore ion homeostasis. Additionally, we sought to determine the capacity and threshold for restoring the disturbances that lead to elevated ICP.

**Materials and Methods**

**Experimental Protocol**

Twenty-four adult male Sprague–Dawley rats weighing 340 to 375 g were divided equally into four experimental groups: CAO for 15 minutes (Group 1); 60 minutes of CAO (Group 2); TBI (Group 3); and TBI combined with 15 minutes of CAO (Group 4). The animals were initially anesthetized with halothane, intubated, and mechanically ventilated via an endotracheal tube with 1.5 L/minute N₂, 0.8 L/minute O₂, and 0.6% halothane. Arterial blood gas levels were maintained throughout the experiment (PO₂ 100–130 mm Hg and PCO₂ 35–40 mm Hg).

Bilateral femoral arteries and a single vein were cannulated to obtain measurements of MABP and arterial blood gas levels, as well as to deliver fluids. The MABP was maintained at 100 mm Hg. The animals were hydrated with saline at a rate of 10 ml/kg/hr, and their temperature was maintained at 37°C by using a rectal temperature probe and a heating lamp.

**Surgical Procedures**

In animals undergoing CAO, a midline neck incision was made, and all overlying neck muscles were carefully reflected to expose the common carotid arteries. Snare ligatures consisting of No. 3-0 Ethicon (Johnson & Johnson, Somerville, NJ) were looped around the common carotid arteries. The animal was positioned in a stereotactic frame (David Kopf Instruments, Tujunga, CA), and ear bars were used to secure the head. A midline incision in the scalp was made, and all overlying tissue was reflected. Four burr holes were made for placement of the Na⁺ and K⁺ ion-selective electrodes, the reference electrode, and ICP sensor.

**Induction of Ischemia**

Transient forebrain ischemia was produced using a model of CAO combined with hypotension. Hypotension was achieved by withdrawing arterial blood and increasing the level of halothane anesthetic to reduce MABP by 40 mm Hg. Halothane was increased 45 seconds prior to occluding the CAs. The timing of the ischemic insult began when MABP reached 40 mm Hg. The brain was reperfused by removing the CA snares, infusing the arterial blood, and decreasing the halothane to return MABP to baseline values.

**Traumatic Brain Injury**

The impact-acceleration model of closed-head injury was used to induce TBI. Briefly, a midline scalp incision was made, and the skin and periosteum were reflected. A 10-mm round stainless-steel disk was positioned midline between the bregma and lambda and fixed to the skull vault with superglue. The rats were placed prone on a foam bed with a known spring constant under a hollow Plexiglas tube. A sectioned brass weight of 450 g was dropped from a height of 2 m onto the center of the metal disk. After injury, the animal was reconnected to the ventilator. In animals undergoing TBI and CAO of 15 minutes’ duration (Group 4), the same ischemia protocol was followed as for those undergoing 15-minute CAO alone (Group 1); following impact-acceleration injury, the rats were placed in the stereotactic frame and ischemia was initiated.

**Ion Analysis**

Custom-fabricated ion-selective electrodes (Microelectrodes, Inc., Bedford, NH) were used to monitor [K⁺] and [Na⁺]. The electrodes were calibrated using mock–cerebrospinal fluid solutions with concentrations of K⁺ and Na⁺ ranging from 0 to 50 and 160 to 75 mM, respectively. Following calibration, the ion-selective electrodes were inserted into the parietal cortex, 1.5 mm below the dura mater. After allowing 1 hour for stabilization, a 10-minute baseline was es-

![Graph depicting data obtained in Group 1. Note the changes in MABP, ICP, [Na⁺], and [K⁺], during ischemia-reperfusion. Mean data are presented ± SDs.](image)
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established. In those animals undergoing impact-acceleration injury, the electrodes were temporarily removed to induce TBI.

Statistical Analysis of Ionic Changes

Each animal served as its own internal control. To allow for variability of animals and determine significant changes in Na\(^+\) and K\(^+\), a 95% confidence interval (± two SDs) was established in each experiment by using the first 10 minutes to establish baseline values. Repeated-measures one-way analysis of variance was used to determine the time points at which ICP deviated from the respective baselines. A one-way analysis of variance was used to determine statistical significance among groups. Probability values of less than 0.05 were considered statistically significant. Data are presented as the mean ± SD.

Results

Ischemic Injury

Physiological Parameters. The MABP was significantly reduced during the ischemic period (40 ± 12 and 39 ± 7 mm Hg [p < 0.05] in Groups 1 and 2, respectively; Figs. 1 and 2). It returned to preischemia values in both groups following the ischemic insult. The ICP appeared to alter slightly from baseline, but it did not significantly differ from control values in Group 1 (Fig. 1). In Group 2, ICP transiently increased after ischemia and remained elevated (13 ± 1 mm Hg) for 10 minutes (p < 0.05) but then gradually returned to baseline values (Fig. 2).

Potassium. Ischemia resulted in a rapid accumulation of K\(^+\), requiring 2 ± 0.25 and 1 ± 0.5 minutes to reach significance in Groups 1 and 2, respectively (Figs. 1 and 2). Extracellular potassium remained elevated for the duration of the insult, with the highest values attained at the end of ischemia (Figs. 1 and 2). The maximal [K\(^+\)] values varied depending on the duration of the ischemic insult (41.94 ± 13.65 and 66.33 ± 6.63 mM in Groups 1 and 2, respectively; p < 0.05) (Table 1). Clearance rates for [K\(^+\)] also varied with the duration of the ischemic insult (1.8 ± 0.84 and 3.75 ± 0.5 minutes in Groups 1 and 2, respectively; p < 0.05) (Table 1). Following the restoration of MABP, [K\(^+\)],

![Graph demonstrating data obtained in Group 2. Note the changes in MABP, ICP, [Na\(^+\)], and [K\(^+\)], during ischemia-reperfusion. Mean data are presented ± SDs.](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Preinjury</th>
<th>Max</th>
<th>Postinjury</th>
<th>Clearance (t(_{1/2})) (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.56 ± 0.32</td>
<td>41.94 ± 13.65</td>
<td>3.44 ± 0.46</td>
<td>1.80 ± 0.84</td>
</tr>
<tr>
<td>2</td>
<td>4.55 ± 0.38</td>
<td>66.33 ± 6.63</td>
<td>3.82 ± 0.34</td>
<td>3.75 ± 0.50</td>
</tr>
<tr>
<td>3</td>
<td>4.43 ± 1.10</td>
<td>30.42 ± 11.67</td>
<td>4.66 ± 1.01</td>
<td>5.00 ± 2.64</td>
</tr>
<tr>
<td>4</td>
<td>4.51 ± 1.44</td>
<td>65.12 ± 13.13</td>
<td>20.44 ± 15.63</td>
<td>17.67 ± 3.51</td>
</tr>
</tbody>
</table>

* Groups included 15 minutes of CAO (Group 1); 60 minutes of CAO (Group 2); TBI alone (Group 3); and TBI followed by 15 minutes of CAO (Group 4). Abbreviation: t\(_{1/2}\) = half-life.
† Values are presented as the means ± SDs.
normalized to preischemic levels (3.44 ± 0.46 and 3.83 ± 0.34 mM in Groups 1 and 2, respectively).

Sodium. Intergroup baseline [Na+]e levels determined prior to injury were not significantly different (148 ± 11 and 159 ± 13 mM in Groups 1 and 2, respectively) (Table 2). At the onset of ischemia, all animals exhibited an initial elevation of [Na+]e and a subsequent rapid decline (Figs. 1 and 2). Extracellular sodium was reduced for the duration of the ischemic insult, with the lowest values measured at the end of the ischemic insult (Table 2). Contrary to what was observed with [K+]e, the minimum intergroup values did not significantly differ (64 ± 18 mM [Group 1] and 72 ± 11 mM [Group 2]; p > 0.05). Restoration of [K+]e exhibited a biphasic response, initially accumulating above normal and then gradually declining to baseline.

Traumatic Brain Injury

Physiological Parameters. Baseline MABP was 111 ± 1 mm Hg and exhibited a significant elevation to 124 ± 4 mm Hg (p < 0.05) during the first 30 minutes after TBI (Fig. 3). Intracranial pressure was also significantly elevated (mean 15 ± 2 mm Hg) for 30 minutes immediately after trauma (p < 0.05).

Potassium. The baseline [K+]e value was not significantly different in the ischemia groups (4.43 ± 1.1 mM). The maximal [K+]e values were observed immediately after TBI

### TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Preinjury</th>
<th>Min</th>
<th>Postinjury</th>
<th>Clearance (t1/2) (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>148 ± 11</td>
<td>64 ± 18</td>
<td>153 ± 19</td>
<td>4.00 ± 0.82</td>
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<tr>
<td>2</td>
<td>159 ± 13</td>
<td>72 ± 11</td>
<td>168 ± 14</td>
<td>4.25 ± 2.75</td>
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<tr>
<td>3</td>
<td>140 ± 15</td>
<td>63 ± 33</td>
<td>143 ± 17</td>
<td>4.20 ± 1.64</td>
</tr>
<tr>
<td>4</td>
<td>147 ± 15</td>
<td>42 ± 12</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Values are presented as the means ± SDs. — = the [Na+]e level could not be calculated; it was never restored postinjury.

![Graph depicting data obtained in Group 3. Note the changes in MABP, ICP, [Na+]e, and [K+]e. Mean data are presented ± SDs.](image)
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(30.42 ± 11.67 mM) (Fig. 3). This peak, however, was significantly less than that measured in Group 2. Following TBI, [K\textsuperscript{+}] gradually returned to baseline, where it remained for the duration of the experiment; this value was not significantly different from pretrauma values (post-TBI 4.66 ± 1.01 mM; p > 0.05). The clearance rate of [K\textsuperscript{+}] in Group 2 had a calculated half-life of 5.26 ± 2.64 minutes, a significantly longer duration than that observed in Group 1 (1.8 ± 0.84 minutes; p < 0.05). Although the maximal level of [K\textsuperscript{+}], measured with TBI was less than that seen in both ischemia groups, the clearance rate was not significantly different from that in Group 2 (3.75 ± 0.5 minutes; p > 0.05) (Table 1). 

Sodium. Immediately following injury [Na\textsuperscript{+}] exhibited its lowest value (63 ± 33.59 mM) but gradually returned toward baseline (Fig. 3). The calculated half-life of [Na\textsuperscript{+}], 4.2 ± 1.64 minutes, was similar to the calculated times in both ischemia groups (Table 2). As was observed in the ischemia groups, [Na\textsuperscript{+}], briefly rose above baseline and then gradually returned to pre-TBI values (143 ± 17 mM).

Traumatic Brain Injury and Secondary Ischemia (Group 4)

Physiological Parameters. Blood pressure was significantly reduced during the ischemic period but returned to control values when ischemia was terminated. Intracranial pressure exhibited a marked rise following ischemia and remained significantly elevated for the duration of the experiment (Fig. 4). The mean ICP during the 1st hour of reperfusion was 34.3 ± 14.2 mm Hg (p < 0.05).

Potassium. The baseline [K\textsuperscript{+}] value was 4.51 ± 1.44 mM. The maximal [K\textsuperscript{+}] value obtained at the termination of the ischemic insult, 65.12 ± 13.13 mM, was significantly greater than that in animals undergoing trauma only (Group 3) (p < 0.05) (Fig. 4 and Table 1). This value, however, was comparable with the [K\textsuperscript{+}] level measured after 60 minutes of CAO (66.33 ± 6.63 mM; p > 0.05). The clearance rate of K\textsuperscript{+} was significantly longer than that observed in other experimental groups (half-life 17.67 ± 3.51 minutes) (Table 1). Moreover, [K\textsuperscript{+}], failed to return to baseline values and remained significantly elevated for the duration of the experiment (Fig. 4 and Table 1).

Sodium. The mean baseline [Na\textsuperscript{+}], was 147 ± 15 mM. Similar to that observed in animals undergoing ischemia alone (Group 3), the greatest change in [Na\textsuperscript{+}], was demonstrated at the termination of the ischemic insult (42 ± 12 mM) (Fig. 4). A half-life for [Na\textsuperscript{+}], could not be calculated because [Na\textsuperscript{+}], was not restored (Table 2).

Discussion

Analysis of the results of this study shows that during ischemia and TBI there is a marked accumulation of [K\textsuperscript{+}], with an accompanying reduction in [Na\textsuperscript{+}], which are restored to baseline after injury. Secondary ischemic insults, however, impede the restorative capacity of the injured brain and prevent the return of ion homeostasis, which contributes to the development of raised ICP.

In the uninjured brain, ion gradients are established and maintained by active and passive processes in cells. Traumatic brain injury and ischemia trigger a cascade of events, including mechanical deformation, neurotransmitter release, metabolic energy perturbation, and membrane depolarization, that lead to ion dysfunction.2,3,13,35,44 Although the initiating events may differ with the type of injury, the
driving force for ion movement is the same and results from ions diffusing down their electrochemical gradients. In an attempt to restore ion homeostasis there is increased activity of transport processes and subsequent energy consumption.\textsuperscript{47,48} When transport processes are damaged or overpowered, however, the ability to maintain these gradients is surpassed. A compounding factor is that during this time of increased energy demand, there may be reduced ATP production due to decreased delivery of metabolic substrate or impaired mitochondria activity. As a result, there is a mismatch between the energy produced and the energy needed to restore ion homeostasis.

The ability to measure the degree and time course of altered ion homeostasis is crucial to understand brain injury and swelling. It is well documented that $[K^+]_i$ increases during brain injury.\textsuperscript{10,25,26,35,48} Based on simple pathophysiology, the inability to maintain ion gradients should be accompanied by a concomitant movement of $Na^+$. Although numerous studies have been focused on $[K^+]_i$, few have been conducted to monitor $[Na^+]_i$.\textsuperscript{16,42} In this study we present the first results of simultaneous measurements of $[Na^+]_i$ and $[K^+]_i$ in TBI as well as secondary ischemia. We clearly found that the ionic disturbances in TBI are identified by an increase in $[K^+]$, with a concomitant decrease in $[Na^+]$.

**Secondary Insults**

The pathological cascades caused by trauma and secondary injury are the result of numerous cascades, and as a result, defining the sequence of events is an arduous task. In earlier studies of various brain injury models investigators reported the deleterious effects of posttraumatic secondary insults.\textsuperscript{21,28,34} Secondary insults prolong the time of BBB disruption and contribute to the regional breakdown of the BBB.\textsuperscript{41} In other studies, Kita and Marmarou\textsuperscript{23} and Ito, et al.\textsuperscript{28} have demonstrated posttraumatic increases in brain edema and rapidly rising ICP, which contribute to secondary damage in the injured brain.\textsuperscript{0,22,41,43} Taken in concert with the present findings, when trauma is followed by secondary ischemia, the brain is unable to restore ion homeostasis, ion dysfunction persists, and is accompanied by elevated ICP. Cerebral blood flow values were not obtained in the present study. The present experimental design required four burr holes, two bilaterally. Implementation of CBF monitoring would require additional, larger burr holes. Omission of CBF monitoring, therefore, allowed for a larger skull surface for the impact-acceleration injury; however, we cannot determine if the failure to recover from ion dysfunction was the result of reduced CBF, mitochondrial injury, and impaired ATP production, or if it was the result of inadequate energy supply.

Both trauma and ischemia are sublethal in isolation, causing minimal neuronal damage after 24 hours.\textsuperscript{49} When these injuries are combined and trauma is followed by a secondary ischemic insult, there is increased neuronal damage. The model used is one of severe TBI and has been well characterized for neuronal damage, edema formation, and BBB disruption.\textsuperscript{5,5,30,49} The ischemia durations were chosen based on previous unpublished observations, which revealed that 15 minutes of CAO-based ischemia consistently resulted in the restoration of ion homeostasis, followed by reestablishment of EEG activity. Ischemia of 30 and 45 minutes varied with the ability to restore EEG.

We confirmed in the present study that ion homeostasis is completely reversible after ischemia and TBI. When trauma is associated with a secondary insult such as ischemia, however, the injury is severe and ischemic dysfunction persists. Additional studies are needed to determine if the inability to restore ion dysfunction is the result or cause of increased neuronal damage.

**Energy-Dependent Processes**

The production of ATP in both ischemia and trauma necessitates the delivery of a substrate in sufficient amounts so that levels of ATP meet the demands of the injured cells. Restoration of CBF provides O$_2$, and substrate to the injured brain; however, mitochondrial function is a prerequisite for the ability to produce ATP. Although mitochondria are extremely sensitive to ischemic injuries, ATP production can be restored after ischemia.\textsuperscript{15,25,36}

The results of TBI studies confirm the preservation of cortical oxidative capacity and the capacity to produce ATP.\textsuperscript{14,45,46} In a study involving the same model of TBI, mitochondrial oxidative capacity was elevated within 1 hour of trauma. Moreover, the cytosolic phosphorylation ratio was reduced, representing an increased demand or hypermetabolic state of the injured brain. Analysis of the results of this study suggests that the mechanisms of ion restoration following injury involve energy-dependent processes. The observed so-called overshoot during $[Na^+]_i$ restoration is indicative of active transport mechanisms. Furthermore, the clearance of $[K^+]_i$ suggests transport dependency with clearance rates following first-order kinetics, characteristic of an enzyme-mediated ATP-dependent process.

**Conclusions**

To the best of our knowledge, this is the first report to document $[Na^+]_i$, and $[K^+]_i$, transients that result from TBI. Although the exact mechanisms still need to be defined, ischemia and TBI initiate ionic disturbances identified by an increase in $[K^+]$, and a concomitant reduction in $[Na^+]$. Secondary ischemic insults exacerbate brain injury and impair the ability to restore ion homeostasis that is crucial for proper neuronal function, and they contribute to raised ICP.

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