Background and Purpose—Thiopental has been shown to protect against cerebral ischemic damage; however, it has undesirable side effects. We have examined how thiopental alters histological, physiological, and biochemical changes during and after hypoxia. These experiments should enable the discovery of agents that share some of the beneficial effects of thiopental.

Methods—We made intracellular recordings and measured ATP, sodium, potassium, and calcium concentrations from CA1 pyramidal cells in rat hippocampal slices subjected to 10 minutes of hypoxia with and without 600 μmol/L thiopental.

Results—Thiopental delayed the time until complete depolarization (21 ± 3 versus 11 ± 2 minutes for treated versus untreated slices, respectively) and attenuated the level of depolarization at 10 minutes of hypoxia (−33 ± 6 versus −12 ± 5 mV). There was improved recovery of the resting potential after 10 minutes of hypoxia in slices treated with thiopental (89% versus 31% recovery). Thiopental attenuated the changes in sodium (140% versus 193% of prehypoxic concentration), potassium (62% versus 46%), and calcium (111% versus 197%) during 10 minutes of hypoxia. There was only a small effect on ATP (18% versus 8%). The percentage of cells showing clear histological damage was decreased by thiopental (45% versus 71%), and thiopental improved protein synthesis after hypoxia (75% versus 20%).

Conclusions—Thiopental attenuates neuronal depolarization, an increase in cellular sodium and calcium concentrations, and a decrease in cellular potassium and ATP concentrations during hypoxia. These effects may explain the reduced histological, protein synthetic, and electrophysiological damage to CA1 pyramidal cells after hypoxia with thiopental.

Key Words: anoxia ■ barbiturates ■ cerebral ischemia ■ hypoxia ■ rats
techniques used in the present study have been reported by us before and will be described briefly.8-10,13 Transverse hippocampal slices were prepared from adult male Sprague-Dawley rats (aged 90 to 120 days) that were anesthetized with 2% isoflurane before decapitation.14 The slices were incubated at room temperature for 45 minutes, and then the temperature was increased to 37°C and maintained at that level for the rest of the experiment. Artificial cerebrospinal fluid (aCSF) contained (mmol/L) NaCl 126, KCl 3, KHPO4, 1.4, NaHCO3, 26, glucose 4, MgSO4, 1.3, and CaCl2, 1.4, at pH 7.4, and was equilibrated with 95% O2/5% CO2. Hypoxia was generated by switching to aCSF preequilibrated with 95% N2/5% CO2; this led to an oxygen concentration of 30 mm Hg in the aCSF superfusing the slice. There is a gradient of oxygen in the slice during hypoxia such that the center of the slice has less oxygen. Unless indicated in the text, the duration of hypoxia is 10 minutes. In the thiopental-treated groups, slices were exposed to 600 μmol/L thiopental 15 minutes before the hypoxia, during, and 10 minutes after the hypoxia. Each animal yields up to 20 slices; slices from the same animal are distributed among the different experimental groups.

**Electrophysiology**

The hippocampal slices were submerged 1 mm below the aCSF surface and superfused at a rate of 3 mL/min. A bipolar stimulating electrode was placed in the Schaffer collateral pathway before impaling a CA1 neuron with a glass micropipette filled with 4 mol/L KAc (70 to 120 MΩ). Only neurons with stable resting potentials of at least −55 mV for 15 minutes with high-amplitude short-duration action potentials that showed spike frequency accommodation and were activated by short latency Schaffer collateral stimulation were examined. These parameters are typical of CA1 pyramidal cells, and our recordings were typically stable for >1 hour.

**ATP, Sodium, and Potassium Measurements**

Slices were mounted on nylon mesh attached to a Plexiglas grid and placed in beakers containing aCSF. Slices from the same animal were distributed to beakers subjected to either control or experimental treatments. The aCSF in the beakers was aerated with 95% O2/5% CO2 to generate hypoxia, the aCSF in a beaker was aerated with 95% N2/5%CO2. Sodium, potassium, and ATP levels in tissue from the CA1 region were measured before, during, 30 minutes after, and 90 minutes after the hypoxia. ATP concentrations were measured from the microdissected CA1 region of slices frozen in liquid nitrogen and lyophilized.14,15 To measure sodium and potassium concentrations, slices were placed in agitated ice-cold (4°C) isotonic sucrose for 10 minutes at the end of the experiment. This procedure was used to wash ions from the extracellular space.8 The CA1 regions of the slices were microdissected; CA1 regions from different slices from the same animal were pooled so that there was enough tissue to measure sodium and potassium. The pooled tissue was dried at 85°C for 48 hours and weighed. Diluted nitric acid (0.1N) was added to the tissue and allowed to incorporate [3 H]leucine into protein after hypoxia is due to altered uptake. The change in [3 H]leucine incorporation into protein after hypoxia is due to altered uptake. The change in [3 H]leucine that we measured is therefore a good measure of new protein synthesis. Slices were dehydrated and embedded in methylacrylate resin. Sections (5 μm) were mounted on coated slides, dehydrated, cleared, dried, and dipped in Kodak NTB-2 liquid emulsion.12,13 The slides were exposed for 7 days at 4°C so that the emulsion was in the linear range for densitometric analysis and the silver grain density was proportional to the amount of radioactivity incorporated into newly synthesized protein. Autoradiographs were viewed with dark-field illumination, digitized (CCD camera and LG-3 PCI card, Scion Corp), and measured densitometrically by use of NIH Image software. Densities from experimental groups were compared with densities of control sections contained on the same slide exposed to identical conditions after the experiment.

**Protein Synthesis and Morphology**

Forty-five minutes after the end of hypoxia, 4.5 μCi/mL [3 H]leucine was added to each beaker, and the slices were allowed to incorporate [3 H]leucine into protein for an additional 75 minutes. Slices were washed in ice-cold buffer for 3 minutes to remove extracellular [3 H]leucine and were fixed overnight in 4% paraformaldehyde in 0.1 mol/L phosphate buffer at 4°C. [3 H]Leucine incorporated into protein, but not free [3 H]leucine, is fixed by paraformaldehyde. Anoxia/aglycemia, a more severe insult than used in the present study, does not alter [3 H]leucine uptake in the period after anoxia/aglycemia.12 It is unlikely that the decrease in [3 H]leucine incorporation into protein after hypoxia is due to altered uptake. The change in [3 H]leucine that we measured is therefore a good measure of new protein synthesis. Slices were dehydrated and embedded in methylacrylate resin. Sections (5 μm) were mounted on coated slides, dehydrated, cleared, dried, and dipped in Kodak NTB-2 liquid emulsion.12,13 The slides were exposed for 7 days at 4°C so that the emulsion was in the linear range for densitometric analysis and the silver grain density is proportional to the amount of radioactivity incorporated into newly synthesized protein. Autoradiographs were viewed with dark-field illumination, digitized (CCD camera and LG-3 PCI card, Scion Corp), and measured densitometrically by use of NIH Image software. Densities from experimental groups were compared with densities of control sections contained on the same slide exposed to identical conditions after the experiment.

Morphological assessment was accomplished on adjacent tissue sections stained with a modified Richardson’s stain and viewed with bright-field illumination at ×200.13 The cells in the CA1 pyramidal layer were classified into 3 categories by use of a scoring method described previously.13 Class A neurons are healthy in appearance with intact distinct membranes, a clear uniform nucleus, and clear cytoplasm. Class B neurons are either less distinct with a less prominent nucleus or more darkly stained with a distorted shape. Class C neurons lack a distinct nuclear boundary and have either a vesiculated cytoplasm or are notably swollen. The histologist was blinded to the experimental treatment.

The morphological data were analyzed with a Kruskal-Wallis test followed by the Dunn multiple comparison test. Unless explicitly stated, all other data were analyzed parametrically with an ANOVA followed by the Dunnett multiple comparison test or Student t test to determine significance (Prism, GraphPad Software). A value of P<0.05 was considered significant.

**Results**

**Electrophysiology**

Intracellular recordings were made from CA1 pyramidal cells before, during, and after hypoxia. The mean resting potential before hypoxia was −65 mV; hypoxia caused a hyperpolarization, followed by a slow depolarization, and finally a rapid and complete depolarization (Figures 1 and 2). This final depolarization was concomitant with a complete loss of excitability. Thiopental caused a slight depolarization in the period before hypoxia; however, during hypoxia it reduced the rate of depolarization and increased the time until com-
Figure 1. Effect of thiopental (600 μmol/L) on membrane potential during hypoxia. The mean±SE of 12 untreated neurons and 9 thiopental-treated neurons subjected to continuous hypoxia is graphed. During initial phases of hypoxia, thiopental neurons were more depolarized than untreated neurons; after ~8 minutes of hypoxia, thiopental neurons were less depolarized than untreated neurons.

ATP
Thiopental (600 μmol/L), when present before, during, and after hypoxia, significantly reduced the fall in ATP during hypoxia to 18% of its normoxic concentration; this is comparable to a fall to 8% in untreated slices (Table 1). In both thiopental-treated and untreated tissue, ATP concentrations recovered to ~70% and 60% of their normal levels after 30 and 90 minutes of reoxygenation, respectively. This represents a significant recovery of ATP, even though it remained significantly lower than its concentration before hypoxia. Thiopental not only reduced the fall in ATP during hypoxia but improved the recovery of ATP after hypoxia.

Figure 2. Effect of thiopental on electrophysiological responses in 2 CA1 pyramidal cells before, during, and after 10 minutes of hypoxia. A, Continuous recording from a neuron in an untreated slice is displayed; hypoxia is indicated by solid bar below trace. Resting potential remained at 0 mV after hypoxia even though the slice was reoxygenated. Intermittent traces above this recording are evoked responses from a different experiment in which the Schäffer collateral pathway was stimulated with a bipolar electrode. The placement of these intermittent traces indicates the corresponding time in the lower continuous recording when these traces were obtained. B, Conditions were as described above, but thiopental (600 μmol/L) was present before hypoxia and washed out after hypoxia. Both resting potential and evoked response in the CA1 pyramidal cells recovered. Vertical calibration bar is 20 mV for both the evoked responses and the continuous traces; horizontal calibration bar is 100 milliseconds.

Sodium and Potassium
The cellular concentration of sodium increased to 193% of its normoxic level during 10 minutes of hypoxia; with thiopental, this increase was significantly attenuated to 140% (Table 1). The increase due to hypoxia was significant for both the untreated and the thiopental-treated groups. There was complete recovery of the sodium concentrations 30 and 90 minutes after the hypoxic period in both untreated and thiopental-treated tissue.

Potassium concentrations decreased during hypoxia, falling to 46% of their normoxic concentration in untreated tissue and to 62% of these levels in thiopental-treated tissue (Table 1). Thiopental significantly attenuated the fall in potassium during hypoxia. In the period after 10 minutes of hypoxia, potassium levels returned to normal in both the thiopental-treated and the untreated slices.
Thiopental reduced the changes in sodium and potassium during hypoxia, but even in untreated tissue, these ions returned to their normal concentrations by 30 minutes after hypoxia and remained normal 90 minutes after hypoxia.

**Calcium**

During 10 minutes of hypoxia, the concentration of intracellular calcium increased to 197% of its baseline normoxic concentration (Figure 3). Thiopental blocked the increase in calcium during hypoxia (111% of baseline).

The calcium concentration of untreated tissue 35 minutes after hypoxia was not significantly different from its prehypoxic concentration (110% of baseline). In slices treated with thiopental during hypoxia, calcium was reduced to 55% of its baseline concentration 35 minutes after hypoxia (Figure 3).

Thiopental significantly attenuated the rise in calcium during hypoxia, and the calcium concentration returned to its prehypoxic level more quickly after hypoxia. The calcium concentration in thiopental-treated slices continued to fall significantly below its prehypoxic control level.

**Morphology**

In normoxic slices, 48% of the neurons appear morphologically healthy (class A), whereas 28% of the neurons in these CA1 layers had clear pathological changes (class C) (Table 2 and Figure 4). Twenty-four percent of the cells were of intermediate appearance (class B).

Hypoxia caused persistent morphological changes in CA1 neurons (Figure 4). Only 10% of the CA1 neurons exhibited healthy morphology (class A), whereas 71% of the neurons exhibited a clear pathological morphology (class C) by 2 hours after 10 minutes of hypoxia (Table 2). There was a significant increase in the number of CA1 pyramidal cells with a pathological morphology and a decrease in cells with a healthy morphology after 10 minutes of hypoxia.

There were more class A neurons in the thiopental-treated slices compared with slices subjected to hypoxia in the absence of thiopental (34% versus 10%). There were also correspondingly fewer class C neurons in the thiopental-treated slices (45% versus 71%). Treatment with 600 μmol/L thiopental during hypoxia significantly improved neuronal morphology after hypoxia (Table 2).

**Protein Synthesis**

Normoxic slices exhibit robust protein synthesis (69% of the CA1 layer with silver grains, Table 2). Hypoxia resulted in a significant decrease in [3H]leucine incorporation to 20% of normoxic incorporation (Table 2). Thiopental treatment during hypoxia significantly attenuated the inhibition of protein synthesis such that it recovered to 75% of normoxic levels. The protein synthesis in slices with thiopental present during

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**TABLE 1. Effect of Hypoxia and Thiopental (600 μmol/L) on Metabolite Concentrations in CA1 Region of Rat Hippocampal Slices**

<table>
<thead>
<tr>
<th>Metabolite Concentration, nmol per mg dry wt</th>
<th>ATP</th>
<th>Sodium</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td>5.0±0.16 (24)</td>
<td>127±10 (7)</td>
<td>170±11 (7)</td>
</tr>
<tr>
<td>Normoxia (thiopental)</td>
<td>4.9±0.14 (23)</td>
<td>113±4 (6)</td>
<td>176±5 (6)</td>
</tr>
<tr>
<td>5-min hypoxia</td>
<td>. . .</td>
<td>184±12* (5)</td>
<td>147±8 (5)</td>
</tr>
<tr>
<td>5-min hypoxia (thiopental)</td>
<td>. . .</td>
<td>141±5† (5)</td>
<td>141±3* (5)</td>
</tr>
<tr>
<td>10-min hypoxia</td>
<td>0.4±0.04* (24)</td>
<td>245±13* (8)</td>
<td>78±10* (8)</td>
</tr>
<tr>
<td>10-min hypoxia (thiopental)</td>
<td>0.9±0.04† (24)</td>
<td>177±13† (6)</td>
<td>106±6† (6)</td>
</tr>
<tr>
<td>30-min posthypoxia</td>
<td>2.9±0.07* (24)</td>
<td>150±9 (7)</td>
<td>178±3 (7)</td>
</tr>
<tr>
<td>30-min posthypoxia (thiopental)</td>
<td>3.5±0.07† (24)</td>
<td>132±11 (6)</td>
<td>161±7‡ (6)</td>
</tr>
<tr>
<td>90-min posthypoxia</td>
<td>3.1±0.06* (23)</td>
<td>158±8 (7)</td>
<td>168±7 (7)</td>
</tr>
<tr>
<td>90-min posthypoxia (thiopental)</td>
<td>3.6±0.08*† (23)</td>
<td>158±16 (6)</td>
<td>168±6 (6)</td>
</tr>
</tbody>
</table>

Values are mean±SE. For ATP values, numbers in parentheses indicate the number of slices; each group contained 6 rats. For sodium and potassium values, numbers in parentheses indicate the number of rats; slices from individual rats were pooled.

*P<0.01 compared with corresponding normoxic group by Dunnett multiple comparison test.

†P<0.01 and ‡P<0.05 vs. untreated group at that time period by t test.
hypoxia was not significantly different from normoxic incorporation.

Discussion

The use of in vitro preparations to model clinical pathology is fraught with difficulty, and the interpretation of such experiments is frequently confounded by the use of nonphysiological conditions in vitro. We use a hippocampal slice model that we have substantially modified to mimic in vivo conditions. For example, we maintain slices at 37°C and use physiological concentrations of magnesium (1.4 mmol/L), calcium (1.4 mmol/L), and glucose (4 mmol/L) in the artificial cerebrospinal fluid. An important distinction between our model and tissue culture models of hypoxia/ischemia is that our tissue is from adult animals, which are more sensitive to hypoxia/ischemia. The adult slices maintain their macroscopic cellular architecture with respect to the major inputs and outputs to the CA1 pyramidal cells, which are examined in the present study. An important distinction between our results and in vivo and clinical results is that we are examining the direct effects of pharmacological agents on neurons; there are no vascular or systemic effects in our in vitro preparation. In spite of this, we have found many similarities between our results and those from in situ preparations.

During hypoxia, the slices are continuously perfused with aCSF containing 4 mmol/L glucose; this not only provides a source of energy via anaerobic glycolysis but will wash metabolites and transmitters from the extracellular space and maintain extracellular pH. Thus, the hippocampal-slice hypoxic procedures that we use would more closely model focal or incomplete global ischemia than complete global ischemia.

The concentration of thiopental used in the present study was previously found to provide robust protection against hypoxic damage in brain slices7,8 and approximates a high barbiturate coma dose, which Nussmeier et al13 demonstrated improves neuropsychiatric outcome after cardiopulmonary bypass surgery.

<table>
<thead>
<tr>
<th></th>
<th>Protein Synthesis, %</th>
<th>Morphology, %</th>
<th>Resting Potential, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type A</td>
<td>Type B</td>
</tr>
<tr>
<td>Normoxia</td>
<td>69±7 (19)</td>
<td>48±5 (19)</td>
<td>24±2</td>
</tr>
<tr>
<td>Normoxia/thiopental</td>
<td>72±5 (18)</td>
<td>40±6 (16)</td>
<td>24±2</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>14±5* (17)</td>
<td>10±4‡ (16)</td>
<td>19±2</td>
</tr>
<tr>
<td>Hypoxia/thiopental</td>
<td>58±7‡ (17)</td>
<td>34±7§ (16)</td>
<td>21±2</td>
</tr>
</tbody>
</table>

Values are mean±SE. Numbers in parentheses are the number of slices; each group contained slices from 5 rats. Protein synthesis is calculated as percent silver grain density. Morphology is calculated as percent of total neurons (type A, healthy; type C, damaged; see Materials and Methods for criteria). Resting potential is calculated as percent of neurons that recover their resting potential.

*P<0.001 vs normoxia and †P<0.001 vs hypoxia by Newman-Keuls test (ANOVA, P<0.001) for protein synthesis data.

‡P<0.001 vs normoxia and §P<0.05 vs hypoxia by Dunn test (Kruskal-Wallis, P<0.001) for morphological data; all comparisons are between values in the same column.

|                   |                      |                      |                      |
|                   |                      |                      |                      |

Changes During Hypoxia

In agreement with others, we have found that there is a hyperpolarization followed by a slow then a rapid depolarization during hypoxia:17 thiopental blocked the hyperpolarization and delayed the onset of the rapid depolarization. It also reduced the cellular sodium concentration during 5 and 10 minutes of hypoxia and the loss of cellular potassium during 10 minutes of hypoxia. These changes may explain the delayed rapid hypoxic depolarization and the reduced final level of depolarization at 10 minutes of hypoxia with thiopental. The early hyperpolarization during hypoxia in untreated slices could be due to a calcium-activated potassium conductance18; since thiopental attenuates the rise in calcium during hypoxia, this would be blocked. Barbiturates have been shown to enhance the depolarizing GABA response,19,20 which would also attenuate the early hyperpolarization.

During hypoxia, there is an increase in cellular sodium and calcium.21 Blocking the increase in sodium with lidocaine22 or calcium with 10 mmol/L magnesium23 improves electrophysiological recovery. However, since the changes during hypoxia are linked, it is not possible to change only one variable. Blocking sodium selectively with either lidocaine (10 μmol/L) or tetrodotoxin (600 nmol/L) not only reduces sodium influx during hypoxia but also attenuates the fall in ATP. This low concentration of lidocaine did not block the hypoxic increase in calcium, which suggests that some of the protective efficacy of thiopental is due to its ability to block sodium influx during hypoxia, independent of its effect on calcium.

Thiopental is, thus far, unique among the agents that we have examined in that it causes only a small improvement in ATP concentrations at 10 minutes of hypoxia.9 Other agents that improve recovery enhance ATP to a greater extent24; thiopental exacerbates the ATP depletion at 3.5 minutes of hypoxia. It appears that, at least at the cellular level, thiopental does not protect by preserving ATP levels but by attenuating ionic changes during hypoxia.

In agreement with previous reports, we found that the intracellular calcium concentration increased during hypox-
The inhibition of this increase in calcium by thiopental during hypoxia could block calcium-activated processes such as protein kinase C, proteases, phospholipases, and nucleases. Indiscriminant activation of these processes may lead to permanent damage after hypoxia and ischemia.

There are a number of mechanisms that contribute to the increase in cytosolic calcium. Voltage-sensitive calcium channels and N-methyl-D-aspartate channels probably open during the hypoxic depolarization, and the Ca\(^{2+}\)-ATPase pump and the Na\(^{+}\)-Ca\(^{2+}\) antiport will be inhibited. The Na\(^{+}\)-Ca\(^{2+}\) antiport may act to load the cell with calcium as the gradients will favor calcium influx and sodium efflux.

Thiopental delays the hypoxic depolarization and directly blocks sodium influx; this will help maintain the sodium electrochemical gradient and attenuate the increase in cytosolic calcium during hypoxia.

Reducing or delaying the hypoxic depolarization has also been shown to reduce neuronal damage. Barbiturates have been shown to block excitatory amino acid–induced depolarization and sodium flux, and N-methyl-D-aspartate and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid induced damage.

The significant attenuation of changes in sodium, potassium, calcium, and membrane potential during hypoxia with thiopental may explain the enhanced recovery after hypoxia. It is likely that a combination of these effects contribute to the protective efficacy of thiopental.

Changes After Hypoxia

In agreement with our studies, ischemia or hypoxia in the slice produce early and severe morphological damage. In vivo studies report histological damage after hypoxia or ischemia in the first hours after an insult. This damage correlates with a decrease in key cytoskeletal proteins, which can be prevented by leupeptin, a calcium-activated protease inhibitor, and by the calcium channel antagonist nilvadipine. Thiopental may prevent the morphological changes by blocking the increase in cytosolic calcium during hypoxia and reducing the calcium concentration after hypoxia.

There was a dramatic reduction in the calcium concentration after hypoxia in hippocampal slices treated with thiopental during hypoxia. Even though thiopental was washed out shortly after hypoxia, the decrease in calcium continued. It is possible that thiopental, which is highly lipophilic, remained in the tissue and that this explains its continued effect.

Protein synthesis inhibition persists after hypoxia despite the recovery of energy substrates and the reestablishment of ion gradients. Indeed, protein synthesis failure is a hallmark characteristic of ischemic damage in vivo and has been linked to the eventual necrosis of neurons. Calcium changes have been implicated in protein synthesis damage due to ischemia in vivo and in vitro. The blockade by thiopental of the calcium increase during hypoxia and the reduction of calcium concentrations after hypoxia might be key factors in maintaining protein synthesis after hypoxia.

Thiopental improved recovery of the resting and action potential after hypoxia. We have previously shown that thiopental improved recovery of the evoked postsynaptic population spike after hypoxia; the response did not recover without thiopental. This is consistent with the present results, which suggest that the electrode is not coming out of the cell but that there is a real loss of the resting and action potentials from these CA1 pyramidal cells. The intracellular electrophysiological damage we measure is consistent with protein synthetic and histological indicators of persistent damage.

By 30 minutes after hypoxia, both the sodium and potassium concentrations returned to normal in the untreated tissue. Thus, long-term alterations in sodium and potassium cannot explain damage after hypoxia; if these ions are important, then their increase during hypoxia must trigger
more persistent changes. In untreated tissue, there was a depolarization that persisted after hypoxia even though the sodium and potassium gradients returned to normal. One possible explanation is that there is a maintained conductance that is large enough to depolarize the neurons but small enough to allow the cell to maintain normal intracellular sodium and potassium levels with the Na\(^+\)-K\(^-\) pump. The pump would use more ATP to maintain normal sodium and potassium concentrations, and the reduced ATP levels after hypoxia may, in part, be due to this enhanced pumping. Thiopental significantly improved ATP levels after hypoxia; this could be due to the recovery of the resting potential, leading to reduced posthypoxic ion flux and thereby reduced Na\(^+\)-K\(^-\) pumping.

Thiopental reduced and delayed the depolarization of CA1 neurons and attenuated the changes in intracellular calcium, sodium, and potassium concentrations during hypoxia. It also reduced calcium concentrations in the period after hypoxia. Our results support the hypothesis that the attenuation by thiopental of these ionic changes prevents the long-term electrophysiological, morphological, and protein synthetic damage. These primary ionic effects of thiopental may prevent activation of secondary pathways that lead to long-term damage. An agent or a combination of agents that shares the ionic effects of thiopental, further improves ATP concentrations, and overcomes some of the clinical disadvantages of thiopental may provide improved therapy for stroke.

Acknowledgments

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References

Numerous investigations in animals have established that barbiturates increase neuronal tolerance to temporary focal ischemia. Early investigation by Michenfelder\(^1\)\(^2\) evaluating the effect of barbiturates on cerebral metabolism provided data that led many to postulate that barbiturate protection of the brain was mediated primarily by a metabolic suppressant effect. Recently, this belief has been challenged, and it appears that any potential benefit of barbiturates on the ischemic brain may involve numerous mechanisms that are not precisely understood.\(^3\)\(^4\)

In this report by Wang and colleagues, a rat brain slice model of neuronal hypoxia was used to assess the effect of clinically relevant concentrations of thiopental on physiological, biochemical, and histologic parameters during and after hypoxia. They observed that thiopental attenuated cellular depolarization during hypoxia and improved recovery after hypoxia. Thiopental also ameliorated the decrease in ATP during hypoxia, maintained ionic gradients, and improved neuronal morphology and protein synthesis after hypoxia.

In the clinical setting, potential disadvantages of barbiturates include myocardial depression and systemic hypotension that may worsen an ischemic episode, and excess sedation that may preclude a neurological examination and necessitate mechanical ventilation. The apparent rationale for this investigation was to “examine the direct effects of thiopental on neurons”—ostensibly to further delineate the cellular mechanism(s) of barbiturate neuroprotection. A more precise understanding of the mechanism(s) of barbiturate neuroprotection should allow for development of therapeutic options that share the beneficial neuroprotective properties of barbiturates without their adverse effects.

The authors have provided data that represent a valuable contribution to our understanding of the effect of thiopental on neurons. However, readers should be cautioned to consider the limitations of data derived from a neuronal preparation without a vasculature and mammalian systemic interaction. Moreover, mechanisms of neuroprotection by barbiturates were not rigorously evaluated by Wang et al. One mechanism of potential interest is glutamate excitotoxicity. In a previous study of a hippocampal rat brain slice model,\(^5\) the above laboratory evaluated the effect of thiopental on N-methyl-D-aspartate (NMDA)- and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionyl (AMPA)-induced neuronal damage. Their data clearly indicated that thiopental can attenuate both NMDA- and AMPA-mediated toxicity (as demonstrated by a greater preservation of the CA1 population spike in response to stimulation of Schaffer collaterals).

Although this article is a comprehensive assessment of the physiological, biochemical, and histologic effects of thiopental on hypoxic neurons, further study, testing an independent manipulation on a postulated pathway of neuroprotection and its effect on injury, is required before any inference as to mechanisms of protection by barbiturates should be made.

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