Experimental Brain Ischemia: Protection From Irreversible Damage With a Rapid-Acting Barbiturate (Methohexital)

BY F. M. YATSU, M.D., I. DIAMOND, M.D., C. GRAZIANO, AND P. LINDQUIST

Abstract: Experimental brain ischemia was produced in rabbits by coupling systemic hypotension and hypoxia. The animals were paralyzed with succinylcholine and ventilated mechanically. Durally implanted electrodes were used to monitor the severity of ischemia. Five minutes of an isoelectric EEG produced irreversible brain damage in most of the rabbits. Four rabbits receiving the rapid-acting barbiturate, methohexital, in the dosage of 5 mg/kg at the onset of the isoelectric EEG showed complete protection from detectable ischemic damage and recovered dramatically. It is suggested that suppression of brain metabolism with barbiturates during cerebral ischemia is worthy of clinical assessment.

Additional Key Words experimental model of stroke isoelectric EEG systemic hypotension hypoxia

Introduction

We wish to report a relatively simple and reproducible experimental model of brain ischemia which produces irreversible brain damage, and the therapeutic efficacy of administering a rapid-acting barbiturate to this model in preventing irreversible damage. This finding has potential clinical implications for patients experiencing brain ischemia from a variety of causes.

The critical neurochemical features of cerebral ischemia are: (1) deprivation of substrates, i.e., glucose and oxygen, and (2) accumulation of metabolites due to vascular stagnation, i.e., organic acids such as lactic acid. Various experimental models of brain ischemia have advantages and limitations.

Methods

The animals used were random-breed New Zealand white rabbits weighing between 3.6 and 5.3 kg. The rabbits were given intravenous cyclohexylamine (ketamine), 22.2 mg/kg, and then intubated with a cuff endotracheal tube. After intubation, the rabbits were paralyzed with intravenous succinylcholine (20 mg) and mechanically ventilated on a Harvard pump with 90 to 100 cc strokes at 25 to 35 cycles per minute. This maintained oxygenation as measured by arterial oxygen and pH. Cortical electrodes were used to record the electrical potential of the cortex. Stainless-steel screw electrodes were implanted.
parasagittally into the cranial dura one centimeter apart at the level of the anterior motor and posterior sensorimotor cortex. The stainless-steel electrodes displayed less than 10,000-ohm resistance, and the electroencephalogram (EEG) was recorded with a standard 8-channel Grass Electroencephalography-Model 5 (Quincy, Massachusetts) which also recorded the electrocardiogram (EKG). Resting EEG activities were comparable to those previously reported. The femoral artery was catherized for constant blood pressure recording through a transducer and visual readout utilizing the Electronics for Medicine PR-7 (White Plains, New York). The femoral artery catheter allowed for the collection of blood for gases and pH. Body temperature was recorded by a rectal thermometer (Yellow Springs Instruments, Yellow Springs, Ohio) and was maintained by a heating pad. All operative procedures utilized local anesthesia (0.5% Xylocaine) to control pain for the paralyzed animal. Wound areas were reinfiltrated every 30 minutes to assure proper anesthetic effect.

Utilizing a tilt table which angles the head up at 30°, trimethaphan (Arfonad) was given intravenously at 5 to 10-mg increments until the mean arterial blood pressure stabilized at 30 to 35 mm Hg. The total dose varied from 20 mg to 70 mg over a duration of three to six minutes. After achieving a stable hypotensive level, 4% oxygen with 96% nitrogen was given through the Harvard pump. Pressors (adrenalin 0.33 mg, and/or isoproterenol-HCl [Isuprel] 0.03 mg) were required frequently, particularly in the control rabbits. Body temperature slowly declined, even though an attempt was made to stabilize it.

To facilitate recovery from the curare-like effects of trimethaphan, the rabbits were given neostigmine (0.25 mg) and atropine (0.1 mg). Upon return of normal respiratory movement, the animals were extubated and placed in an incubator. Careful and attentive care in the postischemic period was uniformly maintained for each animal. Four experimental rabbits given 5 mg/kg methohexital (Brevital) and five control rabbits were studied. The last control and treated rabbits were performed in a double-blind fashion.* The methohexital was given intravenously within 10 to 20 seconds of the appearance of an isoelectric EEG as defined above. The dosage of 5 mg/kg was chosen since this total amount was reported tolerated in humans undergoing EEG-activation. This dosage given to rabbits not subjected to hypotension showed only mild decrease in EEG amplitude. When a total dose of 10 mg/kg was given, moderate suppression of EEG activity occurred, but without an isoelectric EEG. The electrical activity normalized within eight minutes.

**Results**

The results of our studies on the effect of the rapid-acting barbiturate methohexital are given in tables 1 and 2. Noteworthy are the relatively similar characteristics between the five control rabbits and the four treated rabbits up to the time of EEG recovery.

The ease of maintaining a stable hypotensive blood pressure was similar in both groups: three to six minutes in the controls and three to five minutes in the treated. The duration between the introduction of 4% oxygen and the appearance of an isoelectric EEG was approximately four minutes on the average in the controls, while the average in the treated rabbits was almost half that time at approximately two minutes. Whether this difference in time, which occurred prior to the administration of methohexital, is a significant factor in the outcome will require further studies. Our impression from previous animals is that this period of time is not as critical as the length of the isoelectric EEG.

Striking differences in the two groups of experimental animals were readily apparent with regard to spontaneous EEG recovery after introduction of 100% oxygen, and the duration required for ventilatory support. In methohexital-treated rabbits, EEG-recovery time was 11 minutes (range = less than one minute to 23 minutes), whereas the control rabbits' average

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*The practicality and reproducibility of our model was assessed in 25 rabbits prior to this study.
TABLE 1
Comparison Between Methohexital-Treated and Control Rabbits Subjected to Brain Ischemia

<table>
<thead>
<tr>
<th>Experimental rabbit</th>
<th>Weight (kg)</th>
<th>Cyclohexalmine (mg)</th>
<th>Sucnlyxoline (mg)</th>
<th>Trimethaphan (mg)</th>
<th>Time to reach hypotension (min)</th>
<th>Time between 4% O2 and isoelectric EEG (min)</th>
<th>Methohexital after onset of isoelectric EEG (min)</th>
<th>Duration of isoelectric EEG (min)</th>
<th>Time between 100% O2 and spontaneous EEG activity (min)</th>
<th>Time between 100% O2 and discontinuance of respiratory support (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1*</td>
<td>4.3</td>
<td>80</td>
<td>30</td>
<td>40</td>
<td>4</td>
<td>4</td>
<td>None</td>
<td>5</td>
<td>44</td>
<td>109</td>
</tr>
<tr>
<td>C-2</td>
<td>4.5</td>
<td>80</td>
<td>20</td>
<td>30</td>
<td>3</td>
<td>3</td>
<td>None</td>
<td>5</td>
<td>11</td>
<td>191</td>
</tr>
<tr>
<td>C-3</td>
<td>5.3</td>
<td>80</td>
<td>40</td>
<td>25</td>
<td>4</td>
<td>4</td>
<td>None</td>
<td>5</td>
<td>22</td>
<td>96</td>
</tr>
<tr>
<td>C-4</td>
<td>5.0</td>
<td>80</td>
<td>20</td>
<td>40</td>
<td>3</td>
<td>4</td>
<td>None</td>
<td>5</td>
<td>32</td>
<td>63</td>
</tr>
<tr>
<td>C-5</td>
<td>3.7</td>
<td>80</td>
<td>20</td>
<td>70</td>
<td>6</td>
<td>4</td>
<td>None</td>
<td>5</td>
<td>37</td>
<td>147</td>
</tr>
<tr>
<td>Average</td>
<td>4.6</td>
<td>80</td>
<td>26</td>
<td>41</td>
<td>4</td>
<td>4</td>
<td>None</td>
<td>5</td>
<td>29</td>
<td>121</td>
</tr>
<tr>
<td>range</td>
<td>(3.7-5.3)</td>
<td>(20-40)</td>
<td>(25-70)</td>
<td>(3-6)</td>
<td>(3-4)</td>
<td>(0)</td>
<td>(0)</td>
<td>(11-44)</td>
<td>(63-191)</td>
<td></td>
</tr>
<tr>
<td>M-1†</td>
<td>4.5</td>
<td>80</td>
<td>20</td>
<td>70</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>&lt;1</td>
<td>156</td>
</tr>
<tr>
<td>M-2</td>
<td>4.1</td>
<td>70</td>
<td>24</td>
<td>35</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>23</td>
<td>60</td>
</tr>
<tr>
<td>M-3</td>
<td>3.6</td>
<td>60</td>
<td>30</td>
<td>38</td>
<td>5</td>
<td>&lt;1</td>
<td>5</td>
<td>5</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>M-4</td>
<td>4.3</td>
<td>80</td>
<td>30</td>
<td>20</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>90</td>
</tr>
<tr>
<td>Average</td>
<td>4.1</td>
<td>73</td>
<td>26</td>
<td>41</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>11</td>
<td>83</td>
</tr>
<tr>
<td>range</td>
<td>(3.6-4.5)</td>
<td>(60-80)</td>
<td>(20-30)</td>
<td>(20-70)</td>
<td>(3-5)</td>
<td>(1-3)</td>
<td>(0)</td>
<td>(0)</td>
<td>(1-23)</td>
<td>(27-156)</td>
</tr>
</tbody>
</table>

*C = Control rabbits.
†M = Methohexital-treated rabbits.
### TABLE 2

**Comparison of Blood Gases and Temperature in Methohexital-Treated and Control Rabbits Subjected to Brain Ischemia**

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>pH</th>
<th>Po&lt;sub&gt;2&lt;/sub&gt;</th>
<th>P&lt;sub&gt;CO&lt;/sub&gt;</th>
<th>Temp</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>7.45</td>
<td>465.00</td>
<td>39.00</td>
<td>37.40</td>
<td>Rabbit had delayed marked neurological deficit at 2 to 3 days such as inability to assume normal position. It became relatively normal by 4 to 5 days.</td>
</tr>
<tr>
<td>C-2</td>
<td>7.40</td>
<td>490.00</td>
<td>39.00</td>
<td>37.20</td>
<td>Animal died within 24 hours. Rabbit unable to assume normal position or right itself before death.</td>
</tr>
<tr>
<td>C-3</td>
<td>7.35</td>
<td>385.00</td>
<td>36.00</td>
<td>37.00</td>
<td>Rabbit had severe neurological deficit at 24 hours. It was unable to stand, right itself or withdraw from painful stimuli.</td>
</tr>
<tr>
<td>C-4</td>
<td>7.30</td>
<td>360.00</td>
<td>35.00</td>
<td>36.80</td>
<td>Animal died 2.5 hours after discontinuance of mechanical ventilator. It showed severe impairment such as inability to stand or right itself; it also showed extremity spasms.</td>
</tr>
<tr>
<td>C-5</td>
<td>7.29</td>
<td>445.00</td>
<td>52.00</td>
<td>37.10</td>
<td>Rabbit died shortly after discontinuance of mechanical ventilator with little motor recovery.</td>
</tr>
<tr>
<td>M-1</td>
<td>7.44</td>
<td>420.00</td>
<td>36.00</td>
<td>37.40</td>
<td>No observable neurological damage.</td>
</tr>
<tr>
<td>M-2</td>
<td>7.40</td>
<td>415.00</td>
<td>36.00</td>
<td>36.80</td>
<td>No observable neurological damage.</td>
</tr>
<tr>
<td>M-3</td>
<td>7.35</td>
<td>410.00</td>
<td>35.00</td>
<td>35.80</td>
<td>No observable neurological damage.</td>
</tr>
<tr>
<td>M-4</td>
<td>7.29</td>
<td>400.00</td>
<td>34.00</td>
<td>35.60</td>
<td>No observable neurological damage.</td>
</tr>
</tbody>
</table>

*Temperature in degrees centigrade.*
EEG-recovery time was 29 minutes (range = 11 minutes to 44 minutes). Ventilatory support in the former group was an average of 83 minutes, while the control group was an average of 121 minutes.

After administration of succinylcholine, the animals slowly lost body temperature and, in spite of the use of a heating blanket, maintenance of temperature was particularly difficult, especially after the ischemic insult. The control animals' temperatures were about 1°C higher than those of the treated group.

Within two to three hours of each experiment, all methohexital-treated rabbits were alert and their motor, sensory and equilibratory functions were intact. Each of the untreated control rabbits either died following attempts at resuscitation or showed severe neurological deficits, such as inability to stand or hop. One animal (Control C-1) improved to a normal level in four to five days after manifesting delayed neurological impairment at two to three days. Another animal (C-3) became progressively worse the day following the experiment, with increased paralysis of its legs.

Discussion
In 1836, Astley Cooper reported pioneering efforts in the production of experimental cerebral ischemia. He found that bilateral carotid and vertebral artery ligations were tolerated in dogs but not in rabbits. Despite subsequent application of modern techniques, including monitoring devices, no experimental model as yet satisfactorily reproduces cerebral ischemia associated with selective diminution of cerebral blood flow (CBF) and with discrete neurological deficits. In addition, the models do not allow precise quantitation of the reversible and irreversible stages of ischemic brain damage.

Examples of models simulating brain ischemia include decapitation,4 exsanguination,5 selective arterial occlusion or embolization,6 and circulatory arrest.11-12 The cellular insults of ischemia can be defined neurochemically as the effective deprivation of substrates (glucose and oxygen) plus the accumulation of metabolites, particularly lactic acid. Our model attempts to duplicate these neurochemical insults by combining hypotension and hypoxia, each of which has recently been extensively detailed by Siesjö and Nilsson.13

Although the periods vary of achieving a stable hypotensive level (three to six minutes) and of the onset of an isoelectric EEG after the introduction of 4% oxygen (one to three minutes), the duration of an isoelectric EEG as an index of "functional ischemia" has provided a reproducible criterion for reversible or irreversible damages. For example, after three minutes of an isoelectric EEG, the introduction of 100% oxygen and normalization of blood pressure without requiring pressors uniformly leads to complete recovery of rabbits without detectable motor deficits. On the other hand, it was previously observed that five minutes of an isoelectric EEG cause severe neurological impairment as manifested by a slowly recovering and poorly organized EEG, severe motor dysfunction such as an inability to hop or right itself, or inability to survive. Interestingly, one of the experimental control rabbits (C-1) was unable to hop or right itself after two to three days, but after four to five days it was able to do so normally. While this indicates probable biological variation, it raises questions as to the validity of using motor function as a measure of neuronal impairment. Poor motor performance in fact may be a reflection of systemic metabolic factors. Despite this latter possibility, two control rabbits (C-2 and C-3) showed persisting motor disability, indicating that this parameter can be an index of neuronal damage. Ability to detect motor deficit in experimental brain ischemia provides a more satisfactory and refined endpoint than survival alone.

It is suggested, therefore, that this model of combined hypotension and hypoxia with EEG monitoring provides a satisfactory model for investigations of irreversible ischemic brain damages and the neurochemical factors which herald these changes.

Reduction of brain metabolism in order to gain "lead time" or provide cerebral protection during periods of compromised cerebral circulation has received both research and clinical attention. Specific methods evaluated in the past include hypothermia4-10 and volatile anesthetics.17-18 Systematic evaluation of therapeutic suppression of brain metabolism has been hampered by the lack of an experimental model which satisfactorily corresponds to the clinical conditions of cerebral ischemia. For
example, the relatively short time-constant, in terms of minutes, for the appearance of irreversible ischemic brain damage, recognized clinically, is not duplicated with most of the experimental techniques employed. In addition, the therapeutic effects are usually evaluated on the basis of survival, which disregards graded and differential ischemic damages.

In our experiments of rabbit ischemia, complete and dramatic recovery of function was associated with the administration of intravenous methohexital (5 mg/kg). These rabbits were challenged with hypotension-hypoxia and demonstrated an isoelectric EEG for five minutes. Recovery correlated best with an earlier return to the EEG activity (average of 11 minutes for the methohexital-treated rabbits as opposed to 29 minutes in the controls) and the earlier ability to wean the former rabbits from the respirator (average of 83 minutes compared to 121 minutes in the controls). The average rectal temperature at the conclusion of five minutes of an isoelectric EEG was 1°C lower in the barbiturate-treated rabbits, but this temperature differential would not appear to be significant. Other factors such as cardiac status, as gauged by rate, rhythm and QRS complexes, blood pressure response, blood gases during and after the experimental procedure, blood pHs, or the amount of trimetaphan, succinylcholine, or cyclohexylamine administered, do not differ significantly in the control and treated groups. Although systemic factors may play a role in the eventual outcome, EEG recovery indicates a crucial role of brain metabolism in predicting recovery. Furthermore, in the last pair of experiments, cortically evoked light potentials were assessed at one-minute intervals. These evoked potentials developed sooner in barbiturate-treated rabbits.

The mechanism of the protective effect of methohexital on brain metabolism is suggested by the results of Gatfield et al., who found elevated levels of high energy phosphates after barbiturate therapy in brain ischemia produced by decapitation. Their findings suggest that preservation of brain functions in our model may operate through a maintenance of energy phosphate levels secondary to metabolic suppression. The resulting higher Energy Charge (E.C.), as defined by Atkinson, may preserve labile functions susceptible to irreparable damage. The extensive experiments of Siesjö and Nilsson do not support this conclusion, however. In their experiments of asphyxia in rats produced with three minutes of respiratory arrest, barbiturate anesthesia was associated with a significant decrease in the Energy Charge (E.C. = [ATP + 0.5 × ADP]/[ATP + ADP + AMP]) from the control value of 0.9 to 0.48. The results of the tissue energy states were interpreted to contradict the protective value of barbiturates in experimental asphyxia reported by Secher and Wilhjelm and by Wright and Ames. In addition, if the temperature were lowered by 2°C, the E.C. was more nearly normal at 0.8 suggesting that mild hypothermia was critical in conveying cerebral protection with barbiturates. Whether the mild hypothermia potentiated the action of barbiturates in elevating the E.C. is unknown, since blood and brain barbiturate levels were not determined.

In our model, which assesses functional ischemic impairment, the protection from irreversible brain damage with intravenous barbiturate appears to underscore dramatically its crucial role in maintaining tissue viability. Although the molecular mechanisms, which are being currently studied, are uncertain, the protective effect experimentally of intravenous barbiturate indicates that it has promise in its application to patients experiencing cerebral ischemia.

References
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Stroke, Vol. 3, November-December 1972


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Stroke. 1972;3:726-732
doi: 10.1161/01.STR.3.6.726

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