Cerebral Protection with Barbiturates

Relation to Anesthetic Effect

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SUMMARY The effect of racemic mephobarbital and its optical isomers on survival time of mice exposed to 5% O2 was studied. There was an increase in survival time from 4.2 minutes to 12.6 minutes for 100 mg/kg of the anesthetically active (–) isomer and the racemic form, but no increase for 100 mg/kg of the inactive (+) isomer. Since it has been shown that there is no difference in brain concentrations between the isomers, we conclude that the barbiturate protective effect is bound to the anesthetic effect. All mice convulsed, and since the non-anesthetized animals convulsed earlier and stronger than the anesthetized, it was possible that barbiturate protection was accounted for by its anticonvulsant effects. Diazepam 7.5 mg/kg, while reducing convulsions to the same degree as barbiturates without producing anesthesia, only increased survival time to 6.2 minutes. Thus, the barbiturate protective effect is distinct from the anticonvulsant effect. It seems to be bound to a stereospecific receptor for both protection and anesthesia.

BARBITURATES have been reported to protect the brain in a variety of animal models of hypoxia and ischemia (both regional and global). Protection has been demonstrated when the drug is administered both before and during the hypoxic event. The mechanism of protection is unknown, but it has been suggested that it could be accounted for by the decrease in cerebral oxygen consumption (CMRO2) that occurs during barbiturate anesthesia. However, other anesthetics which also reduce CMRO2 do not provide any apparent protection. It has, therefore, been suggested that the anesthetic effect may be incidental to the protective effect and that a more basic mechanism such as free radical scavenging may be involved.

Accordingly, we sought to examine the effects of a barbiturate which could cross the blood-brain barrier without producing anesthesia. For this purpose, the optic isomers of mephobarbital (methylphenobarbital or N-methyl-5-ethyl-5-phenylbarbitaril) were studied. The (–) isomer is an anesthetic, while the (+) isomer is without anesthetic activity. This is not a function of drug distribution or metabolism, as the inactive (+) isomer maintains an equal or higher brain concentration than the active (–) isomer for at least 1 hour after i.v. injection (see fig. 1). The protective effect of these was therefore tested in a simple and reproducible model of acute global hypoxia in mice.

Since hypoxic mice are known to convulse, the effect of diazepam 7.5 mg/kg was also studied. Diazepam is an effective anticonvulsant that does not change CMRO2.

Materials and Methods

White male Sprague Dawley mice weighing 23–32 g with free access to tap water and food pellets were studied. The racemic form of mephobarbital and the (–) and (+) isomers were obtained in crystalline form, dissolved in equimolar amounts of NaOH and diluted to a 1% solution with 0.9% NaCl.

The optical rotation [α]8°(c) (ETOH) of the (–) and (+) isomers was –8.8° and +8.5° respectively, and the melting point of both 101°. Diazepam was used in the commercially available solution, diluted to a 0.075% solution with 0.9% NaCl.

The animal model used has previously been described by Wilhelmi and Arnfred. All mice were weighed and injected intraperitoneally with one of the drugs. The racemic form and both isomers were given in a dose of 100 mg/kg. The active (–) isomer was also given in a dose of 50 mg/kg and diazepam in a dose of 7.5 mg/kg. Controls were given an injection of 0.9% NaCl. In each experiment, one mouse was placed in each of 5 airtight 2 l flow-through chambers mounted in parallel and supplied with room air. The ambient temperature was maintained at 32–34°C. Thirty minutes after injection the supply of room air was turned off and the chambers flushed with N2 8 l/min together with 15 l/min of a mixture of 5.06% O2 and 94.94% N2 from a pre-mixed tank (O2 concentration analyzed by Haldane's method). After 1 minute, the N2 was turned off, and after 2 minutes, the flow of 5.06% O2 in N2 was reduced to 3 l/min. (Pilot studies showed O2 concentration in all chambers to reach 5% after the first minute and stay at 5% through the study). The survival time, i.e., the interval between the start of reduction in the O2 concentration and the end of respiration, was measured for each animal.

Each barbiturate type and dose was examined in 25 animals with 15 simultaneous control animals. There were 1–2 controls in each single study of 5 animals, and these were systematically rotated among the 5 chambers. The action of diazepam was studied in 16 animals with 9 controls. The data were analyzed by Student's t-test for unpaired data.

Results

Since there was no significant difference in survival time between the individual control groups for the different drugs (the means varying between 4.10 minutes and 4.38 minutes with p values >0.7) all controls were grouped together.

The mean survival times with standard error of the mean are shown in table 1 and figure 2.

All drugs except the inactive (+) isomer of mephobarbital significantly increased survival time when compared to controls (p < 0.001). The increase with 100 mg of the racemic form (195%) and 50 mg and 100 mg of the active (–) isomer (126% and 197% respectively) were significantly greater than with diazepam (47%) with p < 0.001. The difference between 50 mg of the (–) isomer and the racemic
sensitive group hidden in one isomeric form is more easily can only happen under very specific circumstances if a sen-
tive group hidden in one isomeric form is more easily show any difference in chemical or physical behavior. With the inactive (+) isomer, survival time was unaltered (3.84 minutes) and, in fact, numerically less than that in any of the individual control groups. These animals all convulsed terminally as did the control animals. Animals either anesthetized (with the racemic form or the active isomer) or unanesthetized, but given diazepam also had terminal convulsions, but of much less intensity.

**Discussion**

Clinical doses of barbiturates produce up to a 55% reduction in CMRO₂ in man and animals. This effect is dose related and appears to be secondary to a reduction in cerebral function, as there is no further reduction in CMRO₂ with increasing doses after an isoelectric EEG has been produced by the drug. If this is the sole basis for cerebral protection, barbiturates should reduce the detrimental effect of acute or chronic hypoxia on the brain only as long as the brain is actively functioning. Furthermore, the dose required for protection should not exceed clinical doses, and other anesthetics should protect to the degree they reduce CMRO₂ provided they do not add other detrimental factors. However, studies of other anesthetics have failed to show significant protection. In addition, some investigators report a need for extremely large doses of barbiturates to provide protection. Finally, recent studies indicate that barbiturates protect even when the hypoxic event is sufficient to deplete most of the brain energy stores. Thus, the mechanism of protection remains unknown and may well not be secondary to the anesthetic or CMRO₂ effects.

To resolve these discrepancies, it has been suggested that protection may be related to a more basic subcellular mechanism such as free radical scavenging. If this is the case, a non-anesthetic barbiturate which crosses the blood-brain barrier should be protective. Accordingly, we studied the active and inactive isomers of mephobarbital. Assuming there is no optically active substrate, these isomers do not show any difference in chemical or physical behavior. They have been shown to maintain the same brain concentrations following i.v. injection, and it is unlikely that there would be any difference in their effects as radical scavengers. This can only happen under very specific circumstances if a sensitive group hidden in one isomeric form is more easily accessible in the other. If, therefore, part of the protective effect is due to scavenging or some other non-anesthetic mechanism, the inactive (+) isomer should give some increase in survival time.

In this study, the anesthetically inactive isomer did not increase the survival time of the mice while the active isomer gave up to a 197% increase at the chosen dosages. We, therefore, conclude that the protective effect is bound to a stereospecific receptor that probably is the same for protection and anesthesia. It is unlikely that radical scavenging could be the protective mechanism, at least in this model.

One problem with the model used is that all mice die with convulsions of 3–5 seconds duration. These are agonal and of greater intensity in unanesthetized than anesthetized mice. Thus, it could be possible that some or all of the protective effects of barbiturates simply result from the anticonvulsant effect. Wilhjelm et al. tested many volatile anesthetics and, while chloroform, trichloroethylene and methoxyflurane reduced the convulsions, they only increased survival time 8–12%. Since volatile anesthetics also have an

**Table 1 Mean Survival Time SEM for Control Mice and Treated Mice**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage mg/kg</th>
<th>N</th>
<th>Mean survival time in min. SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>—</td>
<td>69</td>
<td>4.25</td>
</tr>
<tr>
<td>Racemic</td>
<td>—</td>
<td>69</td>
<td>4.25</td>
</tr>
<tr>
<td>Mephobarbital</td>
<td>100</td>
<td>25</td>
<td>12.55</td>
</tr>
<tr>
<td>(+) Mephobarbital</td>
<td>100</td>
<td>25</td>
<td>3.84</td>
</tr>
<tr>
<td>(-) Mephobarbital</td>
<td>50</td>
<td>25</td>
<td>9.59</td>
</tr>
<tr>
<td>Diazepam</td>
<td>7.5</td>
<td>19</td>
<td>6.23</td>
</tr>
</tbody>
</table>

N = number of animals.
effect on CMRO₂ and cerebral blood flow (CBF). We elected to test the effect of an anticonvulsant on survival time by using diazepam. In rats ventilated with O₂/N₂ 7.5 mg/kg of diazepam has shown to have no effect on CMRO₂.

As expected, diazepam gave some increase in survival time, but significantly less than the anesthetic forms of the barbiturate. The anticonvulsant effect cannot, therefore, explain the protective effect.

If increase in survival time were a function of only the amount of the active isomer given, the results with 100 mg/kg of the active isomer would be bound and, thus, a larger dose would be required for a similar effect. 2) There also must be an optimal dose of barbiturate at which a maximal effect is found. Increasing doses progressively increase survival time until severe respiratory and cardiovascular depression occurs. Thereafter, dose increases will decrease survival. It is possible that a dose of 100 mg/kg of the active (—) isomer is beyond the optimal dose. This, in fact, is suggested to be the case by the relatively large standard error of the mean survival time in this group.

In conclusion, we find that mephobarbital protects mice to a large extent bound to plasma proteins. Adding the inactive (+) isomer would therefore partly displace the active isomer from plasma proteins and increase the free fraction available for the brain, whereas in the absence of the inactive isomer a greater proportion of the active isomer would be bound and, thus, a larger dose would be required for a similar effect. Furthermore, it is shown that if the dose is increased beyond the point at which the maximal effect is found, the survival time decreases. This suggests that there is a balance between the protective and toxic effects of the barbiturate.

References

10. Michenfelder JD: Interdependency of cerebral functional and metabolic effects following massive doses of thiopental in the dog. Anesthesiology 41: 231-236, 1974
17. Nordström C-H, Rehnrona S, Siegdk BK: Effects of phenobarbital in cerebral ischemia. Part two: Restoration of cerebral energy state, as well as glycolytic metabolites, citric acid cycle intermediates, and associated amino acids after pronounced incomplete ischemia. (Unpublished)
18. Knabe J: Personal communication. (Dr. Knabe is Professor of Pharmacological Chemistry, Universitat des Saarlandes, Germany)
19. Pihl A: Personal communication. (Professor Pihl is Head of Department of Biochemistry, Norsk Hydro's Institute for Cancer Research, Oslo, Norway)
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