Physostigmine-Induced Cerebral Protection Against Hypoxia

A.M.E. Scremin, M.D. AND O.U. Scremin, M.D.

SUMMARY Physostigmine, at 0.1, 0.2 and 0.3 mg/kg, was tested for effect on the survival of mice exposed to 5% O₂ - 95% N₂. Some treated animals survived for one hour under the hypoxic atmosphere (2 out of 14 at 0.1 mg/kg and 8 out of 28 at 0.2 and 0.3 mg/kg), an event never observed in untreated controls. The physostigmine-treated animals that died before the hour showed a dose-related increase in survival time from 4.3 min (untreated controls) to 27.6 min (0.3 mg/kg physostigmine). This effect of physostigmine may be related to its reported ability to increase cerebral blood flow and decrease cerebral oxygen consumption.

AN EXPERIMENTAL protocol commonly used to study hypoxia consists of measuring the survival time of animals breathing a lethally hypoxic atmosphere.1, 2 Under this condition death is due primarily to impairment of cerebral function, ultimately resulting in respiratory arrest. This procedure has been used to test the ability of drugs and anesthetics to diminish brain damage during hypoxia. Several agents which are known to decrease cerebral oxygen consumption have been shown to prolong survival of animals in this experimental situation.3, 4 Hypercapnia, which increases CBF, also prolongs survival time under hypoxia.5, 6 Although the mechanism of the "cerebral protection" observed under these conditions is still a matter of debate, it has been proposed that this protection may be mediated by a decrease in cerebral metabolism, an increase in cerebral blood flow or a combination of both, that may improve the balance between O₂ consumption and O₂ delivery to the brain.

A cholinergic mechanism has been proposed in the mediation of the cerebral vasodilatation associated with cortical desynchronization,7 hypotension,8 and hypercapnia.9 On the other hand, cholinergic drugs are known to inhibit brain evoked potentials and reflex activity.10-14 We have recently shown that physostigmine, a cholinesterase inhibitor that crosses the blood-brain barrier, induces an increase in cerebral blood flow or a combination of both, that may improve the balance between O₂ consumption and O₂ delivery to the brain.

Physostigmine-induced cerebral protection against hypoxia.

Materials and Methods

Male white mice, weighing 20-25 g were used. Animals were allowed free access to tap water and food pellets. Physostigmine sulfate (Merck) was dissolved in 0.9% NaCl and injected intraperitoneally at the doses of 0.1, 0.2 and 0.3 mg/kg. In pilot experiments, 0.3 mg/kg was found to be the highest dose that could be given without incidence of respiratory arrest as a toxic manifestation of physostigmine. Control animals were injected intraperitoneally with an equivalent amount of 0.9% NaCl without physostigmine. Fifteen to twenty min after the physostigmine or saline injection, the animals were introduced individually and alternately (one control, one treated) into a 2 liter glass chamber through which a continuous flow of 5 liters/min of a mixture of 5% O₂-95% N₂ was maintained. The O₂ concentration inside the chamber was continuously monitored with a Beckman OM-11 Oxygen Analyzer. Temperature inside the chamber was maintained at 25 ± 0.5° C by means of a heating lamp. The interval between the moment of introduction of the animal into the chamber and the last respiratory effort (survival time) was measured for each animal. The observation time was limited to one hour and the animals that remained alive after this time were removed from the chamber and retested 24-48 hours later without physostigmine treatment.

Results

Physostigmine induced a dose-related increase in the survival time of animals under hypoxia (table). Two out of 14 animals treated with 0.1 mg/kg and 8 out of 28 animals treated with 0.2 or 0.3 mg/kg of physostigmine survived for one hour, an event never observed in untreated controls. These animals were fully awake and although they exhibited minimal locomotor activity while exposed to hypoxia, they showed good motor coordination; one even managed to climb a 10 cm vertical pole inside the chamber after 58 min of exposure. Immediately after removal from the chamber these mice recovered the normal pattern of motor activity and were behaviorally indistinguishable from non-exposed animals. These same mice, when retested 24-48 hours after the exposure which they survived (but this time without physostigmine treatment), died in a period of time not statistically different from that of the untreated, previously unexposed controls (table).

All animals that succumbed during the test showed basically the same sequence of events while experiencing hypoxia: exploratory activity; motor incoordination; irregular, deep and less frequent breathing; tonic-clonic convulsions; gasping; respiratory arrest.
TABLE 1 Survival of Mice During Exposure to 5% O₂ - 95% N₂

<table>
<thead>
<tr>
<th>Condition</th>
<th>N</th>
<th>Survived 1 hr</th>
<th>Number of animals that survived a previous exposure</th>
<th>Survival time (min) of animals that died before the hour (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td>10</td>
<td>0</td>
<td>3.8 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Physostigmine 0.1 mg/kg</td>
<td>14</td>
<td>2</td>
<td>6.7 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Physostigmine 0.2 mg/kg</td>
<td>14</td>
<td>4</td>
<td>13.9 ± 1.6*</td>
<td></td>
</tr>
<tr>
<td>Physostigmine 0.3 mg/kg</td>
<td>14</td>
<td>4</td>
<td>27.6 ± 4.4*</td>
<td></td>
</tr>
</tbody>
</table>

N = Number of animals.
SEM = Standard error of the mean.

Discussion

The ability of physostigmine to prolong survival under hypoxia may be related to the cholinergic effects on cerebral blood flow (CBF) and cerebral oxygen consumption. Topical application of acetylcholine to cerebral vessels in vivo induces vasodilatation. Physostigmine topically applied to the cerebral cortex considerably enhances the vasodilatation associated with cortical activation. Also, intravenous administration of physostigmine induces an increase in cerebral cortical blood flow and internal carotid blood flow in nitrous oxide-anesthetized rabbits. Cholinergic drugs are known to inhibit somatosensory evoked potentials and spinal reflexes. Thus, the inhibition of certain types of neural activity and the increase in CBF induced by physostigmine may mediate an increase in oxygen availability in the brain. An indication that this may be the case is provided by the recent observation that the cerebral venous O₂ content increases following intravenous administration of physostigmine due to a decrease in cerebral O₂ consumption and a concomitant increase in CBF. These effects of physostigmine may underly the cerebral protection of the drug against hypoxia.

The same mechanism probably applies to the previous observation by Gibson and Blass of a delay in the death of methemoglobinemic and hypoglycemic mice induced by physostigmine salicylate.

Anesthetics have been reported to protect the brain from hypoxia, the effects being greatest for barbiturates. Wilhelmi reported an increase in survival time of mice breathing 5% O₂ from 3.1 min (control) to 8 min (50 mg/kg thiopentone). With the same experimental model, Steen and Michenfelder obtained a survival time of 4.25 min in control animals and 12.55 min after 100 mg/kg mephorbarbital. These effects of barbiturates, observed at doses that produce general anesthesia, are considerably weaker than the one here reported for physostigmine. Moreover, the protective effect of barbiturates is inseparable from its anesthetic effect while that of physostigmine is observed in animals that remain fully alert.

In conclusion, physostigmine prolongs survival of mice breathing a lethal hypoxic atmosphere. This action might be related to the previously reported cerebral vascular and metabolic effects of this drug.

References

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