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. 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. Hypercapnia, which increases CBF, also prolongs survival time under hypoxia. 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, hypotension, and hypercapnia. On the other hand, cholinergic drugs are known to inhibit brain evoked potentials and reflex activity. 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.

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.

From the Departments of Anesthesiology and Physiology, University of California, Los Angeles, School of Medicine, Los Angeles, CA 90024.

Supported by NIH Grant H. 17903 and American Heart Association — Greater Los Angeles Affiliate Grant 4371G. O.U. Scremin is a Senior Investigator, American Heart Association — Greater Los Angeles Affiliate.
Experimental model, Steen and Michenfelder\(^2\) obtained a survival time of 4.25 min in control animals and 8 min (50 mg/kg thiopentone). With the same exdibuturates. Wilhjelm\(^1\) reported an increase in survival time of 4 min in mice induced by physostigmine salicilate.\(^{19}\)

Effects of physostigmine may underly the cerebral protection of the drug against hypoxia. 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 \textit{in vivo} induces vasodilatation.\(^7\) - \(^18\) Physostigmine topically applied to the cerebral cortex induces vasodilatation in nitrous oxide-anesthetized rabbits.\(^7\) - \(^18\) Anesthetics have been reported to protect the brain from hypoxia, the effects being greatest for barbiturates. Wilhjelm\(^1\) reported an increase in survival time of mice breathing 5% O\(_2\) from 3.1 min (control) to 8 min (50 mg/kg thipentone). With the same experimental model, Steen and Michenfelder\(^2\) 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.

### Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of animals that survived 1 hr</th>
<th>Survival time (min) of animals that died before the hour ( ( \overline{x} \pm S.E.M. ) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td>10</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td>Physostigmine 0.1 mg/kg</td>
<td>14</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>Physostigmine 0.2 mg/kg</td>
<td>14</td>
<td>6.7 ± 0.9</td>
</tr>
<tr>
<td>Physostigmine 0.3 mg/kg</td>
<td>14</td>
<td>13.9 ± 1.6*</td>
</tr>
<tr>
<td>Untreated controls that survived a previous exposure</td>
<td>10</td>
<td>27.6 ± 4.4*</td>
</tr>
</tbody>
</table>

\( N = \) Number of animals.

\( * \) Differ significantly (\( p <0.01 \)) from both control groups (Student-Newman-Keuls test for multiple comparisons among means).

### References

Physostigmine-induced cerebral protection against hypoxia.
A M Scremin and O U Scremin

Stroke. 1979;10:142-143
doi: 10.1161/01.STR.10.2.142

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/10/2/142

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/