Protection against high-pressure oxygen seizures by amino-oxyacetic acid

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Beckman, D. L., and S. G. Iams. 1978. Protection against high-pressure oxygen seizures by amino-oxyacetic acid. Undersea Biomed. Res. 5(3):253–257.—A variety of autonomic blocking agents, general anesthetics, and anticonvulsants have been shown to offer protection from seizures caused by hyperbaric oxygen. Amino-oxyacetic acid (AOAA) has been shown to offer rats only minimal protection from such seizures. This study investigated whether AOAA protected cats and mice from hyperbaric-oxygen-induced seizures. Cats and mice were exposed to 100% oxygen at 5 ATA until seizures occurred or for a period of up to 60 min. Approximately half of the animals were pretreated with AOAA either 30 or 240 min before oxygen exposure. Results showed that the interval between exposure and grand mal seizures increased significantly in cats pretreated 30 or 240 min before exposure with 17 to 25 mg/kg AOAA; the number of cats remaining seizure-free for 60 min also increased markedly. However, mice received little protection even at doses up to 40 mg/kg. At higher doses the AOAA itself caused seizures even in the absence of hyperbaric oxygen.

hyperbaric oxygen
anti-convulsant
mouse
GABA

Many factors have been found to reduce the incidence of seizures and pulmonary injury caused by hyperbaric oxygen (Clark and Lamberts 1971). These include pretreatment with a variety of anti-epinephrine, sympathetic blocking, ganglionic blocking, and general anesthetic agents (Johnson and Bean 1957; Bean and Zee 1965). Similar agents also were found to protect rats from seizures and lung damage caused by mechanical central nervous system injury (Bean and Beckman 1969).

Amino-oxyacetic acid (AOAA) is an anti-convulsant whose action has been well documented (Busnel and Lehmann 1963; Da Vanzo, Matthews, and Stafford 1964; Roa, Tews, and Stone 1964; Kuriyama, Roberts, and Rubenstein 1966; Wood and Peesker 1973). The mechanism of AOAA’s action, however, remains obscure. Because gamma amino butyric acid (GABA) is an inhibitory neurotransmitter found in the central nervous system, it was thought that it might play a role in the anticonvulsant activity of AOAA. GABA levels in the brain did increase substantially, but only after a 2- to 3-h interval after AOAA injection; therefore any initial protection cannot be attributed to increased GABA levels. Wood and Watson (1965) reported that AOAA was "without effect on the number of rats convulsing or dying from exposure to hyperbaric oxygen, although in some cases the number of animals severely
convulsing was decreased." This partial protection from severe convulsions reportedly occurred within 5 to 60 min after AOAA injection, but it did not occur 240 min after injection, which tends to rule out increased brain GABA levels as the means of protection.

The present series of experiments was performed to determine whether AOAA would protect cats and mice from epileptiform convulsive seizures caused by exposure to 100% oxygen at 5 ATA.

METHODS

Twenty-seven young adult cats and 40 mice of both sexes were exposed to 99.5-100% medical grade oxygen in the hyperbaric chamber described by Bean (1931). Half of the animals were pretreated with AOAA (ip) either 30 or 240 min before exposure. Animals were exposed to the point of severe epileptiform convulsive seizures or, in the absence of any seizures, for 60 min. Compression and decompression were performed over 5-min periods. Humidity and carbon dioxide levels were controlled by maintaining a constant flow-through of at least 5 liters/min. During the exposure the animals were continuously observed for seizure activity. Controls and pretreated animals were usually put in the chamber together to ensure uniform exposure conditions. Two cats or 10 mice constituted a typical exposure group. Amounts of AOAA injected varied from 4, 10, 17, 20, 25, to 40 mg/kg for the dose-response studies; exposure followed pretreatment by 30 min. In an additional series of experiments using the cats from the first series, AOAA pretreatment preceded the exposure to hyperbaric oxygen by 240 min so that it could be determined whether the gradual rise in GABA levels in the brain conferred more or less additional protection.

RESULTS

Results from this study show that treating cats with AOAA 30 min before 100% oxygen exposure at 5 ATA confers marked protection against seizures, but AOAA does not protect mice.

All eight control cats in this series developed severe convulsive seizures; the average exposure time to seizure was 13 ± 3.1 (se) min. Seizure-free time increased significantly and progressively with increasing doses of AOAA in cats given 17- and 25-mg/kg doses (Table 1).

<table>
<thead>
<tr>
<th>AOAA, mg/kg</th>
<th>0</th>
<th>4</th>
<th>10</th>
<th>17</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cats</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Time to seizure, min (mean ± se)</td>
<td>13 ± 3.1</td>
<td>16 ± 2.9</td>
<td>22.5 ± 3.4</td>
<td>35 ± 5.4*</td>
<td>55 ± 17.8*</td>
</tr>
<tr>
<td>Seizure-free animals, percent</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>80</td>
</tr>
</tbody>
</table>

* = Significantly different from nontreated, hyperbaric-oxygen-exposed controls (P < 0.01). Student-t test was used for statistical analysis.
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The percentage of cats at each dose level that remained seizure-free for the 60-min exposure also increased markedly with increasing amounts of AOAA (Table 1). In the series of cats pretreated 240 min before oxygen exposure (Table 2), the percent remaining seizure-free increased with correspondingly increasing doses of AOAA. All of the oxygen-exposed nontreated controls developed severe seizures. Seizure-free time also increased significantly and progressively with increasing amounts of AOAA (Table 2). However, little if any additional protection was afforded by increasing the pretreatment time from 30 to 240 min; this can be seen at the 10- and 25-mg/kg levels in Tables 1 and 2. However, in the mice, seizure-free time and the percent of animals remaining seizure-free did not change significantly with higher doses of AOAA (Table 3).

Early signs of severe seizures such as pilo-erection, mydriasis, and increased and jerky motor activity were also delayed and ameliorated by AOAA pretreatment in both cats and mice.

Gross lung damage in cats exposed to oxygen was minimal in controls and in the AOAA pretreated cats. Cats have previously been found to be relatively nonreactive to hyperbaric-oxygen-induced lung damage (Beckman and Houlihan 1973). However, 30% of the mice exposed to oxygen to the point of overt seizures showed severe gross lung injury. Approximately one-fourth of these seizures caused death. The delay between death and autopsy in these animals did not exceed 45 min.

**TABLE 2**
PROTECTION AGAINST SEIZURES CONFERRED ON CATS BY AOAA 240 MIN BEFORE HYPERBARIC OXYGEN EXPOSURE

<table>
<thead>
<tr>
<th>AOAA, mg/kg</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cats</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Time to seizure, min (mean ± se)</td>
<td>16.5 ± 2.2</td>
<td>31.5 ± 10.0</td>
<td>50.3 ± 10.0*</td>
<td>60.0 ± 0.0*</td>
</tr>
<tr>
<td>Seizure-free animals, percent</td>
<td>0</td>
<td>33</td>
<td>75</td>
<td>100</td>
</tr>
</tbody>
</table>

* = Significantly different from nontreated, hyperbaric-oxygen-exposed controls ($P < 0.01$).

**TABLE 3**
PROTECTION AGAINST SEIZURES CONFERRED ON MICE BY AOAA 30 MIN BEFORE HYPERBARIC OXYGEN EXPOSURE

<table>
<thead>
<tr>
<th>AOAA, mg/kg</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mice</td>
<td>21</td>
<td>5</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Time to seizure, min (mean ± se)</td>
<td>36.6 ± 4.3</td>
<td>32.2 ± 6.2</td>
<td>43.1 ± 5.6</td>
<td>51.8 ± 6.3</td>
</tr>
<tr>
<td>Seizure-free animals, percent</td>
<td>14</td>
<td>0</td>
<td>44</td>
<td>40</td>
</tr>
</tbody>
</table>
DISCUSSION

Results from this study show that AOAA partially protects cats from convulsive seizures associated with exposure to 100% oxygen at 5 ATA. Furthermore, because this partial protection occurred within 30 min after AOAA injection, when GABA levels had only just started to rise (Da Vanzo, Greig, and Cronin 1961; Kuriyama et al. 1966), it is reasonable to attribute this protection, in part at least, to some mechanism other than increased brain GABA levels. In fact, a number of studies has shown that there is a definite lack of correlation among AOAA pretreatment, GABA levels, and protection from seizures (Da Vanzo et al. 1961; Kuriyama et al. 1966; and Wood and Peesker 1973, 1976).

Wood and Peesker (1976) showed a correlation between seizure activity and a function of GABA metabolism called the RE\textsubscript{GABA} value, which incorporated both the GABA content and activity of glutamic acid decarboxylase in the brain. However, this general relationship did not hold for the anticonvulsant action of AOAA against isonicotinic acid hydrazine.

Several agents offer protection from seizures and pulmonary damage caused by hyperbaric oxygen (Clark and Lambertsen 1971). Hypophysectomy reduced the amount of lung damage and the number and severity of seizures in rats exposed to 5.8–8.1 ATA of oxygen (Bean 1955). Such protection was attributed to the reduction of adrenocortical activity. Adrenalectomy also protected rats and mice from convulsions and pulmonary damage (Bean and Johnson 1955). In addition, adrenomedullectomy protected rats from convulsions and lung damage, which suggests a sympatho-adrenomedullary interaction (Clark and Lambertsen 1971). Several adrenergic blocking agents such as dibemamine and SKF-501 protected rats against pulmonary damage but did not reduce the severity of convulsions, whereas phenoxybenzamine also offered protection against seizures (Clark and Lambertsen 1971). The ganglionic blocking agents hexamethonium and tetraethylammonium provided protection against both lung damage and seizures at 6.4 ATA of oxygen (Clark and Lambertsen 1971). Chlorpromazine and reserpine offered similar protection.

Results from the present series of experiments show that AOAA offers cats significant protection from overt, severe epileptiform seizures caused by exposure to 100% oxygen at 5 ATA. This protection occurs both 30 and 240 min after pretreatment with AOAA, which suggests that AOAA alone offers some initial protection and that the delayed protection may be accounted for by a GABA-related mechanism.

Dr. John P. DaVanzo provided useful consultation and advice for these experiments, and Steven J. Blumenthal performed many of the technical procedures.—Manuscript received for publication December 1977; revision received March 1978.

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avant l’exposition, et que le nombre de chats qui n’ont pas présenté de crise avant 60 min a augmenté aussi. L’effet protecteur n’est pas apparu chez la souris, même à des doses jusqu’à 40 mg/kg. Aux doses plus grandes, le médicament lui-même a provoqué des crises sans exposition à l’oxygène hyperbare. Nos résultats montrent que l’AOAA exerce un effet protecteur contre les crises grand mal sévères provoquées par l’oxygène pur hyperbare à 5 ATA.

anticonvulsant  
chat  
souris  
GABA (acide gamma-aminobutyrique)

REFERENCES


