PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 168, 45–48 (1981)

Protection from Oxygen-Induced Seizures by Clonazepam and Propylene Glycol (41232)

DAVID L. BECKMAN AND DANIEL J. CRITTENDEN

Department of Physiology, School of Medicine, East Carolina University, Greenville, North Carolina 27834

Abstract. General anesthetics, ganglionic blocking agents, anticonvulsants, and antioxidants have been shown to afford protection from seizures caused by exposure to hyperbaric oxygen. In the present study cats were exposed to 5 ATA oxygen in pairs in a hyperbaric chamber until both the control and pretreated cat convulsed or for a maximum 120 min exposure. Small amounts of four common antiepileptic agents and propylene glycol in amounts far less than previously reported (0.1 to 0.2 ml/kg) were initially tested for potential anticonvulsant activity. Two agents, clonazepam and propylene glycol, offered significant protection in delaying the onset of seizures whereas carbamazepine, valproic acid, and trimethadione appeared to hasten the onset of seizure activity. The time to seizures was increased nearly five times by clonazepam and over three times by very small amounts of propylene glycol.

Protection from seizures due to exposure to oxygen at high pressure (OHP) has been widely reported to result from general anesthetics (1), antioxidants (2, 3), GABA inhibitors (4, 5), ganglionic blocking compounds (6), and others (7, 8). Our purpose in the present study was to determine whether low doses of several common antiepileptic agents offered protection from OHP-induced seizures and whether very small doses of propylene glycol (PG) also might protect. Because previous studies showed that 2 ml/kg PG were effective against OHP-induced seizures (1), we were interested in determining whether far smaller amounts also would ameliorate the somatic response in cats exposed to 5 atmospheres absolute (ATA) of OHP. In order to determine whether any residual neurological damage had occurred after OHP a group of cats were observed for signs of ataxia or paralysis as indicators of residual brain damage.

Methods. Thirty-eight young adult cats were exposed in pairs to 5 ATA of 100% oxygen. A pretreated cat and its control were exposed to OHP until both cats convulsed or for a maximum of 120 min. A flow-through system was used with soda lime so that CO_2 levels remained below 1%. The exposure chamber was previously described by Bean (9). A 3-min flush was followed by a 3-min compression to 60 lb gauge pressure. Four antiepileptic agents or PG in amounts far less than previously reported were administered orally in gelatin capsules 120 min prior to exposure. The carriers given to controls were either saline, polyethylene glycol (PEG) 200, or PG. Drugs and doses were as follows: clonazepam, 0.03 and 0.06 mg/kg; PG, 0.1–0.2 ml/kg; carbamazepine, 5.6 mg/kg; valproic acid, 120 mg/kg; and trimethadione, 120 mg/kg.[1] Doses were approximately two to three times the clinically recommended levels. Preliminary groups consisting of two cats given each drug and two nontreated controls were exposed to OHP in order to determine whether there was any evidence of protection. Any agent showing such protection was tested further. Because OHP at 4 ATA or more has been shown to result in residual brain damage in rats and mice but not in guinea pigs, rabbits, or man (10), we also examined a group of 17 cats (chosen randomly) for signs of ataxia or paralysis following OHP exposure. In addition, in order to determine the extent of any pulmonary damage, lungs from 12 cats were examined following OHP. These lungs were

[1] Valproic acid and trimethadione were kindly supplied by Abbot Laboratories, clonazepam by Hoffman–LaRoche, Inc., and carbamazepine by Geigy Pharmaceuticals.

0037-9727/81/090045-04$01.00/0

excised, blotted dry, weighed, dried for 24 hr at 70°, and reweighed. The statistically applicable data in these experiments were confined to tests of two of the drugs which showed initial evidence of protection. Student's *t* test was employed to compare mean times to convulsions of control and pretreated cats (11).

Results. The results showed that clonazepam and also PG in very low doses offered significant protection against OHP-induced seizures. All five controls given only PEG 200 convulsed within the 120-min exposure period as did six additional controls without any carrier. The average time to onset of seizures was 29.4 ± 15.5 min in control cats given PEG 200 whereas 0.06 mg/kg clonazepam delayed the onset of seizures for at least 120 ± 0 min ($P < 0.001$). In a second series, six untreated cats convulsed after 18.5 ± 4.2 min OHP and 0.06 mg/kg clonazepam-pretreated cats only after 101.7 ± 7.9 min ($P < 0.001$) (Table I). Five cats given only 0.03 mg/kg clonazepam in PG did not have significant protection from OHP-induced convulsions. PG alone, however, in very low doses (0.1–0.2 ml/kg) did offer significant seizure protection. Saline-treated controls convulsed after 14.0 ± 3.1 min OHP whereas PG-treated cats remained seizure free for 46.5 ± 17.0 min ($P < 0.01$). Three other antiepileptic anticonvulsants given in low doses to two cats each appeared to offer no protection. These agents, carbamazepine, valproic acid, and trimethadione, actually shortened the time to onset of convulsions.

The general response of most cats to oxygen exposure at 5 ATA usually began with piloerection occurring within 5 min after pressurization. Two types of behavior were then noted that eventually led to seizures. Within 30 min of pressurization, most cats exhibited shaking or head movements which were closely followed by convulsions. On the other hand, about 25% of the cats showed no physical signs at all until after 30 min or more had elapsed and then began very active washing and licking motions. Although many of these cats eventually convulsed, other signs such as shaking and head movements often intervened. In both groups the advent of seizures was initiated with clonic leg jerking. The eyes appeared glassy, the pupils were dilated, and stringy saliva drooled from the mouth. This was followed by sharp, rapid back-to-front

TABLE I. EFFECT OF PRETREATMENT ON OHP-INDUCED CONVULSIONS

Agent	Dose (mg/kg)	Carrier	Time to convulsion (min)		
			n	Mean ± SEM	*P* value
Control	—	PEG[a] 200	5	29.4 ± 15.5	
Clonazepam	0.06	PEG 200	5	120.0 ± 0	<0.001
Control	—	—	6	18.5 ± 4.2	
Clonazepam	0.06	PG[b]	6	101.7 ± 7.9	<0.001
Control	—	—	5	23.8 ± 8.1	
Clonazepam	0.03	PG	5	71.2 ± 23.1	n.s.
Control	—	—	7	14.0 ± 3.1	
PG	0.1–0.2	—	8	46.5 ± 17.0	<0.01
Control	—	—	2	14	
Carbamazepine	5.6–11.2	Saline	2	13	
Control	—	—	2	14	
Valproic acid	120	Saline	2	13	
Control	—	—	2	42	
Trimethadione	120	Saline	2	14	

Note. *n* = number of cats.
[a] Polyethylene glycol.
[b] Propylene glycol.

head jerks that gradually increased in severity and frequency as the cats settled into a squatting position on the floor of the chamber. The ears were laid back with the head lowered between outstretched forelimbs. Gradually the whole body became involved in clonus until violent, explosive muscular discharges caused many of the cats to collide repeatedly with the walls of the chamber. However, some cats that appeared to be on the verge of seizures became nauseous instead and developed dry vomiting behavior with no vomitus actually expelled.

Five control cats which experienced moderate to severe convulsions were apparently completely normal when examined within 48 hr of exposure without any signs of ataxia or paralysis related to residual brain damage. Five additional control cats and seven clonazepam-pretreated cats examined within 6 days of OHP exposure also were completely normal in appearance and behavior.

Lung wet wt/dry wt ratios were unaffected by these OHP-induced seizures. Calculations showed ratios for six normal nonconvulsed controls to average 4.96 ± 0.1 compared to 4.82 ± 0.1 for six cats exposed to OHP until severe seizures had occurred.

Discussion. The results from the present study showed that both clonazepam and PG delayed the onset of OHP-induced seizures. While others (1) reported seizure protection from PG, the doses were higher by a factor of 10–20 times those used in the present study. Our results suggest that the other three antiepileptic drugs tested, carbamazepine, valproic acid, and trimethadione, may not be effective against OHP-induced seizures. All three drugs appeared to hasten rather than delay the onset of seizures (Table I). However, because of the very limited sample size their possible protective action cannot be entirely ruled out.

The effects of clonazepam and PG do not appear to be synergistic, the time to seizures being virtually the same when given together vs clonazepam alone. Clonazepam did, however, offer better protection than PG. Clonazepam at both 0.03 and 0.06 mg/kg produced some loss of motor control and apparent disorientation prior to as well as during exposure. It is possible that prolonged administration would reduce such effects. In contrast, PG appeared to produce no adverse effects on the cats before or during exposure. Larger amounts of PG (1 ml/kg in two cats) also had no adverse effects but in our tests in cats did not increase the time to seizures.

Both PG and clonazepam may protect against OHP-induced seizures by maintaining CNS γ-aminobutyric acid (GABA) activity at near-normal levels. GABA normally acts to prevent convulsions but is depleted during OHP exposure (12). PG maintains GABA activity near normal by conserving brain GABA levels although the mechanism remains unclear (13). Clonazepam appears to render any GABA present in the brain more effective by facilitating GABA receptor function. The benzodiazepine receptor sites in the brain, when occupied by clonazepam, may function by displacing an endogenous GABA-inhibitor substance (GABA-modulin) from GABA receptor sites. GABA-modulin displacement increases the affinity of GABA receptors for their agonist and helps to maintain normal GABA-ergic transmission at times when GABA stores may be depleted (14).

Many other agents have offered at least some protection from OHP-induced convulsive seizures. These have included antioxidants (3), general anesthetics (1, 15), and ganglionic blocking agents (6). While there may be some question concerning the mechanisms through which the sympathetics may influence the response to OHP exposure (16), there is general agreement that the sympathetics do have a definite influence. Adrenalectomy delays the onset of convulsions, thus providing a degree of protection (17). Furthermore, administration of sympathetic blocking agents prior to OHP exposure results in decreased lung damage (6). A direct sympathetic neural influence on the lungs during OHP exposure also has been suggested (18).

Lung damage from mechanical head injury may share a common autonomic mechanism with OHP. This head injury also produces seizures and lung damage in rats (19) and seizures and less lung damage

in squirrel monkeys (20) that are ameliorated by sympathetic blockers. In cats, head injury causes a striking increase in circulating catecholamines (21) but results in only minor gross lung damage. However, both OHP and head injury produce severe gross lung damage in rats (19). While rats and mice exposed to OHP routinely develop severe lung damage (22) the incidence is far less common in cats (23, 24). In the present study there was no increase in lung weights or change in gross lung appearance in cats.

We examined 17 cats for residual brain damage due to OHP exposure. Although two control cats convulsed severely and continuously for over 1 hr and were still paralyzed immediately after decompression and removal from the chamber, they appeared normal within 48 hr. Eight other control cats and seven clonazepam-pretreated cats observed within 6 days of exposure also were normal in appearance and behavior. The observation that cats may be less susceptible than rats and mice to residual brain damage (23) was supported by the absence of ataxia and paralysis 1–6 days postexposure. In contrast rats and mice were found to be highly susceptible to residual brain damage at pressures of oxygen above 4 ATA (10, 25) which was potentiated by the anticonvulsant action of barbiturate anesthesia. The absence of any residual CNS damage in cats resembles the response found in guinea pigs, rabbits, and humans. Our results therefore concur with those of van den Brenk and Jamieson (10) who did not find permanent residual or potentiated brain damage in species larger than the rat. In summary, both clonazepam and small amounts of PG offered seizure protection. PG gave the added advantage of avoiding the mild loss of motor control as seen with clonazepam.

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Received March 23, 1981. P.S.E.B.M. 1981. Vol. 168.