Strain Differences in CNS and Lethal Effects of Hyperbaric Oxygen in Mice[1] (34284)

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Hyperbaric oxygen (HBO) may have therapeutic application in several disease states but pulmonary damage and convulsions are major toxic side-effects which must be carefully controlled (1, 2). A marked individual variation in susceptibility to convulsions (2) makes the occurrence of this toxic effect difficult to predict. Therefore, information concerning factors that influence susceptibility to HBO would be of practical importance. Significant strain differences in susceptibility to electroshock and audiogenic seizures have been demonstrated (3, 4). Hill *et al.* (5) showed strain differences in survival time of mice exposed to HBO. They also reported observations that some strains were resistant to the seizure-inducing action of HBO while other strains were sensitive, with 100% of the animals demonstrating convulsions.

The present experiments were conducted to further quantify differences in susceptibility of four strains of mice to convulsions induced by HBO. The convulsions were classified into two distinct pattern types, those which were purely clonic in nature (clonic convulsions) and those which had a tonic extension component (tonic convulsions). In addition, survival times of the animals were recorded.

Methods. Male mice 5–7 weeks of age were used. Mice of the CF-1 strain were obtained from Carworth Farms, New City, New York. C57BL/6, C3H/anf, and HA/icr mice were purchased from Cumberland View Farms, Clinton, Tennessee. Mice were randomly assigned, housed three to a cage, and given Purina lab chow and water *ad libitum*. They were maintained in the animal quarters for 5–8 days before experiments were conducted. The mice were not disturbed to clean cages during a period beginning 4–5 days prior to the experiment. Approximately 30 min prior to compression, 12 animals (3 from each strain) were placed in individual compartments (4 × 4 × 4 in.) in a rectangular wire cage, transported to the hyperbaric laboratory, and suspended centrally in the pressure chamber. Four such experiments were performed, giving a total of 12 animals in each strain.

A Bethlehem Corporation Chamber (No. 1836, 18 in. diameter and 36 in. long) with viewing ports on each side was used for all exposures. Before compression the chamber was flushed with high flows of 100% oxygen (Air Products, Inc., Greensboro, N. C.). Compression was accomplished at a uniform increase of 1 atm/min for all pressures and the chamber was vented at 2 liters/min during compression in order to prevent CO_2 accumulation. The CO_2 concentrations in samples of the outflow were less than 0.1%. Temperature was maintained between 25 and 27° during compression and varied by no more than 0.5° over 5 different positions in the chamber. Humidity ranged between 40 and 60%. Experiments were concluded after 300 min of exposure.

Clonic convulsions consisted of loss of righting ability, whole body convulsive movements, and jerking movements of the extrem-

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TABLE I. Frequency of Animals with Clonic and Tonic Convulsions during Exposure to HBO for 300 min.

Type of convulsions	Atm abs	Ha/icr	C3H/anf	C57BL/6	CF-1	p^b
Clonic	3	3/12[a]	6/12	0/12	1/12	<0.05
	4	5/12	11/12	9/12	6/12	<0.05
	5	3/12	12/12	7/12	1/12	<0.001
Tonic	3	6/12	6/12	0/12	1/12	<0.01
	4	4/12	6/12	8/12	3/12	NS
	5	5/12	6/12	3/12	1/12	NS

[a] Number of animals convulsing to number of observations.
[b] Chi-square test; NS indicates $p > 0.05$.

ities. The tonic convulsions were characterized by maximum extension of fore and hind limbs lasting 10–15 sec followed by relaxation with clonic movements. The moment of arrival at the desired pressure was used as the beginning point (zero time) in determining the time to onset of convulsions and death. The end point for survival time was cessation of respiration. If animals were still breathing at the end of the experimental period, survival time was recorded as 300 min.

Results. The frequency of animals with clonic and tonic convulsions during exposure to HBO for 300 min is shown in Table I. There was a significant strain difference in the occurrence of clonic convulsions at 3, 4, and 5 atmospheres absolute (atm abs). Generally, the order of sensitivity as measured by this parameter was C3H/anf > C57BL/6 > HA/icr > CF-1. Incidence of clonic convulsions was higher at 4 atm abs than at 3 atm abs in each strain, but increasing the pressure to 5 atm abs slightly decreased the incidence in three of the four strains. This may have been due to an earlier lung damage which could possibly change the dose of oxygen to which the brain cells are exposed (6). Strain differences in susceptibility to tonic convulsions were statistically significant only at 3 atm abs (Table I). However, there were also substantial differences between some of the strains at 4 and 5 atm abs which were not statistically significant. Again, HBO at 4 atm abs induced the highest incidence of tonic convulsions. Overall, the order of strain sensitivity appears to be C3H/anf > HA/icr > C57BL/6 > CF-1. Although with both clonic and tonic convulsions, the order of ranking might vary slightly if only one pressure is examined, it is clear that the C3H/anf strain is most sensitive and the CF-1 strain least sensitive with respect to the occurrence of convulsions induced by HBO.

Since the greater number of mice convulsed at 4 atm abs, the times to onset of clonic convulsions at this pressure were subjected to analysis of variance. Only those animals that had clonic convulsions were used for the analysis. This test revealed a significant strain difference in times to convulsions (Table II). A further comparison of all strains by Duncan's New Multiple Range Test (7) showed significant differences between all of the strains except HA/icr and C3H/anf (Table III). The order of strains from shortest to longest time to convulsions was C3H/anf $\cong$ HA/icr < C57BL/6 < CF-1. As with the previous analyses, C3H/anf mice were most sensitive and CF-1 mice were least sensitive.

During 300 min of HBO exposure at 3 atm abs, 5 of 12 HA/icr mice died and 4 of 12 C3H/anf mice died while none of the mice in C57BL/6 or CF-1 strains died. However, only 1 or 2 of the 12 mice in each strain survived exposure at 4 atm abs and none survived exposure at 5 atm abs. Survival times at 4 and 5 atm abs are given in Table II. Increasing the pressure from 4 to 5 atm abs greatly shortened the survival time. Since analysis of variance demonstrated a significant difference between strains at each pressure, the survival times were further com-

TABLE II. Time to Onset of Clonic Convulsions and Survival Time of Mice of Various Strains Exposed to HBO for 300 min.

Measurement	HA/icr	C3H/anf	C57BL/6	CF-1	p^c
Time (min) to clonic convulsions at 4 atm abs	95 ± 7[a](5)[b]	82 ± 12 (11)	124 ± 15 (9)	145 ± 12 (6)	<0.001
Survival time (min) at					
4 atm abs	158 ± 22 [1][d]	157 ± 20 [1]	230 ± 16 [2]	247 ± 15 [1]	<0.001
5 atm abs	99 ± 10 [0]	112 ± 6 [0]	140 ± 5 [0]	148 ± 7 [0]	<0.001

[a] Mean ± standard error of the mean.

[b] Number of animals showing clonic convulsions; 12 animals from each strain were observed; only those which had clonic convulsions were used for analysis of time to clonic convulsions.

[c] Analysis of variance.

[d] Number of animals surviving 300 min; all 12 animals of each strain were used for statistical evaluation.

pared by Duncan's New Multiple Range Test (Table III). From these comparisons, it appears that the appropriate order of ranking with respect to susceptibility to the lethal effect of HBO would be C3H/anf ≅HA/icr > C57BL/6 ≅ CF-1.

Discussion. The present results clearly demonstrate significant strain differences in the response of mice to HBO. These differences occur with respect to incidence of clonic and tonic convulsions, time to onset of clonic convulsions, and survival time. Of the four strains tested, the C3H/anf strain was most sensitive to the CNS and lethal effects of HBO while the CF-1 strain was least sensitive. Hill *et al.* (5) presented quantitative data demonstrating significant strain differences in survival time of mice subjected to HBO. They also suggested strain differences in susceptibility to HBO-induced convulsions. The present studies corroborate the survival time studies of Hill *et al.* (5) and present data which extend the strain differences in susceptibility to include the convulsive phenomena.

TABLE III. Comparison between Strains of Time to Convulsions and Survival Time.[a]

Comparison	Time to clonic convulsions at 4 atm abs	Survival time at 4 atm abs	Survival time at 5 atm abs
HA vs. C3H	NS	NS	NS
HA vs. C57	<0.01	<0.01	<0.01
HA vs. CF-1	<0.01	<0.01	<0.01
C3H vs. C57	<0.01	<0.01	<0.01
C3H vs. CF-1	<0.01	<0.01	<0.01
C57 vs. CF-1	<0.01	NS	NS

[a] Duncan's New Multiple Range Test; NS indicates $p > 0.05$.

Various reports suggest an involvement of brain biogenic amines (norepinephrine, dopamine, and 5-hydorxytryptamine) in convulsions resulting from exposure to HBO (8–10). Studies on seizures in mice induced by electroshock and auditory stimuli have also revealed strain differences in susceptibility which seem to be correlated with amine levels in the brain (3, 4). Highly susceptible strains had lower brain amine concentrations while less sensitive strains had higher concentrations (3, 4). Such correlations should also be examined with HBO.

Strain differences in the response of lung tissue to HBO may offer additional information concerning the lethal effects of HBO. Also, it has been shown that adrenal gland activity influences sensitivity to the toxic effect of HBO. For example, adrenalectomy delayed the onset of oxygen toxicity in rats and mice (11, 12). This protective effect was counteracted by administration of adrenocortical hormones or epinephrine, indicating that both aspects of adrenal activity are important (11, 12). Such studies suggest that strain differences in the response of endocrine systems or autonomic nervous system may be responsible for some of the results of the experiments reported here. As suggested by Hill *et al.* (5), comparative studies of physio-

logical, biochemical, and pharmacological characteristics of strains with differing sensitivities to the toxic effects of HBO may provide insight into mechanisms involved in the production of these effects.

Summary. Four strains of mice were tested for their response to the toxic effects of HBO. The order of strain sensitivity as measured by various parameters was: (i) incidence of clonic convulsions, C3H/anf > C57 BL/6 > HA/icr > CF-1; (ii) incidence of tonic convulsions, C3H/anf > HA/icr > C57BL/6 > CF-1; (iii) time to onset of clonic convulsions, C3H/anf ≅ HA/icr > C57BL/6 > CF-1; and (iv) survival time, C3H/anf ≅ HA/icr > C57BL/6 ≅ CF-1. The results of this study clearly demonstrate significant strain differences in the response of mice to HBO.

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