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COMPARATIVE EFFECTS OF HYPERBARIC OXYGEN AND PENTYLENETETRAZOL ON LUNG WEIGHT AND NON-PROTEIN SULFHYDRYL CONTENT OF EXPERIMENTAL ANIMALS

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Abstract—Rats convulsed by hyperbaric oxygen (OHP) or by pentylenetetrazol (PTZ) suffer consolidative lung damage. This effect and its moderation by pretreatment of the animals with protective compounds were quantitated by measuring lung weight and lung non-protein sulfhydryl (NPSH) content; liver NPSH content was also measured. Changes in lung weight were also examined in mice, guinea pigs, and rabbits treated with these two agents.

Rats and mice convulsed by either OHP or PTZ suffered a similar degree of lung damage. This effect was absent when exposures did not lead to onset of convulsive seizures. In contrast, guinea pigs and rabbits convulsed by OHP for similar times sustained considerably less lung damage. PTZ-induced convulsions in these two species did not lead to significant increases in lung weight.

Rats convulsed with OHP or PTZ had an identical loss of NPSH (44%) from lung but much less loss (25%) from liver. The liver NPSH loss was duplicated by non-convulsive OHP treatment, while lung NPSH content was relatively unaffected by such exposures. Starvation for 18 hr depleted liver NPSH levels but led to no greater loss of this component under OHP. Lung weight increases and lung NPSH loss due to PTZ were prevented by prior administration of compounds, (e.g. 5-hydroxytryptamine), known to prevent effects of OHP.

The experimental data were contrasted with the results of clinical experience with human patients. The conclusion was reached that the gross aspects of OHP-induced lung damage in animals are probably attributable to some mechanism other than direct oxidative attack upon pulmonary structures.

ACUTE exposure of rats to hyperbaric oxygen (OHP) causes convulsions, spastic paralysis, and hemorrhagic consolidation of the lungs.[1–3] These effects are accompanied by loss of lung dehydrogenase activity,[4] changes in the properties of the lung surfactant material,[5] and oxidation of sulfhydryl groups.[6] While it is not yet clear which effects result from the direct action of oxygen on pulmonary structures and which are indirect manifestations of central nervous system (CNS) damage, recent evidence suggests that "neuroendocrinogenic factors" associated with the autonomic component of seizure are responsible for at least the gross lung damage.[7] According to this view, the pulmonary consolidative changes produced by OHP result from sustained hypertension associated with sympathetic discharge from the CNS.[8] Similar lung damage

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has been observed in rats convulsed by chemical CNS-stimulants such as pentylenetetrazol.[7]

On the other hand, exposure of cells or intact animals to hyperbaric oxygen causes rapid oxidation of the intracellular reduced pyridine nucleotide pool,[9] an effect occurring well in advance of convulsions and evident lung damage. The link between the early biochemical events and the gross symptoms of oxygen poisoning is unknown. However, since a reduced pyridine nucleotide (NADPH) furnishes the reducing equivalents required for the continual reduction of oxidized glutathione by glutathione reductase, lack of such equivalents could conceivably lead to net depletion of reduced glutathione. Since low molecular weight (acid-soluble) sulfhydryl compounds protect SH-dependent enzymes against inactivation by oxygen both *in vivo*[4,10] and *in vitro*,[11] loss of such compounds might form one link between the very early pyridine nucleotide changes and later effects (convulsions, etc.). The non-protein sulfhydryl (NPSH) content of Ehrlich ascites tumour cells is, in fact, oxidized by OHP under certain *in vitro* conditions.[12]

The present studies were undertaken to learn whether loss of NPSH occurs in animals exposed to OHP and, if so, whether this loss precedes the onset of convulsions. Further, we wished to know how closely, if at all, the effects of oxygen were mimicked by the chemical convulsant, pentylenetetrazol, and whether the effects of the chemical could be prevented by agents known to moderate OHP effects. Since considerable variation may occur among different mammalian species in response to pharmacologic agents, these studies employed mice, guinea pigs, and rabbits, in addition to rats. The data are contrasted with results of current clinical experience with OHP toxicity in humans.

MATERIALS AND METHODS

Albino mice (18–24 g) and Canberra black rats (150–200 g) were purchased from commercial suppliers. Guinea pigs (500–700 g) and lop-ear rabbits (1·5–4·0 kg) were bred from stocks maintained at the Cancer Institute.

Animals were exposed to oxygen at 3 or 5 atm absolute (ATA) at room temperature (21–23°) in the pressure vessel described elsewhere.[13] The chamber was flushed with oxygen for 5 min prior to pressurization and a through-flow of gas was maintained during exposure. Decompression was completed in 3–7 min and animals were sacrificed within 10 min of decompression by cervical dislocation or injection of air into an ear vein.

The chemical convulsant, pentylenetetrazol, (Leptazol B.P.; purchased as Cardiazol from Knoll A-G Chemical Works, Ludwigshafen/Rhine, West Germany) was injected i.p. 5 min after injection of protective compounds or saline. This compound is generically identical to the Metrazol used by Bean and co-workers.[7] Since death occurred almost immediately when the dose (0·75 g/rat) used by these workers was administered to our rats, lower doses were used in our studies. Convulsing animals were kept in a chamber through which oxygen was passed at 3 l/min.

Lungs were excised, rinsed in saline, blotted, and weighed as rapidly as possible. For NPSH assay, samples of rat lung or liver were immediately minced in 4 ml of ice-cold 5% (w/v) sulphosalicylic acid in a tared beaker, weighed, and homogenized with a ground-glass homogenizer (Kontes Glass Co., Vineland, N.J.) for 2 min at 1000 rpm. Following centrifugation of the homogenates and Millipore filtration of

the supernatants, the extracts were brought to pH 6·8 with 2 vol of 0·5 M phosphate buffer containing NaOH. One volume of phosphate buffer, pH 6·8, containing the Ellman reagent, 5,5′-dithiobis-(2-nitrobenzoic acid)[14] was added immediately and the resulting color was read at 412 mμ.

RESULTS

Effect of oxygen and pentylenetetrazol on lung weight

Both hyperbaric oxygen (OHP) and pentylenetetrazol (PTZ) caused a marked increase in the lung weight of convulsed rats and mice (Table 1). In agreement with the findings of Bean and co-workers,[7] the chemical caused less increase than oxygen, but the lungs of both species showed the typical consolidative changes described by others.[1,7] Increasing the dosage of PTZ did not cause a further increase in lung weight of mice.

TABLE 1. EFFECT OF HYPERBARIC OXYGEN AND PENTYLENETETRAZOL (PTZ) ON LUNG WEIGHTS

Treatment* (no. of Animals)	Mean onset of convulsion	Lung wt. ± S.E.	% Control wt.
Mouse		g/20 g	
Control (5)	—	0·124 ± 0·006	100
5 ATA O_2, 45 min. (5)	22 min	0·255 ± 0·032	206
PTZ, 0·3 g/kg, 120 min (4)	29 sec	0·179 ± 0·012	144
PTZ + 5 HT, 15 mg/kg† (8)	54 sec	0·123 ± 0·006	99
PTZ + AET, 250 mg/kg† (8)	40 sec	0·166 ± 0·010	134
PTZ + AET, 250 mg/kg‡ (4)	61 sec	0·132 ± 0·005	106
Rat		g/175 g	
Control (5)	—	1·19 ± 0·10	100
3 ATA O_2, 60 min (4)	—	0·92 ± 0·10	77
5 ATA O_2, 30 min (4)	—	1·12 ± 0·05	94
5 ATA O_2, 60 min (3)	40 min	2·57 ± 0·38	216
PTZ, 0·3 g/kg, 25 min (3)	60 sec	2·17 ± 0·16	178
PTZ + 5 HT, 15 mg/kg† (4)	70 sec	1·12 ± 0·20	94
PTZ + Cysteamine, 100 mg/kg† (4)	57 sec	1·53 ± 0·02	128
Guinea pig		g/600 g	
Control (3)	—	4·29 ± 0·34	100
5 ATA O_2, 70 min (3)	45 min	6·82 ± 0·86	165
PTZ, 0·2 g/kg, 25 min (3)	60 sec	4·13 ± 0·17	96
Rabbit		g/kg	
Control (3)	—	3·38 ± 0·18	100
5 ATA O_2, 40 min (3)	22 min	4·06 ± 0·20	120
PTZ, 0·2 g/kg, 25 min (4)	100 sec	4·89 ± 0·84	145

Abbreviations: PTZ = pentylenetetrazol; 5-HT = 5-hydroxytryptamine creatinine sulphate; AET = 2-aminoethyl isothiouronium bromide hydrobromide.

* Except for mice, OHP (5 ATA) and PTZ schedules were chosen to allow a similar mean duration of convulsion (20–25 min) for all species.

† Agent injected i.p. 5 min prior to PTZ.

‡ Agent injected i.p. 30 min prior to PTZ.

Increased lung weight due to hyperbaric oxygen was not the result of direct attack of oxygen on pulmonary structures, since the effect was observed only in convulsed animals. Exposure of rats to 5 ATA for 30 min or to 3 ATA for 60 min neither induced seizures nor caused apparent lung damage (Table 1).

On the other hand, oxygen at 5 ATA produced less lung weight increase in guinea pigs and rabbits than in the smaller species, although all animals suffered typical

seizures and were allowed to convulse for similar times prior to sacrifice (Table 1). The effect in guinea pigs and rabbits, while small, was significant ($P \leqslant 0{\cdot}05$).

In contrast to the results obtained for rats and mice, PTZ-induced convulsions in guinea pigs and rabbits were not accompanied by significant lung damage. Lung weights in PTZ-convulsed guinea pigs remained at control level while rabbits showed a more variable response. The mean value of four rabbits (145 per cent of control) was not significantly different from control; two rabbits were clearly affected (6·97 and 5·49 g/kg) while the other two were not (3·18 and 3·93 g/kg). These results suggest that a "threshold" dosage of PTZ may exist for lung damage in rabbits, although there was no difference among the four in onset, duration, or severity of seizure. The difference between PTZ and OHP guinea pig lungs (but not rabbit lungs) was significant ($P \leqslant 0{\cdot}025$).

Effect of "protective agents" on lung weight

Certain pharmacological compounds reduce or eliminate lung damage in OHP-convulsed animals,[10] and it was of interest to study the effects of these agents on PTZ-induced lung damage. The results indicate that lung damage caused by PTZ is entirely separable from the motor component of seizure, since lung damage was moderated or prevented while the seizure itself progressed as in unprotected animals (Table 1). Complete protection was afforded by 5-hydroxytryptamine creatinine sulphate (5-HT) and by 2-aminoethyl isothiouronium bromide hydrobromide (AET) administered to mice 30 min prior to PTZ injection (Table 1). AET had little protective efficacy when injected 5 min before PTZ, a result of some interest in view of the greater radioprotection by this compound when administered 45 min, as compared to 15 min, prior to irradiation of rats.[10] Cysteamine also decreased the damage caused by PTZ, but did not provide absolute protection.

Compounds which protected against lung damage usually delayed the onset of PTZ-seizure (Table 1). Convulsions in "protected" animals, once initiated, proceeded as in unprotected animals although there was a subjective impression that the former group convulsed with slightly less vigor than did their saline-injected (unprotected) counterparts.

Non-protein sulfhydryl content

Total liver and lung NPSH were relatively unaffected by hyperbaric oxygen as long as the rats were not convulsed (Table 2). Exposure to 3 ATA oxygen for 60 min decreased lung NPSH by 12·8% and liver NPSH by 16·9% while even smaller effects were observed after 30 min at 5 ATA (9·8% decrease in lung, 0·3% decrease in liver).

When rats were convulsed by either hyperbaric oxygen (5 ATA, 60 min) or pentylenetetrazol, a dramatic loss of total lung NPSH occurred. Because of the grossly hemorrhagic condition of lungs from these animals, it was necessary to introduce a correction factor for the influx of blood by using the data of Jamieson and van den Brenk.[6] Under the same hyperbaric conditions used in the present study, these workers found that 42 per cent of the weight gain in rat lungs could be accounted for as whole blood, the remainder of the weight increase representing plasma and protein-free fluid. In the present study, therefore, there would have been 0·58 ml of extra blood in the lungs of OHP-convulsed rats and, (assuming the same percentage to apply to PTZ-treated animals), 0·41 ml of additional blood in the lungs of PTZ-

TABLE 2. EFFECT OF HYPERBARIC OXYGEN AND PENTYLENETETRAZOL (PTZ) ON NON-PROTEIN SULFHYDRYL CONTENT OF RAT LUNGS AND LIVER

Treatment (no. of rats)	Organ wt.	Non-protein sulfhydryl content		
		μmole/gm	μmole/organ	% control
Lung				
Control (5)		1·99 ± 0·05	2·37	100
5 ATA O_2, 30 min (4)		1·88 ± 0·14	2·11	90·2
3 ATA O_2, 60 min (4)	See	2·25 ± 0·17	2·04	87·2
5 ATA O_2, 60 min (3)*	Table 1	0·73 ± 0·05	1·86/1·34†	78·5/56·5†
PTZ, 0·3 g/kg, 25 min (3)*		0·81 ± 0·13	1·67/1·31†	70·5/55·3†
PTZ + 5 HT,* 15 mg/kg (4)*		2·31 ± 0·53	2·55	107·9
Liver (fed animals)				
Control (10)	9·5 ± 0·4	6·00 ± 0·26	57·12	100
5 ATA O_2, 30 min (6)	9·5 ± 0·4	5·98 ± 0·18	56·93	99·7
3 ATA O_2, 60 min (4)	—	5·58 ± 0·48	(47·47)‡	83·1
5 ATA O_2, 60 min (4)*	9·0 ± 0·5	5·13 ± 0·59	46·12	80·7
Liver (18 hr-starved animals)				
Control (8)	7·2 ± 0·1	5·25 ± 0·29	37·59	100
5 ATA O_2, 30 min (6)	7·1 ± 0·2	5·14 ± 0·27	36·70	97·8
3 ATA O_2. 60 min (3)	—	4·19 ± 0·22	(30·00)‡	79·8

Abbreviations: PTZ = pentylenetetrazol; 5 HT = 5-hydroxytryptamine creatinine sulphate.
* Animals convulsed for 20–25 min.
† First figure represents uncorrected value; second figure is corrected for influx of blood as described in text.
‡ Livers not weighed; value calculated on assumption that no significant weight change occurred during exposure.

convulsed rats. The NPSH content of rat blood was 0·89 μmole/ml and in view of the work of Mengel and Kann[15] it is unlikely that this value would change under hyperbaric conditions. Therefore, the measured lung NPSH of oxygen-convulsed and PTZ-convulsed rats was corrected by substracting 0·52 μmole from the former and 0·36 μmole from the latter. This correction gives a "true" lung NPSH content of approximately 56 per cent of control for both treatments (Table 2). The loss of lung NPSH in PTZ-treated rats was prevented by prior administration of 5 HT (Table 2).

Liver NPSH was much less affected than lung NPSH in OHP-convulsed rats, decreasing by only about 18 per cent. This value was not corrected since liver weight was not significantly altered by exposure. Since earlier experiments had shown that OHP affects the NPSH of Ehrlich ascites tumor cells only in the absence of substrate,[12] rats were deprived of food for 18 hr prior to OHP exposures. This pretreatment decreased liver weight by 25 per cent, NPSH concentration by 12 per cent, and total NPSH content by 34% but did not change the relative effect of OHP (Table 2).

Oxygen toxicity in humans

Relatively recently the therapeutic potential of hyperbaric oxygen has been investigated in several pathological conditions in humans. To date, the most widespread use of OHP has been in cancer radiotherapy where it is used to increase extracellular pO_2, thus reversing the radioresistance attributable to tumor hypoxia. In the techniques employed, patients have been exposed to oxygen at 3–4 ATA for relatively

brief periods (30–60 min). The incidence of convulsions in such patients has been very low and any patient developing convulsion is rapidly decompressed.

In the largest series of patients thus far reported in the literature,[16] 614 anesthetized patients received a total of 2569 exposures to hyperbaric oxygen at 4 ATA (2–12 exposures per patient). The average duration of each exposure was 40 minutes at pressure. Only 11 convulsions occurred, and there was no evidence of subsequent clinical or radiological pulmonary damage in any patient, convulsed or not. Further studies disclosed no alteration in the vital capacity of 68 patients who had received 6 consecutive hyperbaric exposures, even as late as 3 months post-treatment.

In other studies, in which unanesthetized patients were exposed to 3 ATA oxygen for similar times, no pulmonary damage was observed; 4 convulsions occurred in 850 exposures.[17] More recently, this same center has recorded no pulmonary damage following 1559 treatments accompanied by 5 convulsions (I. Churchill-Davidson, personal communication).

These results, taken together with the data in Table 1, suggest that pulmonary damage is not a major problem in exposure of humans to OHP. Moreover, human seizures produced by other treatments, e.g. electro-convulsive shock therapy, do not cause pulmonary lesions comparable to those in rats and mice convulsed by OHP or PTZ. The reservation must be made, however, that the clinical situation in man is hardly comparable to the laboratory situation in experimental animals, the convulsions observed in the former case being of limited duration and intensity compared to the latter.

DISCUSSION

Despite the temporal coincidence of seizure and lung damage in OHP- or PTZ-convulsed animals, it is clear that the former is not the immediate cause of the latter. Since lung damage and convulsive activity are entirely separable,[7,18,19] there is no logical alternative but to consider both of these phenomena as caused by some central mechanism.

The present experimental results suggest that lung damage in acute hyperbaric oxygen poisoning results from some mechanism other than a direct oxidative attack on pulmonary structures. This conclusion is supported by recent evidence that arterial pO_2, rather than oxidation of pulmonary structures, is of primary significance in production of lung damage.[20] Although an unequivocal correlation between CNS activity (as measured by EEG signals) and lung damage has not yet been demonstrated,[19] there is ample evidence that CNS changes do occur shortly after pressurization of rats in oxygen,[19,21] and, at least under certain conditions, well in advance of pulmonary consolidative changes.[21] Moreover, the demonstration that both CNS-reactive compounds and hyperbaric oxygen provoke similar lung damage in rodents and that the effects of both are moderated or eliminated by identical doses of the same "protective" agents (Table 1) strongly suggests that CNS damage, while at least partly independent of lung damage, nevertheless plays an important role in the development of the classical symptoms of hyperbaric oxygen poisoning. Unfortunately, the precise nature of the nexus between CNS and pulmonary effects remains obscure.

The conclusion that CNS-damage in acute hyperbaric oxygen-exposures is in some way linked to production of both pulmonary consolidative changes and convulsive activity does not deny that oxygen may produce other, non-CNS-mediated,

effects of considerable importance. Oxygen, especially in chronic exposures, has a well-documented effect on pulmonary morphology in the absence of convulsions.[22] Further, the lung dehydrogenase activity of OHP-treated rats was affected by exposures too short to induce either seizure or obvious pulmonary consolidative changes.[4] At the present time, however, the possibility that even this latter effect may be linked to CNS malfunction cannot be eliminated.

The data presented in Table 1 indicate that considerable species variability exists with respect to lung damage by convulsive OHP or PTZ treatment. Similar tolerance of guinea pigs and rabbits to OHP-induced paralytic sequelae was reported previously.[18] While it is not possible to relate these findings to specific pharmacological or physiological mechanisms (e.g. differences in threshold for lung damage), the existence of such variability must be borne in mind when attempting to extrapolate from small-animal experimental data to clinical situations involving humans.

Although oxidation of SH groups and inactivation of SH-dependent enzymes are widely recognised as significant events in oxygen poisoning,[6,11] such losses need not represent a direct effect of oxygen *in vivo*. In the present experiments, for example, the minimal loss of NPSH in non-convulsed animals suggests that direct oxidation by inhaled oxygen is an inadequate explanation (Table 2). The data of Jamieson, Ladner and van den Brenk[6] may be interpreted in a similar vein, since total lung sulfhydryl was relatively unaffected in rats exposed to oxygen at 5 ATA for 25–30 min and decompressed before convulsive activity would have ensued, but markedly decreased in animals exposed for an additional 10–15 min and probably convulsed. Wolman observed no oxidation of sulfhydryls in brains from rats pressurized to 5 ATA oxygen for as long as 80 min.[23]

Liver NPSH is readily depressed by stress or starvation[24,25] (Table 2), the former effect being eliminated by ganglionic blocking agents.[24] Such a mechanism may have contributed to the decrease in lung NPSH in convulsed rats. Since NPSH compounds protect SH-dependent enzymes against inactivation by oxygen both *in vivo*[4] and *in vitro*,[11] the relatively greater depression of NPSH in lungs of oxygen-convulsed rats (Table 2) may offer a partial explanation for the observation that dehydrogenase activity is markedly reduced in lung and unaffected in liver of rats exposed to OHP.[4]

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