In Vitro, effect of Hyperbaric Oxygen treatment on contractile and relaxant responses of the rat trachea.

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Introduction

Prolonged exposure of mammals to high concentrations of oxygen causes lung damage characterised by gross alveolar oedema and haemorrhage, and, if the partial pressure of oxygen exceeds of about 300 KPa, convultions precede the obvious signs of pulmonary pathology. The aetiology of hyperoxic toxicity is not yet elucitated, although excers production of reactive oxygen species appears to be the underlying event. Recently , Lin and Jamieson demonstrated that in vivo leukotriene B4 (LTB4) could contribute to pulmonary pathology caused by hyperbaric oxygen therapy on bronchomotore tone; leukotriene D_4 (LTD4) and C 4(LTC4) play little if any part in oxygen toxicity.

The aim of present study was to evaluate the effects of hyperbaric oxygen (HBO) exposure on the responses in vitro to contractile and relaxant responses of tracheal preparation of rats exposed for 20 days to 2 and 3 absolute atmospheres of oxygen.

Materials and methods

Male Winstar rats weighing 150-200 g. were used in these experiments. 10 animals were treated for 20 days with hyperbaric oxygen therapy at 2 (5 rats) and 3 (5 rats) atmospheres. 10 rats were considered control group. Hyperbaric oxygen exposures were carried out in a cylindrical steel chamber (40 cm diameter x 65 cm length Galeazzi model-Italy) with thick glass windows allowing for direct observation of animals during the exposure.

Before pressurisation, 100% medical oxygen gas was flushed through the chamber for 5 min to displace the ambient air. Oxygen pressure was then increased at a costant rate to reach the pressure of 2 and 3 atmospheres in 4 min. Sixty minutes was the maximal period that animals spent under compression.

No convulsive responses like quick clonic convulsions with loss of the righting reflex were noted in both groups, during the HBO exposure.

The chamber was constantly ventilated at a rate of 4 l/min to avoid carbon dioxide accumulation during pressurisation. Gases were analysed and 0_2 concentration was >99% (Taylor servomex OA272 oxygen analyser) and carbon dioxide below 0,2% (Med. Gas Analyser LB-2, Model 240 M, Beckman). The rats spent 60 min at 2 and 3 atmospheres of O_2 everyday for 20 days; then were decompressed over a period of 4 min with a steady exhaust flow rate. After the last treatment (20 th day) the rats were killed by cervical fracture and the trachea were rapidly removed. Ring preparations, derived from rat trachea, were set up in a 10 ml organ bath under initial load of 3.5 g. The tissues were equilibrate for 90 min in Tyrode's.

solution at 37° C with 5% CO_2 in O_2. The Tryrode's solution contained (mM): NaCl 139.2, KCI 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, NaHCO 311.9, NaH_2 PO_4 O.4 and glucose 5.5; pH 7.4. Isometric force trasducers (Narco F-60) and Linseis physiographs (MKIV) were used to record the changes in force. Muscle preparations were challenged with ACh (100 μM). The tissues were then washed and when the basal tone had been re-estabilished cumulative concentration-effect curves to Acetylcoline (Ach) were performed or tissues were contracted and a relaxation curve wase produced using Isoprenaline (ISO).

All data were shown as % of the maximal contractile response induced by the contractile agonist. The EC_{50} values were interpolated from each curve and transformed into logharithm (pD_2 values) and the Emax was recorded for the concentration wich induced a maximal effect.

All values are presented as mean ± S.E.M.; statistical analysis was performed using the ANOVA's test for paired values.

	Ach		ISO	
	Emax (g)	pD_2	Emax (% vs Ach)	pD_2
Controls	3.4± 2.2	5.3± 0.2	99± 5.0	5.3± 0.4
2 ATM	2.9± 0.1	5.0± 0.5	98± 4.2	5.5± 0.6
3 ATM	3.16± 0.50	5.2± 0.3	100± 7.2	5.3± 0.6

Table 1 - Emax and pD_2 of Ach and ISO obtained from rat trachea treated with hyperbaric oxygen at 2 and 3 ATM. Each value represents the mean (± SEM) of 4 experiments. Ach = acetylcholine; ISO = isoprenaline.

Results

The treatment with hyperbaric oxygen (2 or 3 atmospheres) for 20 days did not change the contractile and relaxant responses of trachea rings of rats (Tab. 1 and Fig. 1). In fact, our results showed that hyperbaric oxygen therapy, at the same clinical doses (2 and 3 atm), did not modify the responsiveness (Emax) and sensitivity (pD_2) of rat trachea to contractile and relaxant agonists (Tab. 1 and Fig. 1).

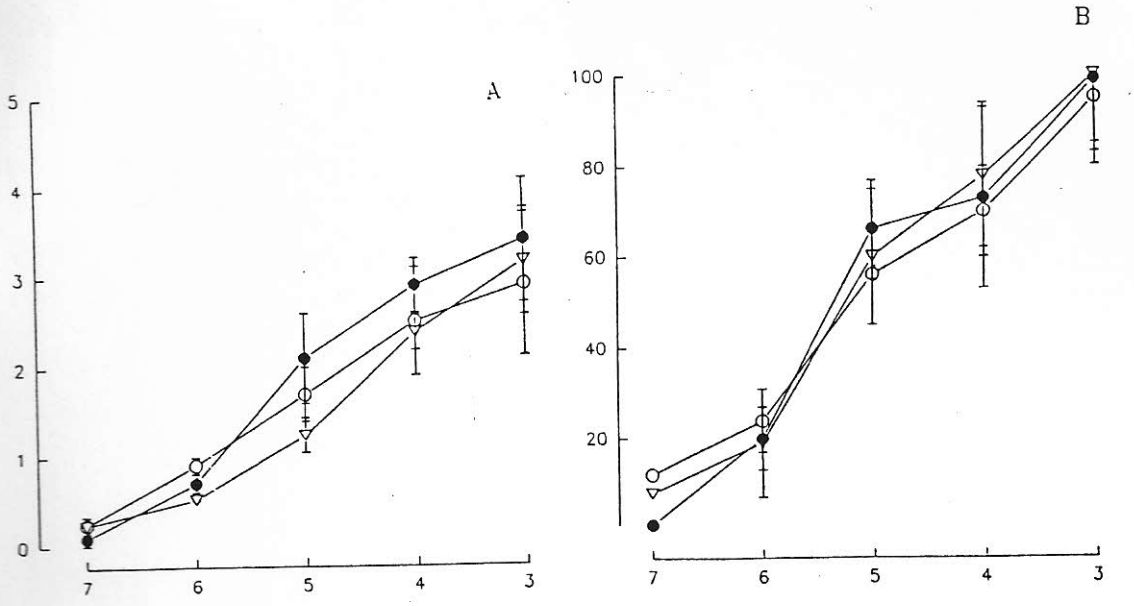

Figure 1
Panel A: effects of hyperbaric oxygen treatment (o 2ATM; Δ 3ATM) on cumulative concentration-effect curve of Ach (•). In abscissa coordinate, molar concetrations of Ach. In ordinate coordinate, g of contraction. Each value in figure represents the mean (± SEM) obtained from 4 experiments. For details see the test.
Panel B: effect of hyperbaric oxygen treatment (o 2ATM; Δ 3ATM) on cumulative concentration -effect curve of ISO (•). In abscissa coordinate, molar concentrations of ISO. In ordinate coordinate, % of relaxation induced by ISO. Each value in figure represents the mean (± SEM) obtained from 4 experiments. For details see the test.

Discussion

Several investigators have suggested that the production of free radicals, by products of metabolism of the endothelial and epithelial cells lining the alveoli, and/or from radicals generated by inflammatory cells, may be responsable of respiratory system damage caused by hyperbaric oxygen. Large increases in the numbers of inflammatory cells, particulary neutrophils, have been reported in hyperoxic damaged lung but whether they contribute to the pathology, or are merely a conseguence of cell damage, is still disputed. Inflammatory mediators such as leukotrienes, which may be generated by neutrophils or macrophages or by endothelial cells in the lungs, could also contribute to pulmonary pathology.
On the contrary, the prolonged exposure to hyperbaric oxygen does not seems to cause a damage charged to responsiveness of rat trachea. In fact, our results demonstrated that tracheal tissues did not exhibit a different response to relaxant and contractile agonists in presence or in absence of hyperbaric oxygen treatment. We purpose to carry on our studies of trachea considering the HBO effects in confront with mediators as leukotrienes, platelet-activating factor (PAF) and endotheline.

References

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