

SHORT COMMUNICATION

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Expiration of ethane in rats under variously elevated inspiratory O₂-concentrations

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Abstract Expired ethane is regarded as a noninvasive indicator of lipid peroxidation. As a model of oxidative stress we have investigated in male Wistar rats (body wt. 309 ± 15 g) the effects of various levels of elevated inspiratory oxygen concentrations on the expiration rate of ethane. After 4 days under 21 vol% O₂ (basic condition) the rats were exposed for 6 or 5 days to 40, 60 or 80 vol% O₂ over 8 or 23 h/day. The variously O₂-enriched air was conducted through the cages and expired ethane adsorbed onto charcoal was thermo-desorbed and measured by gas chromatography. Basic ethane expiration was 3.1 ± 0.8 pmol/100 g body wt. per min. At 40 vol% O₂ over 8 or 23 h/day no increase or a maximum average 47% increase ($P < 0.01$) in ethane expiration occurred on day 4; 60 vol% O₂ over 8 or 23 h/day led to a corresponding increase of 56 or 87% ($P < 0.05$ or $P < 0.01$) on day 3; 80 vol% O₂ over 8 or 23 h/day led to a corresponding increase of 81 or 66% ($P < 0.01$) on days 3 or 2. Our results indicate that with up to 60 vol% O₂ a temporary increase in lipid peroxidation occurs in a dose dependent manner. However, at 80 vol% O₂ no further increase in the maximum ethane expiration occurred. The latter finding and the finding of only transient increase in ethane expiration in probably due to antioxidative counteraction.

Key words Ethane expiration · Lipid peroxidation · Noninvasive methods · Rats · Reactive oxygen species

Introduction

Noninvasive methods for assessing the loading of reactive oxygen species (ROS) in an organism are important because ROS can damage cellular components, such as DNA, RNA, proteins and lipids. Ethane is an end

product of ROS-induced peroxidation of n-3 polyunsaturated fatty acids. Expired ethane is regarded as an indicator of lipid peroxidation (Frank et al. 1980; Filser et al. 1983; Remmer et al. 1989; Kneepkens et al. 1994).

The expiration of ethane has been determined under various types of potential oxidative stress, e.g. in pre-term infants (Varsila et al. 1994), in smokers (Allard et al. 1994), in an irradiated patient (Arterbery et al. 1994), in normal and fasted rats under elevated O₂-concentrations (Habib et al. 1990) and in diabetic rats (Habib et al. 1994). We have found that in variously sized rats under normal conditions (21 vol% O₂) there is a positive correlation between the expiration rates of ethane and the metabolic rates (Topp et al. 1995). In previous studies looking at the effects of elevated normobaric inspiratory oxygen on the expiration of ethane in rats, very high oxygen concentrations (>95 vol% O₂) were used (Roberts et al. 1983; Habib et al. 1990). In the present study we have investigated the effects of various levels of elevated oxygen on the expiration rates of ethane in rats. For this purpose, 40, 60 or 80 vol% O₂ were given normobarically over 8 or 23 h/day for a total period of 5–6 days and the expiration of ethane was determined daily.

Materials and methods

The investigations were carried out in accordance with the German Law on the Protection of Animals. Male Wistar rats (HsdCpb:WU, Harlan Winkelmann, Germany) of mean body wt. 309 ± 15 g, were housed individually in glass metabolic cages (Metabowl Mark III; Jencons, UK; Fig. 1) at 20 °C with 12:12 h light/dark cycles. Drinking water and standard diet (C 1000; Altromin, Germany) were supplied ad libitum. Synthetic air (21 vol% O₂ in N₂; no. 0131, Messer Griesheim, Germany) or various mixtures of synthetic air and O₂ (no. 1404) were delivered at 150 ml/min through the cages (Fig. 1). The gas mixtures were produced using two flowmeters (DK 800 N/REN; Krohne, Germany) per cage. For adaptation the animals were housed in open cages with room air over 4 days. During the following 4 days all animals were housed in open cages, but for 8 h/day synthetic air was delivered through closed cages to determine the basic expiration rates of ethane. The rats were then exposed to 40, 60 or 80 vol% O₂ over 8 or 23 h/day;

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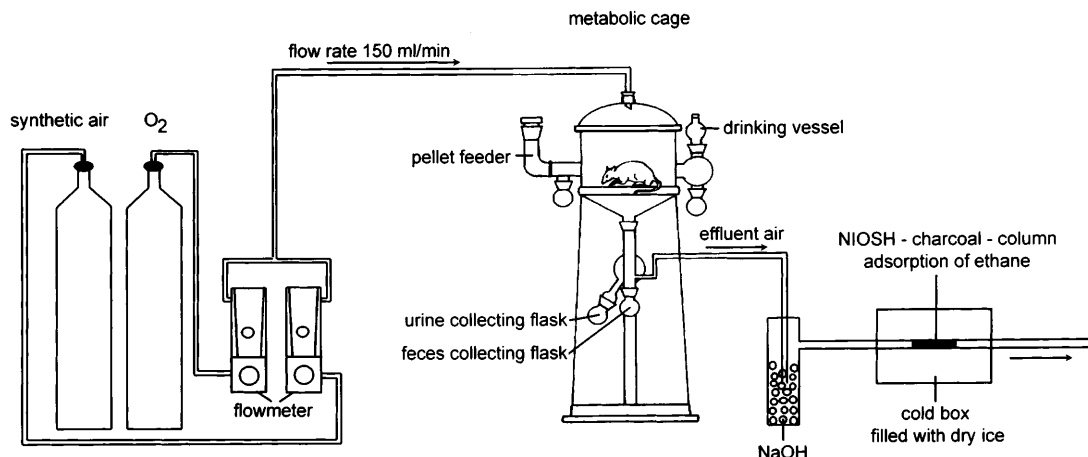


Fig. 1 Experimental set-up for exposure of rats to different oxygen concentrations and for accumulation of expired ethane

for the rest of the time the animals were housed in open cages with room air. These conditions were maintained for 6 days, except 80 vol% O₂ over 23 h/day which was maintained only for 5 days because on days 4 and 5 the animals became lethargic. Generally eight rats were held under each condition except for 80 vol% O₂ over 23 h/day where only four were used (because of the above mentioned indisposition).

For each rat in the various exposure groups a pair-fed control rat was allocated. At the times during which the oxygen enriched air was delivered through the cages of the exposed animals only synthetic air (21 vol% O₂) was conducted through the cages of the pair-fed animals to determine ethane expiration rates. At 21, 40, 60 and 80 vol% O₂ the ethane expiration of each rat was measured every day 120 min after starting the respective condition. In one rat from each of the various elevated O₂-concentrations a second measurement of ethane was carried out immediately after the first one. For the measurements effluent air from the cages was first dried on NaOH pellets (Fig. 1). Expired ethane was adsorbed for 90 min on activated charcoal columns (from coconut; no. 226-01, SKC; for air sampling methods of the National Institute of Occupational Safety and Health, NIOSH, USA) which were cooled (−90 °C) with dry ice. The charcoal was transferred into a 12-ml headspace vial and ethane was desorbed at 280 °C for 10 min. A 100 µl aliquot was injected into the gas chromatograph (HRGC 5160; Fisons, USA) equipped with a flame ionization detector. Ethane was separated on a GS-porapak-Q capillary column (30 m × 0.53 mm; J & W Scientific, USA); chromatographic conditions were injector 130 °C, carrier N₂ 8 ml/min, oven 50 °C for 2 min, then 50 to 230 °C (49 °C/min); detector 190 °C, H₂ 19 ml/min, synthetic air 190 ml/min. A 10 ppm ethane standard (Messer Griesheim, Germany) was used as a reference. Differences between the expiration rates of ethane during the preceding basic conditions at 21 vol% O₂ and during the exposures to the different elevated O₂-concentrations were statistically analysed by a two-factor hierarchical analysis of variance using Statistical Analysis System for Windows 6.11.

Results and discussion

No weight loss of the animals was seen under any of the exposure conditions (results not shown). Recovery of the ethane standard (4100 pmol) after injection into a cage and treated identically to the samples was $80 \pm 2\%$ ($n = 10$). Basic ethane expiration of all animals during the 4 days under 21 vol% O₂ was 3.1 ± 0.8 pmol/100 g body wt. per min. When the rats were exposed over 6 days to 40 vol% O₂ for 8 h/day no increase in ethane expiration occurred, indicating that this loading of O₂ can be tolerated (Fig. 2A). The slight decrease (−25%) in ethane ex-

piration under these conditions onwards from the next to the last day could be the result of an antioxidative counteraction. 40 vol% O₂ over 23 h/day and 60 vol% O₂ over 8 and 23 h/day, led to a dose dependent but only transient increase in ethane expiration with maxima (47, 56 and 87%) on days 4 or 3, respectively. This indicates only a temporary elevation of lipid peroxidation despite ongoing exposure to the elevated oxygen concentrations (Fig. 2-A, B). At 80 vol% O₂ given over 8 or 23 h/day the maxima of ethane expiration of 81 or 66% occurred on days 3 or 2 (Fig. 2C). The results shown in Fig. 2 were always confirmed by the second measurements carried out immediately after the first in one rat from each group (see the Materials and methods). The pair-fed rats showed no changes in the expiration rates of ethane.

Our findings indicate that under variously elevated oxygen concentrations (40, 60 and 80 vol% O₂) the rats can largely compensate for increased levels of ROS up to days 4, 3 and 2, dependent on the oxygen load via antioxidatively acting components such as superoxide dismutases (SOD), glutathione peroxidases (GPx), catalase (CAT), reduced glutathione (GSH), α -tocopherol, β -carotene, ascorbate and ubiquinol. Furthermore, an increase in antioxidatively acting components could be the reason for the only temporary increase in lipid peroxidation despite ongoing elevated O₂-concentrations as well as the fact that no further increase in ethane expiration occurred at 80 vol% O₂. It has been described that 85 vol% O₂ given over 3 days to adult rats led to a significant increase in activity of GPx in the whole lung (Keeney et al. 1992). In another study after 3 days with 80 vol% O₂, significant increases in the lung in the activities of GPx and CAT were recorded (Coursin et al. 1987). Ascorbic acid and ubiquinol can scavenge ROS directly or reduce the α -tocopheroxyl radical to the lipid antioxidant α -tocopherol (Chow 1991; Niki 1991; Ernster and Dallner 1995). Possibly, in rats an elevated ROS loading and increased lipid peroxidation is compensated for by increased synthesis and/or regeneration of ascorbic acid and ubiquinol.

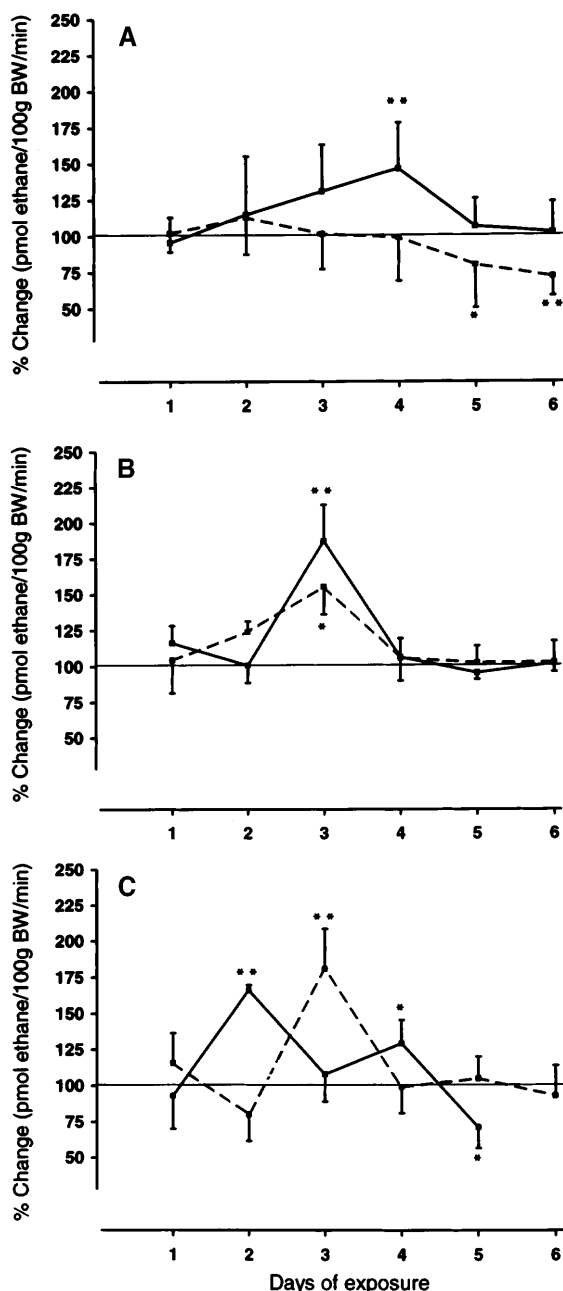


Fig. 2 Expiration rates of ethane in rats exposed to **A** 40 vol% O₂, **B** 60 vol% O₂ and **C** 80 vol% O₂ given over 8 h/day (dashed lines) or 23 h/day (solid lines). The values of each rat were normalized to the corresponding mean value of the preceding basic period (21 vol% O₂ over 4 days) which was set to 100%. Significantly different from the basic period: **P* < 0.05, ***P* < 0.01

We conclude that different degrees of oxidative stress in rats can be temporarily provoked by variously elevated O₂-levels in inspiratory air. Our experimental setup (Fig. 1) allows the simultaneous quantitative collection of urine as a prerequisite for determining further noninvasive indicators of oxidative stress in urine, e.g. thymine glycol and thymidine glycol or 8-oxo-7,8-dihydro-2'-deoxyguanosine stemming from oxidative DNA damage (Adelman et al. 1988; Park et al. 1992). Fur-

thermore, the effects of administered antioxidants and prooxidants on the different noninvasive indicators of oxidative damage could be investigated.

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