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Antioxidants prevent depression of the acute hypoxic ventilatory response by subanaesthetic halothane in men

Luc J. Teppema, Diederik Nieuwenhuijs, Elise Sarton, Raymonda Romberg, Cees N. Olievier, Denham S. Ward * and Albert Dahan

*Departments of Physiology and Anesthesiology, Leiden University Medical Centre, Leiden, The Netherlands and * Department of Anesthesiology, University of Rochester, NY, USA*

We studied the effect of the antioxidants (AOX) ascorbic acid (2 g, I.V.) and α-tocopherol (200 mg, P.O.) on the depressant effect of subanaesthetic doses of halothane (0.11 % end-tidal concentration) on the acute isocapnic hypoxic ventilatory response (AHR), i.e. the ventilatory response upon inhalation of a hypoxic gas mixture for 3 min (leading to a haemoglobin saturation of 82 ± 1.8 %) in healthy male volunteers. In the first set of protocols, two groups of eight subjects each underwent a control hypoxic study, a halothane hypoxic study and finally a halothane hypoxic study after pretreatment with AOX (study 1) or placebo (study 2). Halothane reduced the AHR by more than 50 %, from 0.79 ± 0.31 to 0.36 ± 0.14 $\text{l min}^{-1}\ \%^{-1}$ in study 1 and from 0.79 ± 0.40 to 0.36 ± 0.19 $\text{l min}^{-1}\ \%^{-1}$ in study 2, $P < 0.01$ for both. Pretreatment with AOX prevented this depressant effect of halothane in the subjects of study 1 (AHR returning to 0.77 ± 0.32 $\text{l min}^{-1}\ \%^{-1}$, n.s. from control), whereas placebo (study 2) had no effect (AHR remaining depressed at 0.36 ± 0.27 $\text{l min}^{-1}\ \%^{-1}$, $P < 0.01$ from control). In a second set of protocols, two separate groups of eight subjects each underwent a control hypoxic study, a sham halothane hypoxic study and finally a sham halothane hypoxic study after pretreatment with AOX (study 3) or placebo (study 4). In studies 3 and 4, sham halothane did not modify the control hypoxic response, nor did AOX (study 3) or placebo (study 4). The 95 % confidence intervals for the ratio of hypoxic sensitivities, (AOX + halothane) : halothane in study 1 and (AOX – sham halothane) : sham halothane in study 3, were [1.7, 2.6] and [1.0, 1.2], respectively. Because the antioxidants prevented the reduction of the acute hypoxic response by halothane, we suggest that this depressant effect may be caused by reactive species produced by a reductive metabolism of halothane during hypoxia or that a change in redox state of carotid body cells by the antioxidants prevented or changed the binding of halothane to its effect site. Our findings may also suggest that reactive species have an inhibiting effect on the acute hypoxic ventilatory response.

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Corresponding author L. Teppema: Department of Physiology, Leiden University Medical Centre, PO Box 9604, 2300 RC Leiden, The Netherlands. Email: l.j.s.m.teppema@lumc.nl

A major defence of the mammalian body to acute hypoxia is a rapid increase in pulmonary ventilation called the acute hypoxic response (AHR). This vital chemoreflex is primarily mediated by the carotid bodies located at the bifurcations of the common carotid arteries (Gonzalez *et al.* 1994). During the past decade, considerable progress has been made in unravelling the cascade of events within carotid body type I cells upon exposure to a hypoxic environment, although there are still many areas of controversy (Gonzalez *et al.* 1994; Lopez-Barneo *et al.* 2001). The general picture emerging from most studies is that low oxygen decreases the open probability of potassium channels, which causes membrane depolarization and influx of Ca^{2+} ions. In several species, various types of potassium channels are described that may serve as an oxygen-sensing element to initiate the transduction cascade in hypoxia, for example, K_v channels in rabbits (Perez-Garcia & Lopez-Lopez, 2000; Perez-Garcia *et al.* 2000) and maxi-K and TASK channels in rats (Buckler *et al.* 2000; Riesco-Fagundo *et al.* 2001). Although potassium channels possess redox sensitivity and are sensitive to changes in the concentration of reactive oxygen species (ROS), it is unclear by which mechanism low oxygen is able to decrease the conductance of these channels (Kourie, 1998; Lopez-Barneo *et al.* 1999; Kobertz *et al.* 2000).

Volatile anaesthetics, e.g. halothane, can open potassium channels in various cell types, such as TASK channels in rat carotid body (Patel *et al.* 1999; Buckler *et al.* 2000; Sirois *et al.* 2000; Patel & Honore, 2001*a*,*b*). At the same time, volatile anaesthetics, particularly halothane, are known to depress the acute hypoxic response, an effect that may be mediated through a preferential and potent action on the carotid bodies (Knill & Clement, 1984; Dahan *et al.* 1994).

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It is unknown if the opening of potassium channels by halothane might involve the intracellular concentration of ROS or changes in the cell redox state. However, during hypoxia, halothane undergoes a reductive metabolism in the liver by which radical species are produced and lipid peroxidation is initiated; this reductive metabolism of halothane is thought to be responsible for its mild hepatotoxic effect (de Groot & Noll, 1983; de Groot & Sies, 1989; Spracklin & Kharasch, 1998; Kharasch *et al.* 2000). In guinea-pig liver, peroxidation of lipids following halothane administration can be inhibited by antioxidant treatment with vitamin E (Sato *et al.* 1992).

The above findings on the sensitivity of potassium channels to ROS, the ability of halothane to produce radical species and to open potassium channels and, finally, the role of potassium channels in the hypoxic response, all raise the question of whether halothane may reduce the hypoxic response by producing ROS and/or by influencing the redox state of the carotid body. The aim of the present study in humans, therefore, was to examine the influence of the potent antioxidants α-tocopherol and ascorbic acid on the acute hypoxic ventilatory response with and without halothane.

METHODS

Subjects and apparatus

Thirty-two healthy, non-smoking, male subjects (age 20–35 years) were recruited after protocol approval by the Leiden University Medical Centre Committee on Medical Ethics. All experiments conformed to the declaration of Helsinki. None of the volunteers was taking any medication or ever had surgery under general anaesthesia. All subjects performed a series of test experiments to familiarise themselves with the apparatus and experimental procedures. The subjects were instructed not to eat or drink for at least 8 h before the study. They were not instructed about respiratory physiology, anaesthesia or the intentions of the study. All gave oral and written informed consent before their participation.

After arrival at the laboratory, an intravenous catheter was inserted in the left or right antecubital vein for drug infusion. Subsequently, electrodes for EEG monitoring (BisSensor, Aspect Medical Systems, Newton, MA, USA) were placed on the head at AT_1–FP_1 as specified by the manufacturer, and the subjects rested for 20–30 min before the antioxidant cocktail or placebo was administered. Next a facemask was applied over the mouth and nose. Gas flow was measured with a pneumotachograph connected to a pressure transducer and electronically integrated to yield a volume signal. This signal was calibrated with a motor-driven piston pump (stroke volume 1 l at a frequency of 20 strokes min^{-1}). Corrections were made for the changes in gas viscosity due to changes in oxygen concentration of the inhaled gas mixtures. The pneumotachograph was connected to a T-piece. One arm of the T-piece received a gas mixture (with a flow of 50 l min^{-1}) from a gas-mixing system consisting of three mass-flow controllers (Bronkhorst High-Tec, Veenendaal, The Netherlands). A personal computer provided control signals to the mass-flow controllers so that the composition of the inspired gas mixtures could be adjusted to force end-tidal oxygen concentration (P_{ET,O_2}) to follow a specified pattern in time while the end-tidal carbon dioxide concentration (P_{ET,CO_2}) was kept constant. Part of the nitrogen (5 l min^{-1}) passed through a halothane vaporizer (Dräger 19.2, Lubeck, Germany). During the initial part of the study (control experiments), the vaporizer was kept in the 'off' position. Dräger Nederland BV calibrated the vaporizer before its use in this study.

The oxygen and carbon dioxide concentrations of inspired and expired gases were measured with a gas monitor (Multicap, Datex-Engstrom, Helsinki, Finland) by paramagnetic and infrared analysis. The gas monitor was calibrated with gas mixtures of known concentration delivered by a gas-mixing pump (Wösthoff, Bochum, Germany). The halothane concentration was measured at the mouth with a gas monitor (Multicap Ultima, Datex-Engstrom). This gas monitor was calibrated with a gas mixture of known concentration (Quick Cal, Datex-Engstrom). The arterial saturation of haemoglobin with O_2 obtained via a finger probe (S_{a,O_2}) was measured by pulse oximetry (Satlite Plus, Datex-Engstrom).

The EEG was recorded using an Aspect A-2000 EEG monitor (Aspect Medical Systems, Newton, MA, USA).

Study design

In the first set of studies, designed to test the effect of antioxidant pretreatment on the depression by halothane of the AHR, two separate groups of eight subjects underwent a control hypoxic study, followed by a halothane hypoxic study and finally by a halothane hypoxic study after pretreatment with a cocktail of antioxidants (study 1) or placebo (study 2). In a second set of studies, which was designed to investigate the effect of antioxidant pretreatment on the hypoxic ventilatory response in the absence of halothane, two separate groups of eight subjects underwent a control hypoxic study, followed by a sham halothane hypoxic study and then a sham halothane study after pretreatment with a cocktail of antioxidants (study 3) or placebo (study 4). While the design of the halothane administration was randomized and blinded to the subjects only, both subjects and researchers were blinded to the pretreatment with antioxidants or placebo.

After each hypoxic study blood was drawn from the capillary bed of a hyperaemic finger for the determination of blood acidity (Astrup equilibration technique, Radiometer, Copenhagen, Denmark).

Hypoxia. Hypoxia was induced with a 'dynamic end-tidal forcing' system (Dahan *et al.* 1995, 1996); steps from normoxia (P_{ET,O_2} 15 kPa) into hypoxia (P_{ET,O_2} 6.2 kPa obtained within 4–6 breaths) were applied. Since peak hypoxic responses occur within 3 min (Dahan *et al.* 1995), hypoxia was maintained for 3 min, after which hyperoxia was introduced for 5 min (fractional inspired O_2, F_{I,O_2} > 0.5). The P_{ET,CO_2} was maintained just above individual resting values.

Halothane. During the appropriate studies, the subjects inhaled halothane (Fluothane, Zeneca Ltd, Macclesfield, UK). The flow through the vaporizer was 5 l min^{-1}. This was added to a fresh gas flow of 45 l min^{-1}. Both flows were very precise and generated by mass-flow controllers (Bronkhorst High-Tec). The setting of the vaporizer was 1.1–1.5 % (measured at the outflow tract). After mixing, the measurements were repeated (now 0.1–0.15 %). This procedure ensured that the concentrations employed and measured were in the range as stated in Table 1. The subjects inhaled a halothane concentration that maintained the end-expiratory concentration at 0.11 % for 10 min before the hypoxic study started, resulting in a minimum alveolar concentration

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Table1. Influence of antioxidant and placebo pretreatment on halothane and sham halothane-induced depression of the ventilatory response to hypoxia

	Baseline ventilation ($l\,min^{-1}$)	End-expiratory CO_2 (mmHg)	pH	Halothane (vol. %)	Hypoxic ventilatory response ($l\,min^{-1}\,\%^{-1}$)	Hypoxic ventilatory response (% of control)
Study 1						
Control	12.1 ± 0.5	6.1 ± 0.4	7.41 ± 0.02	0	0.79 ± 0.31	100
Halothane	12.5 ± 3.3	6.1 ± 0.3	7.41 ± 0.02	0.11	0.36 ± 0.14 *	46 ± 11 *
AOX + halothane	14.0 ± 2.1	6.2 ± 0.2	7.42 ± 0.02	0.11	0.77 ± 0.32	96 ± 20
Study 2						
Control	12.5 ± 1.6	6.0 ± 0.2	7.40 ± 0.02	0	0.79 ± 0.40	100
Halothane	12.7 ± 3.2	6.1 ± 0.2	7.40 ± 0.03	0.11	0.36 ± 0.19 *	47 ± 14 *
Placebo + halothane	13.7 ± 4.1	6.0 ± 0.2	7.41 ± 0.02	0.12	0.36 ± 0.27 *†	40 ± 15 *†
Study 3						
Control	13.9 ± 1.9	5.8 ± 0.3	7.43 ± 0.03	0	0.89 ± 0.42	100
Sham halothane	14.5 ± 3.6	5.9 ± 0.2	7.44 ± 0.02	0	0.90 ± 0.44	102 ± 14
AOX + sham halothane	14.5 ± 2.8	5.8 ± 0.3	7.43 ± 0.03	0	1.00 ± 0.54	116 ± 22
Study 4						
Control	14.6 ± 3.3	5.9 ± 0.4	7.42 ± 0.03	0	0.83 ± 0.42	100
Sham halothane	16.9 ± 3.8	5.9 ± 0.4	7.42 ± 0.02	0	0.88 ± 0.45	104 ± 15
Placebo + sham halothane	16.1 ± 2.3	5.9 ± 0.4	7.41 ± 0.02	0	0.89 ± 0.45	110 ± 10

Values are means ± S.D. * $P < 0.01$ *vs.* control of identical study (one-way ANOVA); † $P < 0.01$ *vs.* AOX + halothane of study 1 (Student's paired *t* test). AOX, antioxidant cocktail.

(MAC) equivalent of 0.13 (assuming an age-adjusted MAC of 0.84 % halothane in our young subjects; Gregory *et al.* 1969). Note that because of the short (10 min) exposure time to this end-tidal level of halothane, the brain concentration will be less than 0.11 %, minimizing the occurrence of significant central effects (i.e. within the central nervous system) of halothane. The subjects were under the impression that halothane was given during the sham halothane studies by manipulating an empty vaporizer.

Antioxidant cocktail (AOX). The antioxidant cocktail consisted of 200 mg of oral α-tocopherol (Organon, Oss, The Netherlands) given 1 h before the start of the appropriate hypoxic study, ingested with a cup of yoghurt, and two 1 g intravenous doses of ascorbic acid (Ascorbinezuur CF, 5 ml, Centrafarm, The Netherlands) given 10 and 4 min before the appropriate hypoxic study. Placebos consisted of cellulose tablets and 0.9 % NaCl manufactured by the local pharmacy. The oral placebo was also ingested with yoghurt.

Data and statistical analysis

Analysis was performed on a blinded data set. The breath-to-breath data of the last 10 breaths of normoxia and the last 10 breaths of hypoxia were averaged. Since the relationship between ventilation and arterial oxygen saturation is found to be linear (Dahan *et al.* 1996), we calculated the difference between the mean inspiratory ventilation (V_I) and the S_{a,O_2} data points and expressed the hypoxic ventilatory response or sensitivity as follows (Dahan *et al.* 1996):

$$\frac{V_I\,(\text{hypoxia}) - V_I\,(\text{normoxia})}{S_{a,O_2}\,(\text{normoxia}) - S_{a,O_2}\,(\text{hypoxia})}(l\ min^{-1}\ \%^{-1}).$$

The statistical analysis was performed using SPSS v10.0 for Windows. To detect the significance of differences among the three treatment groups of each study, a two-way analysis of variance was performed. *Post hoc* analysis was by least significant differences and Bonferroni correction. To assess the effect of antioxidant *vs.* placebo pretreatment, Student's paired *t* tests were performed on the appropriate treatment levels of studies 1 and 2 and studies 3 and 4. Values reported are means ± S.D. *P* values less than 0.05 were considered significant.

RESULTS

All subjects completed the protocols without side effects. During all studies, P_{ET,CO_2} values were kept constant at 0.1–0.2 kPa above individual resting values, with no differences between baseline (prehypoxia) and hypoxic P_{ET,CO_2} values and pH. In all hypoxic studies, S_{a,O_2} values were 82 ± 2 %.

The values of baseline ventilatory parameters and the control ventilatory responses to hypoxia (Table 1) are in agreement with earlier observations (Dahan *et al.* 1994, 1996). We observed no effect from low-dose halothane on baseline ventilation. Similarly, antioxidant and placebo pretreatment had no significant effect on baseline parameters (Table 1). Halothane (0.11 % end-expiratory concentration) decreased the ventilatory response to hypoxia by more than 50 %. As shown in Fig. 1, this effect was completely prevented by pretreatment with the antioxidant cocktail (study 1) but not by placebo pretreatment (study 2). Sham halothane did not affect any of the ventilatory baseline and hypoxic parameters, nor did antioxidant (study 3) or placebo (study 4) pretreatment (Table 1 and Fig. 2). The 95 % confidence intervals of antioxidant effect relative to halothane or sham halothane (ratio of (AOX + halothane) : halothane in study 1 and ratio of (AOX + sham halothane) : sham

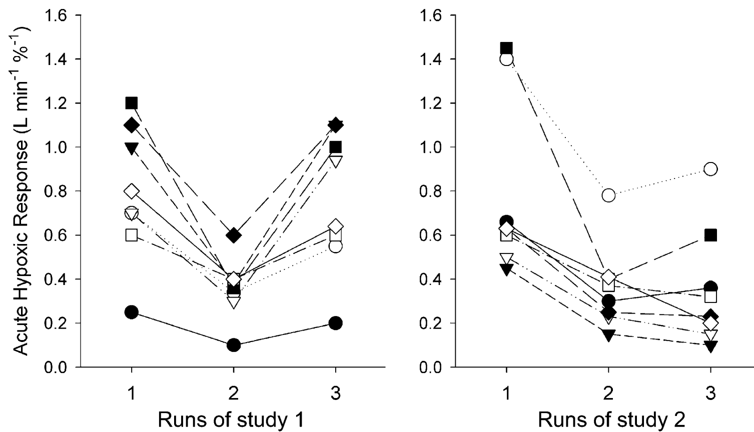

Figure 1. Hypoxic ventilatory responses of individual subjects in studies 1 and 2

Study 1 (left panel): control and halothane hypoxic ventilatory responses and influence of AOX pretreatment on halothane-induced impairment of the hypoxic drive. Study 2 (right panel): control and halothane hypoxic responses and influence of placebo pretreatment on halothane-induced impairment of the hypoxic drive. Note the ability of antioxidant but not placebo pretreatment to prevent depression of the hypoxic response by halothane. Different symbols are used for each subject. Subjects in studies 1 and 2 were different.

halothane in study 3) did not overlap: [1.7, 2.6] and [1.0, 1.2] in studies 1 and 3, respectively (Fig. 3). These data do not indicate that the effect of AOX to abolish halothane's depressant effect are explained by an increase of the AHR by the antioxidants *per se*. However, it cannot be excluded that at higher doses AOX may increase the AHR.

DISCUSSION

We have found that while an antioxidant cocktail had only a small, statistically insignificant effect on the acute hypoxic response (Fig. 3), it did reverse the large depression in the hypoxic response caused by low-dose halothane. To place this result into context, we need to discuss methodological considerations, the possible roles of ROS in the chemoreception process and the possible mechanisms by which halothane depresses the hypoxic ventilatory response.

The measurement of the hypoxic ventilatory response requires isocapnia both across drug treatments and during the hypoxic test. As seen in Table 1, the mean differences in P_{ET,CO_2} for the different treatment conditions in the four studies were closely matched and did not contribute to the changes in the measured AHR.

While we attempted to achieve blinding, the subjects were probably aware of when halothane was being inhaled. However, the depression of the AHR by halothane is large and consistent across subjects (Fig. 1), while the changes in the AHR with the sham halothane protocol are variable and similar to the variation expected with repeated hypoxic tests (Fig. 2). In testing the effects of inhalational anaesthetics, the experimental conditions are very important. We have previously shown that arousing the subject with audiovisual stimulation can reverse the depression of the AHR by isoflurane (van den Elsen *et al.* 1994). Therefore, possible variations in arousal level could be a confounding factor in our experiments. Since one of the researchers continuously observed the raw EEG during the studies and did not see any sign of sleep normally observed in sleep EEGs, we believe that the arousal level was similar in all studies employing halothane (i.e. halothane alone, halothane + AOX and halothane + placebo) as determined from subjective measures (all subjects opened their eyes when spoken to in a soft voice) and EEG measurements.

While we believe that the antioxidant cocktail we used was effective in altering the intracellular and/or extracellular redox state, we have no direct measurement of its efficacy in our subjects. Taking into account that the effects of halothane could be located at several sites – at the outer face of the membrane, within the membrane, in the cytosol or possibly at the mitochondrial level – we rationalized the combined use of water-soluble ascorbic acid, which is a particularly potent antioxidant in plasma and in the cytosol

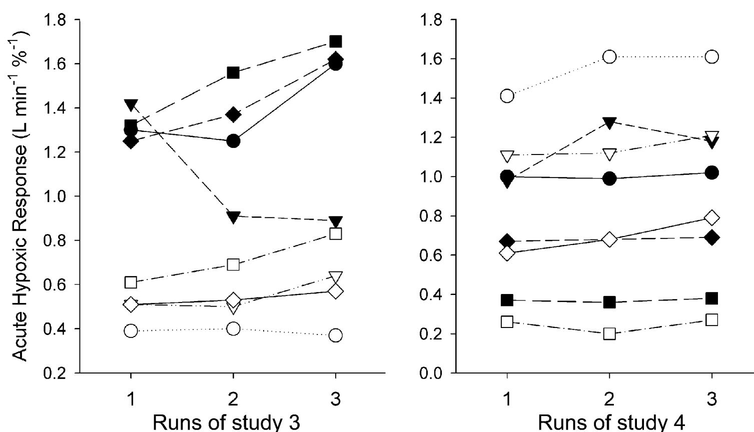

Figure 2. Hypoxic ventilatory responses of individual subjects of studies 3 and 4

Study 3 (left panel): control and sham halothane hypoxic responses and the influence of AOX pretreatment on a sham halothane response. Study 4 (right panel): control and sham halothane hypoxic responses and the influence of placebo pretreatment on a sham halothane hypoxic response. Note the absence of effect of antioxidant relative to placebo on the hypoxic response. Different symbols are used for each subject. Subjects in studies 3 and 4 were different.

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(Frei *et al.* 1989; Carr & Frei, 1999), and α-tocopherol, which, due to its lipid solubility, may be the most important free radical and lipid peroxide scavenger in membranes (Burton *et al.* 1983). Furthermore, it is known that the combined effectiveness of ascorbate and α-tocopherol is synergistic, with the net result that radicals originating from the membrane are removed using two different antioxidants (Packer *et al.* 1979; Niki, 1987). Combined administration of α-tocopherol (2000 i.u. I.M.) and ascorbic acid (2 g I.V.) has been shown to reduce lipid peroxidation in patients undergoing cardiac bypass surgery (Barta *et al.* 1991).

The oxygen transduction cascade in the carotid body (as in the similarly oxygen-sensitive pulmonary artery smooth muscle and the pulmonary neural epithelial cell bodies) has been subject to considerable research over the past decade and, while a much clearer picture of the process has emerged, there are many areas of considerable controversy (see Gonzalez *et al.* 1994 and Lopez-Barneo *et al.* 2001 for recent reviews). The most generally accepted model is that low oxygen decreases the open probability of potassium channels in the membrane of carotid body type I cells, which results in depolarization. This membrane depolarization opens voltage-gated calcium channels, with the resulting influx of Ca^{2+} causing neurotransmitter release, activating the synaptically adjacent carotid sinus nerve. Currently, much interest has focused on the oxygen-sensitive potassium channels in the carotid bodies of several species (Peers, 1997; Lewis *et al.* 2002). The rat and the rabbit have been most commonly studied and they appear to have different types of oxygen-sensitive potassium channels. The rat appears to have both TASK (Buckler *et al.* 2000) and maxi-K channels (Riesco-Fagundo *et al.* 2001) that are oxygen sensitive, while in the rabbit K_v channels seem to serve this role (Perez-Garcia & Lopez-Lopez, 2000; Perez-Garcia *et al.* 2000). However, within this general model, it is not determined how low oxygen closes the potassium channel that seems to initiate the cascade.

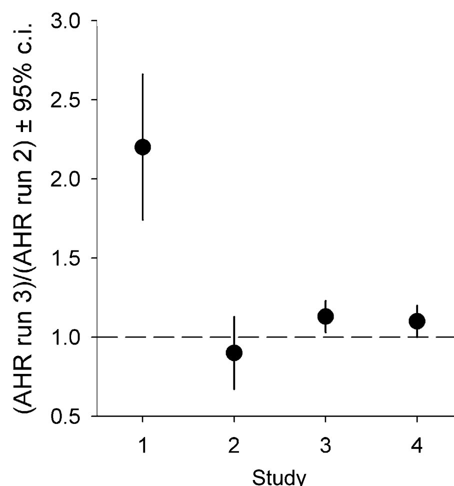

Figure 3. The effect of AOX or placebo on halothane or sham halothane-induced depression of the acute hypoxic response

Values are the ratio of the third hypoxic run to the the second hypoxic run of studies 1–4. ●, means; vertical lines depict the 95 % confidence intervals. A value of 1 indicates no effect of the AOX or placebo pretreatment on halothane's effect on the acute hypoxic ventilatory response. Note that the 95 % confidence intervals of studies 1 (AOX + halothane) and 3 (AOX + sham halothane) do not overlap.

Several studies have indicated that potassium channels show redox sensitivity and considerable sensitivity to levels of ROS (Kourie, 1998; Lopez-Barneo *et al.* 1999; Kobertz *et al.* 2000). It is unclear whether potassium channels possess intrinsic oxygen sensitivity, or whether other elements are required or modulate the O_2-sensing cascade (e.g. cytosolic, possibly membrane-associated, redox couples). Intrinsic oxygen sensitivity could exist in the form of reduction/oxidation of thiol-containing free cysteine residues in K^+ channel β subunits that are required for hypoxic sensitivity (Perez-Garcia *et al.* 1999). One proposed redox model associated with enzymatic production of ROS that may influence potassium channel conductance is the cytochrome P450 system that utilises NAD(P)H as an electron donor. Inhibition of this enzyme system has been shown to prevent the hypoxic inhibition of potassium channels (Hatton & Peers, 1996) but this has not been found in all model systems (Roy *et al.* 2001).

It is clear that within this general framework of hypoxic chemoreception there is considerable variety in specific sensor elements and couplings. Particularly when channels are expressed in heterologous systems, all the elements for the *in vivo* cascade may not be present. In addition, there may be substantive differences between sensing elements of the cascade between the different oxygen-sensitive tissues. Thus, it has been difficult to verify the roles for ROS in carotid body chemotransduction in more physiologically intact preparations. In fact, there is considerable controversy as to whether ROS increase (Leach *et al.* 2001; Waypa *et al.* 2001) or decrease (Lahiri & Acker, 1999) with hypoxia in oxygen-sensitive cells. Experiments in which the redox state of carotid body cells was altered would seem to indicate that ROS might not provide a *direct link* between hypoxia and the membrane depolarization initiated by the closure of the K^+ channel (Roy *et al.* 2001; Sanz-Alfayate *et al.* 2001). Exogenous reductants, however, have been shown to mimic the effect of hypoxia on O_2-sensitive potassium channels in carotid body cells (Benot *et al.* 1993). Thus, whatever the precise mechanism, there is likely to be at least a modulating role for the redox state of the type I cell in O_2 sensing.

The depressant effect of subanaesthetic doses of halothane in humans on ventilation during hypoxia may occur via a preferential and potent action on the carotid bodies (Knill & Clement, 1984; Dahan *et al.* 1994). The mechanism for

this depression is unknown, but inhalational anaesthetics can directly open two-pore domain potassium (TASK) channels in various cell types (Patel *et al.* 1999; Sirois *et al.* 2000; Patel & Honore, 2001*a*,*b*) and in particular in the rat carotid body (Buckler *et al.* 2000). The action of inhalational anaesthetics on TASK channels may be located at a specific region at the junction between the final transmembrane domain and the cytoplasmic C-terminus (Patel *et al.* 1999; Talley & Bayliss, 2002). This site is also involved in neurotransmitter inhibition of the channel but does not contain a motif that is known to be involved in cell signalling mechanisms (Talley & Bayliss, 2002). How changes in ROS and/or redox state could alter the properties of this binding site is unknown. In the lung carcinoma cell line H146, a representative model for pulmonary oxygen-sensitive neuroepithelial body cells, halothane transiently reverses hypoxic inhibition of potassium currents, similar to the reversal caused by the reactive species H_2O_2 (Hartness *et al.* 2001).

The metabolism of halothane itself may also change the redox status of cells. In hypoxia, halothane undergoes a reductive metabolism that in the liver is catalysed by isoforms of cytochrome P450 but in other tissues possibly also by other haem-containing proteins (de Groot & Sies, 1989; Spracklin & Kharasch, 1998; Kharasch *et al.* 2000). Reduction of halothane yields CF_3CHCl radicals able to inactivate cytochrome P450 by covalent binding or, alternatively, to remove hydrogen from polyunsaturated lipids thus initiating lipid peroxidation (de Groot & Noll, 1983; de Groot & Sies, 1989; Kharasch *et al.* 2000). In guinea-pigs, the hepatotoxic effect caused by this reductive metabolism of halothane can be prevented by antioxidant treatment (Sato *et al.* 1992). In humans, haemin induction of haem oxygenase-1, which has an antioxidant role in oxidative stress, has been shown to be effective against halothane-induced liver damage (Odaka *et al.* 2000).

The susceptibility of halothane's depressant effect to antioxidant treatment that we found in this study indicates that the cellular redox state influences the effect of halothane on the oxgyen-sensing mechanism. This could be explained by a modulation by ROS of the coupling of halothane to the potassium channel (or other channels). Whether or not the ROS was generated from halothane's metabolism or from other intracellular processes (Waypa *et al.* 2001), the reduction in ROS with antioxidant treatment could reduce the coupling of halothane to the channel and prevent it from opening the channel.

An alternative way to explain our findings would be to suggest that an increase in the concentration of ROS has an inhibitory effect on the mechanism involved in the acute hypoxic response. In this scenario, the cellular redox state or the signalling from a particular ROS would be the coupling from low oxygen to potassium channel closure. For example, an NAD(P)H oxidase has been proposed as the membrane-bound source of oxygen-sensitive ROS, implying a decrease in ROS in hypoxia (Kummer & Acker, 1995; Semenza, 1999; Jones *et al.* 2000; Kietzmann *et al.* 2000). The increase in local ROS caused by the reductive metabolism of halothane in hypoxia would thus counter the hypoxia-induced decrease in ROS and prevent the hypoxic closure of the K^+ channel. This effect would be most noticeable in hypoxia since halothane's reductive metabolism is increased in hypoxia.

In animal species, the effect of halothane on the hypoxic ventilatory response is variable. In the goat, for example, an end-tidal concentration of 0.5 % halothane does not significantly depress it (Koh & Severinghaus, 1990). In the rabbit and cat, 0.5–1 % halothane reduces the hypoxic response, the effect in the latter species being larger (Davies *et al.* 1982; Ponte & Sadler, 1989). As shown in this and previous studies, the effect of 0.11 % halothane in man is to reduce hypoxic sensitivity by more than 50 %. These species differences could originate from the differences in the type of oxygen-sensitive potassium channel that initiates the transduction cascade (e.g. TASK *vs.* K_v) and their differences in anaesthetic sensitivity or in splice variants of the expressed channel. An alternative explanation could also lie in species differences in the defence against ROS. Goats produce large quantities of ascorbic acid (Chatterjee *et al.* 1975) and may thus be better protected against the adverse effects of free radicals produced by halothane. To a lesser degree this may also be the case for rabbits. Cats produce low quantities of ascorbic acid (Chatterjee *et al.* 1975) and this might explain their higher susceptibility to halothane than rabbits. Humans have lost the ability to synthesize ascorbic acid and may therefore be more vulnerable to the adverse effects of reactive species that are produced by halothane.

It is worth mentioning that in a previous study we were not able to demonstrate a clear depression of the normocapnic AHR by desflurane (Dahan *et al.* 1996). This volatile anaesthetic has a low metabolism, with little production of free radicals (Koblin, 1992). In another study, we found that low-dose propofol, which is known to have antioxidant properties (De La Cruz *et al.* 1999), depressed neither the CO_2 sensitivity of the peripheral chemoreflex loop nor the fast (carotid body-mediated) component of the acute hypoxic response (Nieuwenhuijs *et al.* 2000, 2001). Together with the present findings, these previous data suggest that the (lack of) depressant effects of anaesthetics on the hypoxic response may be related to their pro-oxidant (antioxidant) properties, but further studies are needed to support this hypothesis.

From the data that we present in this study, we conclude that changing the cellular redox state can modulate the depressant effect of halothane on the acute hypoxic response. Furthermore, although our results do not supply direct evidence for an inhibitory role of ROS in the

oxygen-sensing cascade, they could be explained by ascribing at least a modulating role to radical species in the AHR. Our observation that antioxidant pretreatment markedly reduces the depressant effect of halothane on the AHR demonstrates a specific pharmacological reversal of an anaesthetic effect. Further work is needed in both humans and animal preparations to clarify the interaction of cellular redox status, inhalational anaesthetics and oxygen-sensitive potassium channels in the carotid body.

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