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Guinea-pig tracheal responsiveness *in vitro* following general anaesthesia with halothane

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Guinea-pig tracheal responsiveness in vitro *following general anaesthesia with halothane. F.J. Mercier, A. Denjean. ©ERS Journals Ltd 1996.*

ABSTRACT: Halothane and isoflurane induce potent bronchodilation during general anaesthesia and have been used successfully during status asthmaticus. The aim of this study was to determine whether airway hyporesponsiveness was prolonged after halothane administration.

Sixteen guinea-pigs were submitted for 2 h to either 1.5% halothane in oxygen or 100% oxygen, and were killed 24 h later to elicit isometric tracheal contractions in organ baths with various agonists.

Cumulative concentration-response curves to histamine or to KCl and contractions evoked with acetylcholine 1 mM (4.7±0.8 *vs* 4.6±0.5 g) or carbachol 10 µM in calcium-free buffer (4.3±0.6 *vs* 4.4±0.6 g) exhibited no difference between groups. Moreover, when 4% halothane or 4.6% isoflurane were directly bubbled through the organ baths, a significant decrease (13±1% and 37±2%) of maximal contractions evoked with acetylcholine and KCl, respectively, was obtained but these relaxant effects did not persist 30 min after cessation of anaesthetic.

These results indicate that, even though halothane induces transient airway hyporesponsiveness *in vitro*, previous halothane anaesthesia in guinea-pigs does not alter subsequent tracheal responsiveness assessed *in vitro*. Our findings may explain the transient renewal of bronchospasm reported during intermittent periods off halothane in status asthmaticus.

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Volatile anaesthetics, such as halothane and isoflurane, are potent bronchodilators and have been used successfully during status asthmaticus [1–3]. However, the bronchodilating properties of volatile anaesthetics are alveolar concentration-dependent [4–6], and do not remain relevant at very low concentration, at least for isoflurane, either in dogs [7] or in humans [8].

Nonetheless, in dogs, AMYOT *et al*. [9] reported a prolonged (≥24 h) hyporesponsiveness of airway smooth muscle to histamine following general anaesthesia with halothane or sodium thiamylal. As the authors pointed out, this question is of interest both to the clinician and the research scientist. For the clinician, this result implies that general anaesthesia could improve postoperative management of asthmatic patients; however, the transient renewal of bronchospasm reported during intermittent periods off halothane in status asthmaticus do not support this hypothesis [1–2]. For the research scientist, this means that data obtained on airway smooth muscle *in vitro* could be influenced by previous use of anaesthetic agents *in vivo*. This latter point is of particular importance because it has recently been shown that the effects on specific airway resistance of aerosolized bradykinin are different in conscious guinea-pigs and in anaesthetized animals [10].

The aim of the present study, therefore, was to determine whether airway hyporesponsiveness was prolonged after halothane administration by first investigating guinea-pig tracheal ring contractility 24 h after halothane anaesthesia, and then observing the evolution of halothane and isoflurane relaxant effects when these anaesthetics, directly administered on precontracted rings, were discontinued.

Materials and methods

Tissue preparation

Male adult albino guinea-pigs were used for both experiments. They were all stunned by a blow to the head and exsanguinated. This procedure made it possible to avoid the use of anaesthetic agents that might interfere with the aim of the study. The whole trachea was then removed, immediately immersed in physiological saline solution, and cut into 6–8 rings, 3–4 mm in width. Each tracheal ring, with the epithelium left intact [11], was suspended in a vertical 20 mL chamber of an 8-channel computerized organ bath system (IOS®; EMKA Technologies, Paris, France), filled with physiological saline at 37°C and bubbled with 95% O_2 and 5% CO_2 to maintain a pH of 7.4. The composition of the physiological saline was (mM): NaCl 115, KCl 5, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.46, NaH_2PO_4 2.4, dextrose 11. Continuous recordings of the isometric force and subsequent analyses of

data were made using Moise3® software (EMKA Technologies, Paris, France). Preparations were suspended under 2 g resting force and allowed to equilibrate for 90 min with regular (10–15 min) physiological saline washing.

Experimental protocols

Experiment 1. This experiment was conducted with eight pairs of guinea-pigs. During the same experimental day, each pair was randomly assigned to receive *in vivo* either 1.5% halothane in oxygen (post-halothane group) or 100% oxygen (control group). The animal was placed under an airtight glass bell-jar of 5 L capacity, into which a continuous flow of the assigned gas was injected. In one group, 1.5% halothane was added to oxygen *via* a calibrated vaporizer (Fluotec3®; Ohmeda, France), before entering the bell-jar. The anaesthetic partial pressure was continuously monitored by sampling a small amount of gas on the outflow circuit using an anaesthetic monitor (Normac®, Datex, France). A period of 5 min was sufficient to reach a stable value of 1.5%. At that time, the animals appeared to become anaesthetized, as expected: indeed, for guinea-pigs, this value corresponded to an inspired halothane concentration of 1.5 minimum alveolar concentration (MAC) [12]. MAC is defined as the concentration of an inhaled anaesthetic sufficient to keep 50% of subjects from moving in response to a surgical incision [13]. In guinea-pigs, halothane MAC is 1.01± 0.03% (in oxygen) and isofluorane MAC is 1.15±0.05% (in oxygen) [12]. The animals breathed the assigned gas spontaneously for 2 h, and were maintained in the bell-jar for a supplemental 20 min "recovery" period with 100% oxygen. The outcome was uneventful for all of them.

Each guinea-pig was killed 24 h later to assess *in vitro* its tracheal responsiveness with various contractile agonists. A cumulative concentration-response curve to histamine from 10^{-7} to 3×10^{-4} M was performed with 3–4 rings from each animal (fig. 1). After wash-out and re-equilibration for 90 min, a maximal contraction with acetylcholine (ACh) 1 mM was then evoked. The 3 or 4 other rings were used: 1) to first establish a cumulative concentration-response curve to KCl (1, 5, 15, 25 and 65 mM); and 2) to elicit a phasic contraction after wash-out and re-equilibration for 90 min. These phasic contractions were obtained using a method previously described by TAGLIENTE *et al*. [l4]: the rings were incubated for 5 min in a calcium-free buffer prepared by omitting the $CaCl_2$ from the normal physiological saline and by adding ethylene glycol tetra-acetic acid (EGTA) 2 mM. The addition of carbachol 10 µM induced a contraction called "phasic", as it always demonstrated a peak of tension followed by a decay back to resting tension. In guinea-pigs, these unsustained contractions are the only intense contractions reported to be markedly depressed by volatile anaesthetics administered *in vitro* with concentrations in the range of those used *in vitro* to provide anaesthesia (≤2 MAC) [14, 15].

Experiment 2. During the second experiment, volatile anaesthetics were introduced directly *in vitro via* the bubbling gas, to clarify the results found during the first experiment. High concentrations of either halothane or

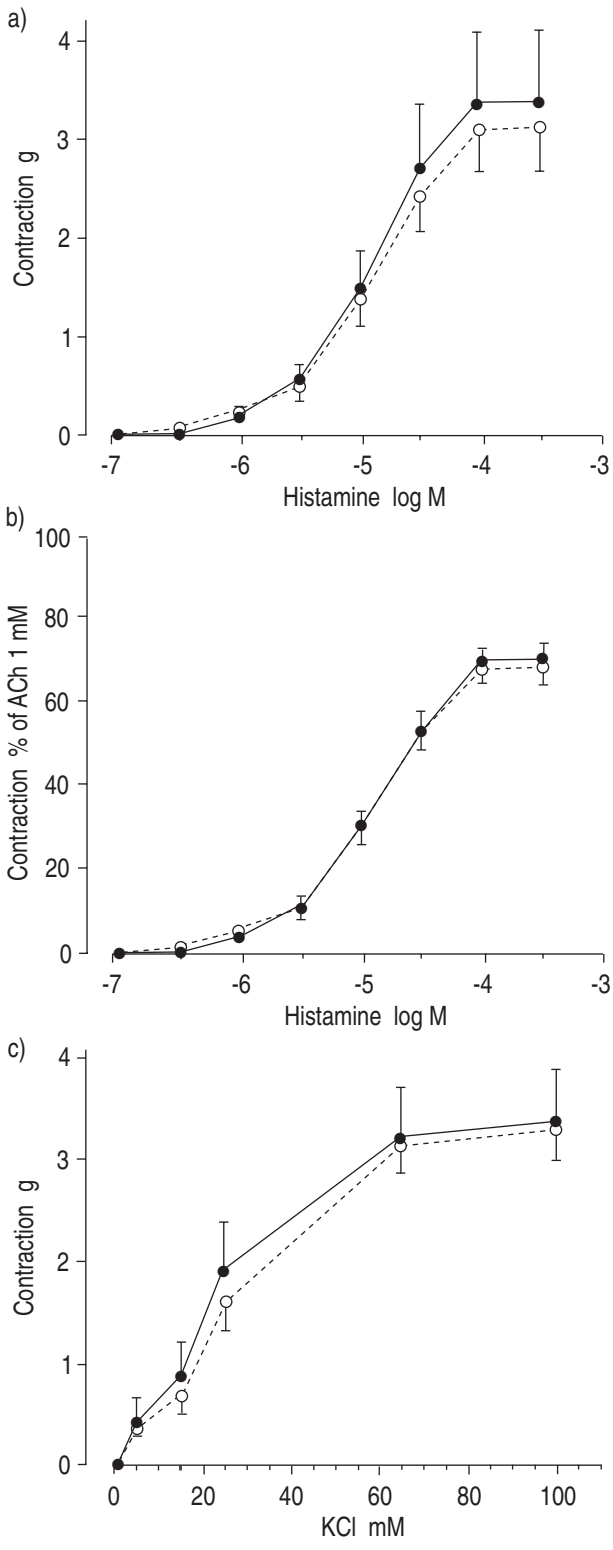

Fig. 1. – Cumulative concentration-response curves to histamine and to KCl obtained *in vitro* 24 h after an *in vivo* 2 h inhalation of either 1.5% halothane in oxygen (—●— ; n=8) or 100% oxygen (--○-- : control; n=8). After halothane anaesthesia, there was no prolonged hyporesponsiveness of guinea-pig tracheal smooth muscle either to histamine (a and b) or to KCl (c). The isometric force developed by tracheal rings is expressed in panel b as percentage of maximal contraction induced by acetylcholine (ACh) 1 mM. Each symbol represents a mean±SEM.

isoflurane were, therefore, used: 4% for halothane and 4.6% for isoflurane, adjusted with the Fluotec3® and Isotec3® calibrated vaporizers, and controlled just before entering the organ baths with the Normac® anaesthetic monitor. These values corresponded to nearly 4 "MAC" for each anaesthetic, as anaesthetic partial pressures in organ baths are close to those needed *in vivo* to obtain a given anaesthetic concentration in tissues [16]. Ten additional guinea-pigs were used for this second part of the study (76 tracheal rings). Three to four rings per animal were tested with ACh 1 mM and the remaining with KCl 65 mM. The maximal contractions (C_{max}) evoked were measured at the plateau 30 min later (fig. 2). Anaesthetics were then introduced for a 30 min period at the end of which the responses (C_{60}) were measured (fig. 2). Recording of the residual effects 30 min after anaesthetic cessation (C_{90}) completed Experiment 2 (fig. 2).

Therefore, Experiment 2 was designed to observe the evolution of the relaxant effects induced by halothane or isoflurane, when directly administered *in vitro*, during a 30 min period after anaesthetic cessation (C_{60}–C_{90}).

Drugs

Halothane was obtained from Belamont (Paris, France) and isoflurane from Abbott (Rungis, France). The other chemicals were acetylcholine chloride, potassium chloride and carbachol from Sigma (St. Louis, MO, USA), and EGTA from Merck (Darmstadt, Germany). The solutions were prepared daily in distilled water and are expressed as final molar concentrations in organ baths.

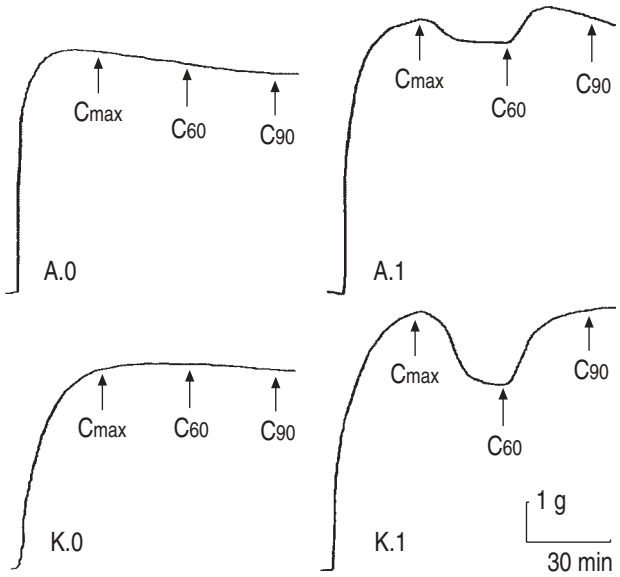

Fig. 2. – Typical tracings of original recordings showing the three sequences of Experiment 2 and the lack of residual relaxant effect of volatile anaesthetics after the cessation of their *in vitro* administration (results in table 1). C_{max}: maximal contraction, obtained within 30 min either with acetylcholine (ACh 1 mM) or potassium chloride (KCl 65 mM); C_{60}: value obtained after a 30 min treatment period ($C_{max} \rightarrow C_{60}$) with either 4.6% isoflurane (Group I), 4% halothane (Group H), or 95% O_2 5% CO_2 (Group C); C_{90}: value obtained after a 30 min anaesthetic cessation period ($C_{60} \rightarrow C_{90}$). A.0: ACh 1 mM in time-control group (Group C); A.1: ACh 1 mM in treated group (Group I for instance); K.0: KCl 65 mM in time-control group (Group C); K.1: KCl 65 mM in treated group (Group H for instance).

Statistical analysis

In the first experiment, halothane or 100% oxygen was administered *in vivo* to the whole animal. Therefore, for each type of response obtained with a given contractile agonist, values provided by the 3–4 rings of the same animal were averaged. This averaged value was then taken as the independent individual variable. The mean dry weight of tracheal rings was similar in both groups (post halothane group: 7.1±1.1 mg *versus* control group 6.9± 0.9 mg), and expression of the results in g tension·g^{-1} weight did not reduce SEM or significantly modify probability values (data therefore not shown).

Statistical comparisons were performed by two-way analysis of variance (ANOVA) for repeated-measures (concentration-response curves) or one-way ANOVA (maximal sustained or phasic contractions) followed by least significant difference (LSD) range tests. Results are expressed as mean±SEM. p-values of less than 0.05 were considered significant.

Results

Experiment 1

There were no significant differences in any type of isometric tension evoked *in vitro* 24 h after an *in vivo* 2 h inhalation of 1.5 MAC of halothane, when compared with the control group treated with 100% oxygen: 1) maximal contractions to ACh 1 mM were not reduced in the post-halothane group (4.7±0.8 *versus* 4.6±0.5 g); 2) concentration-response curves to histamine (fig. 1a), even expressed as percentage of maximal response to ACh 1 mM (fig. lb), and concentration-response curves to KCl were similar in both groups (fig. 1c); and 3) moreover, phasic contractions to carbachol (10 μM) in Ca^{++} free buffer exhibited no difference between the two groups, 4.3±0.6 *versus* 4.4±0.6 g.

Experiment 2

Figure 2 illustrates typical individual tracings of isometric force produced by tracheal rings. Maximal responses to either ACh 1 mM (n=37) or KCl 65 mM (n=39) were recorded 30 min after the introduction of contractile agonists in organ baths. When halothane or isoflurane were subsequently introduced for 30 min, a relaxation was observed for each ring (n=52). However, 30 min after anaesthetic cessation, there was consistently an increase in contractile force (fig. 2).

Analysis of the results (table 1) indicates that: 1) maximal contractions ("C_{max}") in the three groups were initially comparable; 2) both 4 MAC halothane or isoflurane induced a significant and comparable relaxation of tracheal smooth muscle (C_{60}) when compared to the time-control group. The mean relaxation was 13±1% on ACh-induced contractions and 37±2% on KCl-induced contractions; and 3) the increase in contractile force after cessation of halothane or isoflurane led 30 min later (C_{90}) to a level of contractions paradoxically and transiently greater (for ACh-precontracted rings) or comparable (for KCl-precontracted rings) to the ones obtained in the time-control group.

Table 1. – Results of Experiment 2

	Group I	Group H	Group C
C_{max} g			
ACh 1 mM	5.7±0.4	6.4±0.4	5.8±0.4
KCl 65 mM	4.4±0.4	4.6±0.3	4.8±0.3
C_{60} % C_{max}			
ACh 1 mM	84±2***	81±2***	95±1
KCl 65 mM	64±2***	68±3***	105±3
C_{90} % C_{max}			
ACh 1 mM	93±1**	96±1**	88±2
KCl 65 mM	100±2	102±2	102±3

I: isoflurane; H: halothane; C: control; ACh: acetylcholine. Each value is the mean±SEM of at least 10 experiments. ***: $p<0.001$, compared to Group C; **: $p<0.01$, compared to Group C. See figure 2 for experimental design and explanation.

Discussion

In Experiment 1, it was shown that when 1.5% halothane was administered during 2 h to guinea-pigs, no residual inhibitory effect was observed on tracheal smooth muscle sensitivity 24 h post-anaesthesia. Despite apparently similar protocols, the results are inconsistent with those of AMYOT *et al.* [9], who reported in dogs a prolonged (≥24 h) hyporesponsiveness of tracheal smooth muscle strips to histamine following general anaesthesia.

To our knowledge, the results of AMYOT *et al.* [9] have never been challenged. In their study, baseline maximal contractions induced with electrical stimulation were greater in the group who had previously received halothane than in the control group (mean±SD: 166±52 *versus* 107±70 g tension·g^{-1} weight), although this difference was not statistically significant. Using these values stated to be comparable, to normalize for differences in intrinsic contractile properties of tracheal smooth muscle, they found that the maximum effect (E_{max}) of the concentration-response curve to histamine was significantly reduced in the post-halothane group when compared with their control group. However, in our study, maximal contractions induced with ACh 1 mM were very similar in both groups (4.65/4.57=1.02; p=0.93) and the concentration-response curve to histamine (even normalized by maximal ACh-induced contractions) was not inhibited in the post-halothane group. Using KCl concentration-response curves and also phasic contractions, which are very sensitive to halothane [14, 15], we were still unable to detect any residual inhibitory effect.

One difference that might account for the discrepancy could be the choice of the species studied: volatile anaesthetics for instance generate a pronounced relaxation on KCl- (compared with ACh-) induced contractions in guinea-pigs [15], whereas conflicting results exist with regard to the level of their effectiveness on KCl-induced contractions in dogs [17, 18]. Other deliberate differences exist in animal preparation. Our control group differed from the treated group only by halothane administration, whereas in addition to halothane, AMYOT *et al.* [9] used pancuronium, nitrous oxide, tracheal intubation and mechanical ventilation for their treated (but not their control) group. The effects of intubation and mechanical ventilation may be far more likely to cause subsequent changes in airway reactivity than the residual effects of any volatile anaesthetics. Conversely, our animal preparation may also be debated. Concerning the use of 100% O_2, significant pulmonary alterations occur only when exposure times are considerably longer than that used in the present study (160 min) [12], and the airway reactivity that we obtained *in vitro* was in the range of that usually observed without previous exposure to oxygen *in vivo*. Hypoxia was ruled out, as the guinea-pigs breathed nearly 100% O_2 during halothane anaesthesia without any apnoea or cyanosis (which can be observed on the muzzle), and hypoventilation was moderate and readily reversible at the end of anaesthesia. This very good guinea-pig tolerance for volatile anaesthetics (contrary to rats) is in agreement with the almost normal blood gas values reported by SEIFEN *et al.* [12] in similar conditions. In laboratory investigations, it is, in fact, the principal advantage of volatile anaesthetics (compared with pentobarbital *i.p.*), provided that technical conditions allow their use.

Therefore, the results of Experiment 1 strongly support a lack of prolonged hyporesponsiveness of tracheal smooth muscle following general anaesthesia with halothane, at least in guinea-pigs.

Experiment 2 provided further evidence that neither halothane nor isoflurane, directly administered in organ baths, induced residual effects after the cessation of their administration, even when using very high concentrations (≈4 "MAC"). Indeed, halothane or isoflurane relaxed precontracted tracheal rings. This relaxation was weak on ACh-induced contraction, as expected [11, 19] but was consistently obtained. The relaxation obtained on KCl-induced contraction was three times greater, as reported previously [15]. However, these relaxant effects were quickly and completely reversible after interruption of the anaesthetic. The "overshoot" observed for ACh-precontracted rings 30 min after the cessation of halothane or isoflurane probably has no biological significance because it was mild, transient, and not observed on rings precontracted with KCl, a less fierce contractile agonist. Similarly, in dog muscle strips, JONES *et al.* [20] reported that halothane had no significant residual effects on contractile response and aequorin luminescence.

The quick reversal of relaxation of the ring after anaesthetic cessation is consistent with the pharmacokinetic data of BAZIL *et al.* [15], who found that volatile anaesthetics bubbled into physiological saline saturated the solution in 5 min and the immersed tissue in 15–30 min. In our study, this latter period corresponded both to the onset and the reversal of the relaxant effects of volatile anaesthetics.

Thus, after a short period (15–30 min) of tissue washout from volatile anaesthetics, no real residual relaxant effects were observed.

In conclusion, hyporesponsiveness of guinea-pig tracheal smooth muscle, observed when volatile anaesthetics are administrated *in vivo* or *in vitro* is not prolonged after the end of their administration. These results, provided that it can be inferred that they extend to humans, have evident clinical implications for the postoperative period and may also explain the transient renewals of bronchospasm reported during intermittent periods off halothane in status asthmaticus. Our study, contradicting results reported in dogs, also provides a positive

methodological clarification by confirming the validity of many experimental studies performed on guinea-pig tracheal smooth muscle, in which anaesthesia was used to allow a preliminary preparation on the living animal.

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