Role of prostaglandins and nitric oxide on halothane-induced arteriolar dilatation in rat diaphragm

V. DE LARMINAT, J. BOCZKOWSKI, B. DUREUIL, E. VICAUT, R. FARINOTTI, M. AUBIER AND J.-M. DESMONTS

Summary
The effects of anaesthetics on the microcirculation of the diaphragm are incompletely understood. Therefore, we assessed by in vivo intravital microscopy in rats the action of halothane on diaphragmatic arteriolar diameter and the role of nitric oxide and prostaglandins on halothane-induced diaphragmatic arteriolar diameter. We studied 54 rats anaesthetized with thiopentone. Dose–response curves to topically applied Krebs’ solution saturated with halothane at increasing concentrations of 0%, 1%, 3% and 5% were carried out in the presence of an inhibitor of nitric oxide synthesis (Nω-nitro-L-arginine (LNA), 300 μmol litre⁻¹) or inhibitors of prostaglandin synthesis (mefenamic acid 20 μmol litre⁻¹ or indomethacin 20 mol litre⁻¹) or in the absence of any inhibitor. We found dose-dependent arteriolar dilatation which was abolished by mefenamic acid and indomethacin. In contrast, the effect of halothane was not modified by LNA. These data demonstrated that halothane-induced arteriolar dilatation in the diaphragm of the rat was mediated by prostaglandins but not by nitric oxide. (Br. J. Anaesth. 1996; 77: 232–237)

Key words

Halothane is commonly considered as a pure vasoconstrictor. However, animal and human studies in vivo have shown that halothane has different effects in vascular beds [1–3]. Miller, Kistner and Epstein [2] showed in rats that inhalation of halothane induced an increase in blood flow to the brain, kidney and liver, and a decrease in blood flow to the heart and peripheral and respiratory skeletal muscles. In a previous work we showed that inhalation of halothane was unable to dilate the arterioles of the diaphragm [4]. These differences in vascular responses to inhalation of halothane observed in vivo may be related to differences in the sensitivity of vascular beds to this agent [5]. Alternatively, they may reflect differences in local vascular adaptation to the haemodynamic changes induced by halothane. Therefore, we assessed the vascular effects of halothane on diaphragmatic microcirculation.

In this study we assessed the direct effects of halothane on diaphragmatic arteriolar diameters, by topical administration on the surface of the diaphragm in order to avoid its systemic haemodynamic action and evaluated the mechanism(s) of action of halothane on diaphragmatic arterioles. We were interested in the role of the vascular endothelium because it has been shown recently that halothane modulates endothelium-dependent relaxation [6]. Therefore, we assessed the role of endothelial agents, such as nitric oxide and prostaglandins, on halothane-induced diaphragmatic arteriolar diameter by inhibition of nitric oxide synthesis with Nω-nitro-L-arginine (LNA) [7, 8] and prostaglandin synthesis with mefenamic acid or indomethacin [9]. Special emphasis was placed on evaluation of the effects of prostaglandins, as these agents contribute, together with nitric oxide, to modulation of diaphragmatic arteriolar tone [10], a phenomenon which could not be detected in other skeletal muscle microvessels [11, 12].

Materials and methods
Male Sprague–Dawley rats weighing 163 (5) g were anaesthetized with sodium pentobarbitone 50 mg/kg body weight i.p. A patent airway was maintained with a tracheostomy tube and the lungs were ventilated throughout the study with a rodent ventilator (Ugo Basile Apparatus Inc., Italy) at an FiO₂ of 0.5. The left carotid artery was cannulated for continuous arterial pressure monitoring with a Statham P23 DB transducer. All animals whose mean arterial pressure decreased to less than 90 mm Hg were excluded from the study. The right jugular vein was cannulated for administration of 5 ml kg⁻¹ normal saline during surgery. Body temperature was monitored continuously with a rectal probe.


*Address for correspondence: Département d’Anesthésie-Réanimation, Hôpital C. Nicolle-Chu de Rouen, 1, rue de Germont, 76031 Rouen Cedex, France.
probe and maintained constant at 36.5–37.5 °C by a heat lamp and a heating pad (Harvard Apparatus, MA, USA).

The experimental design was in agreement with the recommendations of French Law (Ministère des Affaires Sociales et de la Solidarité Nationale) and all experiments were approved by the local Animal Committee.

PREPARATION OF THE DIAPHRAGM

The diaphragm preparation has been described in detail previously [10, 13]. Briefly, a bilateral thoracotomy was performed in the fifth intercostal space, the sternum remaining intact. The diaphragm was separated carefully from the lungs and the mediastinal tissues. A midline laparotomy was followed by a transverse incision at each side which allowed exposure of the diaphragm in a perpendicular position relative to the body of the animal. The animal was placed in a Trendelenburg position on a rodent operating table (Harvard Apparatus, MA, USA).

When the diaphragm was exposed, its abdominal face was irrigated continuously with a modified Krebs–Henseleit solution containing (mmol litre⁻¹): NaCl 118, KCl 5.9, CaCl₂–2H₂O 2.5, MgSO₄–7H₂O 0.5, NaHCO₃ 28 and glucose 10. This solution was maintained at 37.5 °C and pH, Po₂ and PcO₂ were, respectively, fixed at 7.41 (0.06), 3.1 (0.2) kPa and 5.5 (0.1) kPa by bubbling with a 5 % carbon dioxide–95 % nitrogen gas mixture. Pancuronium 4 μmol litre⁻¹ was added to Krebs’ solution to prevent muscle fasciculation.

The muscle was transilluminated using a 150-W tungsten–halogen lamp connected to a fibreoptic cold light microprobe introduced gently through the left thoracotomy aperture. The diaphragmatic microcirculation was observed with a modified Leitz microscope placed in a position parallel to the area of the abdominal side of the muscle. The image, magnified by a 20 × long-distance objective, was projected into a CCD video camera (Sony DXC-101P) connected to a videotape recorder (Sony VO-9600 P) and a video monitor (Sony PVM-1371 QM). Total magnification from the microscope was projected into a CCD video camera (Sony VO-9600 P) and a video monitor (Sony PVM-1371 QM). Connectivity of the arterial branches arising from the internal mammary and intercostal arteries. Second and third arteriolar orders (A2 and A3, respectively) were analysed according to the number of bifurcations proximal to the studied arterioles, as described previously [15]. Only clearly distinguishable vessels were selected and the number of measured arterioles varied between one and three of each order per preparation. Before any measurement, a 20–30-min period after surgery was allowed to reach a steady state of arteriolar tone.

EXPERIMENTAL DESIGN

Two studies were performed.

Study 1

In seven animals arteriolar diameter was measured at baseline and then Krebs’ solution saturated with increasing concentrations (0 %, 1 %, 3 % and 5 %) of halothane was applied topically in order to perform a cumulative dose–response curve. The different concentrations were administered in a stepwise manner for 2 min each. The diameter of the selected arteriole was measured at the end of each administration. This was followed by a 15-min wash-out period during which the muscle was irrigated with drug-free Krebs’ solution. At the end of this period, the diameter of the same arterioles studied previously was measured and a second cumulative dose–response curve to halothane was performed in order to evaluate the reversibility of the effects of halothane.

Study 2

Twenty-eight animals were allocated to one of four groups according to topical administration on the diaphragm of Krebs’ solution containing LNA 300 μmol litre⁻¹ (n = 7 animals, group LNA), mefenamic acid 20 μmol litre⁻¹ (n = 7 animals, group MA), indomethacin 20 μmol litre⁻¹ (n = 7) or no inhibitor (n = 7 animals, group C). Diameter was measured at baseline and after a 20-min superfusion period. Then, Krebs’ solution saturated with increasing concentrations (0 %, 1 %, 3 % and 5 %) of halothane was applied topically in order to perform a cumulative dose–response curve, as described above. The inhibitors corresponding to each pretreatment group were also present in the halothane-saturated Krebs’ solutions. The diameter of the selected arteriole was measured at the end of each application.

DOSE OF HALOTHANE

Samples of Krebs’ solution equilibrated with halothane were removed at the end of an application period for subsequent measurement of anaesthetic concentration in Krebs’ solution by gas chromatography [16].

REAGENTS

Halothane was obtained from Laboratoires Belamont (Paris, France) and was introduced into 40 ml of Krebs’ solution at 37 °C by bubbling with 5 % carbon dioxide–95 % nitrogen passed through a pre-calibrated vaporizer (flow 0.5 litre min⁻¹) for 15 min. Vaporizers delivered halothane at 0 %, 1 %, 3 % and 5 %. Mefenamic acid and LNA were obtained from Sigma Chemical Co. (St Louis, MO, USA). Mefenamic acid was dissolved in sodium carbonate 1 mg ml⁻¹ and diluted to the final concentration with Krebs’ solution. LNA was dissolved directly in
Krebs’ solution by stirring at room temperature. Indomethacin was obtained from Merck, Sharp and Dohme Chibret Products, France. Drug solutions were prepared freshly daily.

**STATISTICAL ANALYSIS**

All results are expressed as mean (SD). Comparison between arteriolar diameters at baseline in the different groups of animals was performed by one-way analysis of variance. Evaluation of the effects of each inhibitor on baseline arteriolar diameter was performed by a paired t test. Comparison between arteriolar dose–response curves (diameter vs dose of halothane) between the different groups of animals was performed using two-way analysis of variance for repeated measures, one “within” factor, that is factor “dose” and a grouping factor “absence or presence of inhibitor” [17]. \( P < 0.05 \) was considered significant.

**Results**

The experimental interventions did not affect systemic arterial pressure, which was stable over the course of the experiments and within the range 95–120 mm Hg.

**EFFECTS OF HALOTHANE ON DIAPHRAGMATIC ARTERIOLAR DIAMETER**

Halothane administered in concentrations of 0–5 % produced dose-dependent dilatation of diaphragmatic A2 and A3 arterioles (factor “dose” \( P < 0.0001 \) in both arteriolar orders). It must be noted, however, that in both arteriolar orders, dilatation was not observed until 3 % halothane was given (fig. 1).

The effect of halothane was completely reversible. When diaphragmatic muscle surface was washed after administration of halothane, A2 and A3 diameters were not different from baseline values (44.21 (6.50) µm and 25.71 (4.21) µm vs 46.12 (5.21) µm and 27.12 (3.85) µm, for A2 and A3, respectively). Furthermore, the dilator capacity of the arterioles remained intact after halothane, as demonstrated by the similarity between the two dose–response curves to this agent performed with a 15-min washing period interval (fig. 1). Doses of halothane in Krebs’ solution at the different concentrations used in this study are presented in table 1.

**EFFECTS OF HALOTHANE IN THE PRESENCE OF LNA, MEFENAMIC ACID AND INDOMETHACIN**

Baseline arteriolar diameters did not differ between the different groups of animals (table 2). LNA 300 µmol litre\(^{-1}\) mefenamic acid 20 µmol litre\(^{-1}\) and indomethacin 20 µmol litre\(^{-1}\) produced significant reduction in basal diaphragmatic arteriolar diameters (table 2). After LNA superfusion, A2 and A3 diameters were 85 (11) % (\( P < 0.001 \)) and 84 (11) % (\( P < 0.001 \)) of baseline values, respectively. After mefenamic acid superfusion, A2 and A3 diameters were 93 (3) % (\( P < 0.001 \)) and 93 (6) % (\( n = 32; P < 0.001 \)) of baseline values, respectively. After indomethacin superfusion, A2 and A3 diameters were 97 (5) % (\( P < 0.05 \)) and 91 (4) % (\( P < 0.001 \)) of baseline values, respectively. The reduction in diameter was related specifically to the inhibitors as superfusion of the diaphragm during a similar period with drug-free Krebs’ solution did not result in any significant modification in arteriolar diameters (table 2).

Halothane-induced arteriolar dilatation was not modified by LNA (the interaction between “dose” and “absence or presence of inhibitor” was not significant for A2 and A3, when group C was compared with group LNA) (fig. 2). In contrast, halothane-induced increase in A2 and A3 diameters was almost totally inhibited by mefenamic acid

---

**Figure 1** Increase in A2 (top) and A3 (bottom) diameter in response to 0–5 % halothane (Hal.) (mean, sn). First curve (●) = cumulative dose–response curve to administration of halothane (0, 1, 3 and 5 %) performed after a baseline measurement of arteriolar diameter. Second curve (○) = cumulative dose–response curve to administration of halothane performed after a 15-min wash-out period during which the muscle was irrigated with drug-free Krebs’ solution. Halothane induced significant dose-dependent A2 and A3 dilatation (\( P < 0.0001 \)) which was not different between the two dose–response curves.

---

**Table 1** Halothane doses (mg litre\(^{-1}\)) in Krebs’ solution assessed by chromatography (mean (sd)). Number of samples for each concentration of halothane is seven. Halothane was not detected at a 0 % delivered concentration. The increase in the delivered concentration of halothane resulted in a significant increase in halothane concentration in Krebs’ solution.

<table>
<thead>
<tr>
<th>Delivered concn</th>
<th>Dose (mg litre(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>37.31 (7)</td>
</tr>
<tr>
<td>3%</td>
<td>67.1 (8)</td>
</tr>
<tr>
<td>5%</td>
<td>110.1 (1.2)</td>
</tr>
</tbody>
</table>
Microcirculatory effects of halothane

235

(interaction between factor “dose” and factor “absence or presence of inhibitor”, \( P < 0.0001 \) for each arteriolar order, when group C was compared with group MA). A similar result was observed with indomethacin (interaction between factor “dose” and factor “absence or presence of inhibitor”, \( P < 0.0001 \) for each arteriolar order, when group C was compared with group INDO).

Discussion

The main results of this study are that \textit{in vivo}, topically administered halothane in a concentration range of 0–5% produced dose-dependent dilatation of rat diaphragmatic arterioles and halothane-induced dilatation of diaphragmatic arterioles was abolished significantly by mefenamic acid and indomethacin, but not by LNA.

In this study, assessment of the role of nitric oxide and prostaglandins on the arteriolar responses of the diaphragm to halothane was performed indirectly using selective inhibitors of both pathways: LNA for nitric oxide and mefenamic acid and indomethacin for prostaglandins. A possible criticism of this design is that these agents may exert effects on diaphragmatic arterioles via mechanisms other than nitric oxide and prostaglandin synthesis inhibition, respectively. However, this is very unlikely. First, a large body of evidence has demonstrated that LNA is a potent and specific inhibitor of nitric oxide synthesis \textit{in vitro} \cite{7, 18} and \textit{in vivo} in different vascular regions \cite{19, 20}, including diaphragmatic vessels \cite{10, 21}. Furthermore, we demonstrated in a previous study that topically administered LNA, at the concentration used in this study, effectively blocked nitric oxide-mediated dilatation of diaphragmatic arterioles \cite{10}. Second, we found an inhibitory effect of two structurally different inhibitors of prostaglandin synthesis: mefenamic acid and indomethacin \cite{22}. Thus we are confident that the effects of these agents were related specifically to inhibition of the cyclo-oxygenase pathway.

The concentration of halothane in Krebs’ solution perfusing the diaphragm is the determinant of its effect on diaphragmatic arterioles. Therefore, we ensured that the increasing concentrations of halothane resulted in increasing concentrations of this agent in Krebs’ solution.

In this study halothane was administered topically, thus avoiding any significant effect of modification of arterial pressure. Consequently, the dilatation induced by this agent can be regarded as a direct relaxant effect on diaphragmatic arterioles. However, it must be noted that dilatation was observed only when halothane concentrations were greater than
1 %. This is in line with previous in vivo data showing that inhalation of halothane at 0.5–1 % failed to produce diaphragmatic A2 and A3 dilatation [4]. Furthermore, this confirms previous results of several in vitro studies showing that only halothane concentrations greater than 2 % diluted KCl- or phenylephrine-precontracted rat aorta and canine carotid and coronary rings immersed in Krebs’ solution [5, 23, 24]. Interestingly, Muldoon and co-workers [5] showed, in a canine femoral ring model, that 2 % halothane bubbled in Krebs’ solution induced significant contraction of these vessels. Although species differences could explain these controversial results, heterogeneity between the different vessels may also be involved. Absence of vasoconstriction of diaphragmatic arterioles during systemic [4] and topical administration of halothane may be related to the fact that properties of the diaphragmatic vessel may differ significantly from those of peripheral skeletal muscles [10].

Several mechanisms may explain diaphragmatic arteriolar dilatation induced by halothane. For example, it has been reported that halothane has a direct effect on smooth muscle calcium homeostasis and also causes endothelium-dependent relaxation [5]. In this study, we investigated this mechanism by assessing the role of two main vasodilator factors synthesized by the endothelium: nitric oxide and prostaglandins.

The absence of effect of LNA on halothane-induced A2 and A3 dilatation suggests that nitric oxide was not involved in the diaphragmatic arteriolar effects of halothane. In contrast, mfenamic acid and indomethacin induced complete inhibition of halothane-induced diaphragmatic arteriolar dilatation. As both agents increased arteriolar tone, suppression of halothane-induced dilatation may be related to this increase in smooth muscle vascular tone, which in turn may attenuate the relaxant effect of halothane. However, this possibility can be excluded as LNA, which induced a greater increase in arteriolar tone than mfenamic acid and indomethacin, did not modify the dilatation caused by halothane. In addition, complete inhibition of halothane-induced diaphragmatic A2 and A3 arteriolar dilatation by mfenamic acid and indomethacin demonstrated that the diaphragmatic arteriolar effect of halothane was mediated exclusively by prostaglandins. This result is in agreement with that reported by Stone and Johns [23] who showed that indomethacin attenuated the decrease in aortic ring tension observed with increasing doses of halothane. However, other effects of halothane, such as development of pulmonary vasoconstriction in dogs, are not mediated by prostaglandins [25]. It is interesting to note that prostaglandins contribute, together with nitric oxide, to modulation of diaphragmatic arteriolar tone [10], a phenomenon which has not been shown in other skeletal muscle microvessels [11, 12]. Thus it is possible to speculate that modulation of the prostaglandins pathway could be an important mechanism of action of pharmacological agents, such as halothane, in affecting the diaphragmatic microcirculation.

Acknowledgements
We are indebted to Claudine Peiffer MD, for her helpful comments. Supported by grants from Inserm, No. 895004 and Reseau Inserm, No. 49009.

References


