Halothane differentially decreases 5-hydroxytryptamine-induced contractions in normal and chronic hypoxic rat pulmonary arteries

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ABSTRACT

The mechanism of action of halothane is not fully understood in pulmonary circulation and especially in chronic hypertension models. As the 5-hydroxytryptamine (5-HT) pulmonary vasoconstrictor response increases in chronic hypoxic rat, halothane could differentially attenuate this vasoconstriction response on normoxic and chronic hypoxic rats. The effect of halothane on 5-HT-induced contractions on pulmonary arteries isolated from normoxic and chronic hypoxic rats was compared. Rings dissected from proximal pulmonary artery without endothelium were attached to a force transducer to record tone and placed in an organ chamber gassed either by air or air + halothane (1–5%). Contractions induced by (10–4 M) 5-HT were used to test the effect of halothane on rings isolated from normoxic and chronic hypoxic rats. 5-Hydroxytryptamine-mediated contractions were more sensitive to external calcium in normoxic than chronic hypoxic rings. In calcium-free solution, with verapamil or cadmium the amplitude of remaining 5-HT-induced contractions were greater in chronic hypoxic rings. Halothane (1–5%) decreased 5-HT-mediated contractions in normoxic and chronic hypoxic rings. The effect occurred with no change of pD2 for 5-HT and was more pronounced in normoxic rings. The effect of halothane on both rings was abolished in the absence of external calcium or in the presence of verapamil. In the presence of cadmium, 5% halothane had no effect on normoxic rings but still decreased the remaining 5-HT contraction on chronic hypoxic rings. The findings suggested that halothane decreased sarcolemmal calcium entry in pulmonary artery rings by a cadmium-sensitive pathway in normoxic rats and by a cadmium-insensitive pathway in chronic hypoxic rats.

Keywords cadmium, pulmonary hypertension, volatile anaesthetic.

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Prolonged alveolar hypoxia occurs in people living at high altitude and is the main characteristics of chronic obstructive lung disease. The principal consequence of chronic hypoxia (CH) is a sustained pulmonary vasoconstriction followed by vascular remodelling and pulmonary artery hypertension (PAHT). This subsequent PAHT leads to right ventricular hypertrophy, right heart failure and ultimately to death (Dawson 1984, Pierson 2000). Morphological changes associated with CH include hypertrophy and hyperplasia of smooth muscle cells and increased deposition of extracellular matrix components (Rabinovitch et al. 1979, Meyrick & Perkett 1989). Functional changes occur in the pulmonary vasculature as a consequence of CH, including changes in ion channel density and altered vasoreactivity in response to the mediator (Shimoda et al. 2000). Indeed, vasoconstrictor response and plasma level of 5-hydroxytryptamine (5-HT) are increased in PAHT (MacLean 1999).

The overall cardiovascular effect of halothane and other volatile anaesthetics is to decrease mean arterial pressure associated with peripheral vasodilation (Stevens & Kingston 1992). In the lung, halothane inhibits hypoxic pulmonary vasoconstriction by a direct action on pulmonary vascular smooth muscle cells (Marshall & Marshall 1990). The mechanism of action of halothane is, however, not fully understood in the pulmonary circulation and in chronic hypertension...
models. Whether halothane directly attenuates pulmonary artery vasoconstriction caused by 5-HT is not known; but if so, halothane could act by decreasing internal Ca\(^{2+}\). This could explain its vasodilating effect on the pulmonary circulation and have important clinical implications, especially in the pulmonary artery from chronic hypertension models in which the effect of halothane on 5-HT may be different.

The purpose of this study was to examine the direct effect of the volatile anaesthetic halothane on 5-HT-induced contractions of isolated pulmonary artery rings obtained from both normal and chronic hypoxic rats which developed pulmonary hypertension. We recorded the tone of isolated rings and compared the effects of halothane on the two populations of rings. We also determined the importance of external Ca\(^{2+}\) on 5-HT-induced contractions in the presence or in the absence of halothane.

A preliminary report of part of this study has been published in abstract form (DeCrescenzo et al. 2000).

**MATERIALS AND METHODS**

**Exposure to hypoxia**

All animal experiments were conducted according to the ethical standards of the Ministère Français de l’Agriculture. Control adult male Wistar rats (250 g) were placed in normobaric room air. Another group of adults was placed in a hypobaric chamber where the barometric pressure was monitored and held at 380 mmHg, the temperature was 24 °C and the estimated ambient partial pressure of O\(_2\) was 75 mmHg. This same value of oxygen tension in alveoli was considered as the threshold value to induce chronic hypoxic pulmonary hypertension (Anand 1994). All rats were provided with rat chow and water *ad libitum*. Rats were removed from the chamber after 3 weeks and were killed just before the study. Pulmonary hypertension was assessed by calculating the ratio of right ventricle (RV) and left ventricle + septum (LV + S) mass and by calculating mean pulmonary artery pressure. Rats exposed to 3 weeks of hypoxia showed increases in the ratio RV/(LV + S) from 0.30 ± 0.02 (*N* = 8) to 0.62 ± 0.11 (*N* = 15; *P* < 0.01). The increase of this ratio was associated by an increase of mean pulmonary artery pressure in rats exposed to hypoxia from 10.2 ± 2.2 mmHg (*N* = 6) to 27.6 ± 9.8 mmHg (*N* = 5).

**Tissue preparation**

Rats were anaesthetized with pentobarbital sodium (30 mg kg\(^{-1}\) i.p.) and heparinized (100 U). The thorax were opened and lungs rapidly removed and placed in cold (4 °C) physiological saline solution (PSS). Proximal intrapulmonary arterial branches were rapidly isolated and gently cleaned of parenchyma and adhering connective tissue under a dissection microscope. Ring segments ~3 mm in outer diameter and ~4 mm in length were cut from these vessels. All the rings were denuded of endothelium by gently rubbing the intimal surface with forceps. Endothelial destruction was determined in a series of experiments by assessing the disappearance of the relaxant response to the endothelium activator, acetylcholine. This technique was systematically repeated for all other experiments.

**Recording of mechanical activity**

Mechanical responses were measured by methods previously described (Vandier et al. 1997). Briefly, the arterial rings were attached between a rigid support and the arm of a force transducer (UF1 Pyoden control; Canterbury, UK) by slipping the ring over two stainless steel wires. One of the wires was anchored to the rigid support and the other was connected to the transducer. The rings were stretched and then underwent stress relaxation for an 80-min equilibration period to reach the residual passive tension. The protocol of the experiments to determine the optimum stress was as follows: a cumulative stress load was applied in a stepwise fashion and, after a period of stress relaxation for each stress, a high K\(^{+}\) (80 mM) was applied. Next, the initial stress relaxation was determined in order to obtain the optimal response to high K\(^{+}\). Contraction amplitudes were expressed in milli-Newtons (mN) and were measured from the resting tension.

**Solutions and drugs**

Physiological saline solution contained (in mM): NaCl 138.6, KCl 5.4, HEPES 10, MgCl\(_2\) 1.2, NaH\(_2\)PO\(_4\) 0.33, glucose 11, CaCl\(_2\) 1.8. The pH was adjusted to 7.4 with NaOH. The zero Ca\(^{2+}\) solution (0 Ca solution) was PSS with no Ca\(^{2+}\) and 1 mM EGTA added. The 5-HT (10\(^{-4}\) M), verapamil (10\(^{-5}\) M) and cadmium (10\(^{-7}\) M) were dissolved in PSS. The solution was bubbled with air (PO\(_2\) ~150 mmHg). All drugs used were from Sigma (Sigma-Aldrich Chimie, St-Quentin-Fallavier, France).

The volatile anaesthetic was delivered via calibrated agent-specific vaporizers (fluotec 3) in line with the air/gas aerating the solutions directly in the experimental chamber (4 mL). The halothane concentration (volume percentage) was between 1 and 5% at 37 °C.
Protocols

Effect of repeat applications of 5-HT. In order to see if successive applications of 10^{-4} M 5-HT desensitized pulmonary artery rings isolated from both chronic hypoxic (CH rings) and normoxic (NX rings) rat to 5-HT, six successive applications of 10^{-4} M 5-HT were applied separately by a wash out period of 30 min. Normally, no more than four successive applications were applied during the experiments.

The first contraction induced by the application of 5-HT was referred to as control contraction.

The role of external Ca^{2+} on the response of exogenous 5-HT. To assess the contribution of external Ca^{2+} influx to the 5-HT-induced contractions on pulmonary rings isolated from both chronic hypoxic and normoxic rat, 10^{-4} M 5-HT (concentration required for maximum response) was applied in 0 Ca solution (10 min before 5-HT), preceded and followed by an application of 5-HT in the presence of normal Ca^{2+} (1.8 mM).

The first contraction induced by application of 5-HT in normal Ca^{2+} was referred to as control contraction.

Effect of verapamil and cadmium on the response of exogenous 5-HT. The contribution of Ca^{2+} entry pathway and mainly L-type Ca^{2+} channel entry was examined on 5-HT-induced contraction in pulmonary rings isolated from chronic hypoxic and normoxic rat. An amount of 10^{-7} M 5-HT was applied as a solution containing 10^{-5} M verapamil or 10^{-4} M cadmium (10 min before), preceded and followed by an application of 10^{-4} M 5-HT in the presence of drugs. The first contraction induced by application of 5-HT in the absence of drugs was referred to as control contraction.

Effect of halothane on the response of exogenous 5-HT. Cumulative dose–response curves for 5-HT (10^{-7}–10^{-3} M) were performed in the absence or presence of 5% halothane (concentration which induced maximum effect). The halothane was applied directly to the bath solution 10 min before 5-HT (see below) for pulmonary rings isolated from chronic hypoxic and normoxic rat. The 5-HT-induced contractions were increased by adding aliquots as soon as the effect of the previous concentration had reached a plateau.

Effect of increasing concentrations of halothane on the response to exogenous 5-HT. Given that the 10^{-4} M 5-HT-induced contractions were not well maintained for a long period, a concentration of anaesthetic (1–5%) was applied to the strip 10 min before contractile response to 10^{-4} M 5-HT. The anaesthetic was not cumulatively applied during the experiments and two concentrations of anaesthetics were tested on each pulmonary artery ring isolated from both chronic hypoxic and normoxic rat.

The various concentrations of halothane (1–5%) were applied directly to the bath solution, 10 min before 10^{-4} M 5-HT-induced contractions. The two contractions resulting from two different concentrations of halothane were preceded and followed by an application of 10^{-4} M 5-HT in the absence of halothane.

The first contraction induced by application of 5-HT in the absence of halothane was referred to as control contraction.

Effect of halothane on the response of exogenous 5-HT in the absence of external Ca^{2+}. In order to assess if the vasodilation effect of halothane was still present on pulmonary rings, isolated from chronic hypoxic and normoxic rat, in the absence of external Ca^{2+} 10^{-4} M 5-HT was applied twice in 0 Ca solution. First in the absence and secondly in the presence of 5% halothane. The two contractions were preceded and followed by an application of 5-HT in the presence of normal Ca^{2+} (1.8 mM).

The first contraction induced by application of 5-HT in normal Ca^{2+} and in the absence of halothane was referred to as control contraction.

Effect of halothane on the response of exogenous 5-HT in the presence of verapamil or cadmium. Given that the above experiments revealed that the volatile anaesthetic had a vasodilation action that was blocked in the absence of external Ca^{2+}, the effects of halothane on verapamil and cadmium-treated rings were also examined. A 10^{-4} M 5-HT was applied twice in the presence of drugs, first in the absence and secondly in the presence of halothane (5%). These two contractions were preceded and followed by an application of 5-HT in the presence of normal Ca^{2+} (1.8 mM). Control experiments were also performed using the same protocol but in the absence of halothane. The first contraction induced by application of 5-HT in the absence of drugs and of halothane was referred to as control contraction.

Statistical analysis

The 5-HT-induced contractions were expressed as mean ± standard deviation (SD). The contractions were expressed as absolute active tension or as percentages compared with the control contraction induced by 5-HT for each different protocol.

Statistical analysis were performed between rings used the percentage of control 5-HT contraction in order to compensate for variations in muscle mass. For 5-HT dose–response measurements, the contractile data were normalized relative to the maximal response.
and then fitted to obtain pD$_2$ values (–log EC$_{50}$) for individual dose–response curves.

Data were fitted using the Boltzmann function: 
\[ y = A_2 + \frac{(A_1 - A_2)}{1 + \exp((x - \bar{x})/d))} \] 
with $A_1$ the basal tension (0%), $A_2$ the maximum tension recording (100%), $x$ the study 5-HT concentration, $\bar{x}$ the effective dose for 50% contraction and $d$ the slope factor. Statistical analysis was made by paired or unpaired Student’s $t$-tests or one-way analysis of variance (ANOVA) (for comparison between more than two means) followed by Student’s $t$-tests (when appropriate and for comparison between two means). The significance level was set at 5%.

The number of experiments (n) referred to the number of rings and N the number of animals.

All data were collected with a computerized data acquisition system using Genie software (ADVENTECH, Sunnyvale, CA, USA) and analysed using Origin software (MICROCAL SOFTWARE, Northampton, MA, USA).

RESULTS

Repeat applications of 5-HT

In order to test whether successive applications of $10^{-4}$ M 5-HT desensitized pulmonary artery rings, six successive applications of $10^{-4}$ M 5-HT were applied. In NX (n = 15, N = 4) and CH rings (n = 12, N = 3), the amplitude of five successive contractions was not significantly different from the first (one-way ANOVA, $P > 0.05$) (data not shown).

Experiments carried out on NX rings

The importance of external Ca$^{2+}$ on 5-HT-induced contractions in NX rings was determined. The removal of external Ca$^{2+}$ significantly (Table 1, $P < 0.05$) and reversibly decreased the amplitude of 5-HT-induced contraction in NX rings (see protocol in Materials and methods). The contribution of a Ca$^{2+}$ entry pathway was examined on 5-HT-induced contractions in the NX pulmonary rings using verapamil and cadmium. In these rings isolated from normoxic rats, the application of both $10^{-5}$ M verapamil and $10^{-4}$ M cadmium in the bath solution significantly ($P < 0.05$) decreased the amplitude of the contraction (Table 1). The action of verapamil and cadmium was not fully reversible in NX rings (data not shown). Using one-way ANOVA and unpaired $t$-test, it was also found that cadmium had the same effect and verapamil decreased the amplitude of contraction in NX rings more than when external calcium was removed.

The influence of halothane on contractions evoked by $10^{-4}$ M 5-HT on NX rings was tested. Halothane, applied 10 min before 5-HT, decreased the amplitude of 5-HT contractions. A typical example using 5% halothane has been given in Figure 1a. This effect was significant ($P < 0.05$) at each concentration used (Fig. 1b). In the range of concentration used (1–5%), the maximum effect of halothane was observed at 5%. This maximal concentration was then used for all subsequent experiments.

In normoxic tissue, wash out of halothane was accompanied by partial restoration of contractile force and the 5-HT contraction measured after each halothane concentration was between 60 and 74%.

In order to study the effect of halothane upon receptor sensitivity to 5-HT, dose–response curves in the presence and absence of halothane were produced (Fig. 2). The NX rings were submitted to successive increasing concentrations of 5-HT and then after 10 min of halothane exposure, the same protocol was repeated on the same ring. The mean pD$_2$ values for 5-HT in the presence and absence of halothane were not significantly different (5.2 ± 0.5 vs. 5.6 ± 0.4; $P > 0.05$).

| Table 1 The importance of external Ca$^{2+}$ on 5-Hydroxytryptamine (5-HT)-induced contractions in normoxic (NX) and chronic hypoxia (CH) rings |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| 5-HT-induced contraction (%) | 0 Ca (%) | $\varphi$, N | Verapamil (%) | $\varphi$, N | Cadmium (%) | $\varphi$, N |
| NX rings | 21.1 ± 7.6* | 12; 4 | 10.8 ± 5.1* | 5; 2 | 20.3 ± 11.3* | 8; 2 |
| CH rings | 42.5 ± 18.9* ** | 8; 4 | 52.5 ± 14.4* ** | 6; 2 | 62.3 ± 3.5* ** | 9; 3 |

This table shows the relative amplitude of 5-HT contraction in the absence of external Ca$^{2+}$ (0 Ca) or in the presence of $10^{-5}$ M verapamil or $10^{-4}$ M cadmium in NX and CH rings. The control 5-HT contraction was measured in saline solution and results expressed as percentage of maximal contraction in each condition (0 Ca or verapamil or cadmium). Resulted are expressed as mean ± SD. *$P < 0.05$ indicated significant difference using $t$-test within each population of rings. Comparison was made between control contraction (100%), referred to as the reference mean) and contraction in 0 Ca. The same experiment was also carried out within each population but in presence of verapamil or cadmium. **$P < 0.05$ indicated significant difference using unpaired $t$-test between each population and within each condition. For one condition (0 Ca for example) comparison was made between contractions obtained in NX and CH rings. The same comparison was also performed between the two populations but in presence of verapamil or cadmium.
In order to assess if the effect of halothane was because of a decrease of extracellular Ca\textsuperscript{2+} entry, experiments in the absence of external Ca\textsuperscript{2+} or in presence of verapamil or cadmium were performed. A typical example of result obtained using cadmium has been given in Figure 3a. In 14 NX rings, in the absence of external Ca\textsuperscript{2+}, 5-HT-induced contractions were not significantly different (P > 0.05) in the presence or absence of 5% halothane (Fig. 3b). Furthermore, verapamil (n = 8, N = 3) and cadmium (n = 11, N = 5) blocked the effect of halothane on NX rings and in the presence of these drugs, the 5-HT-induced contractions were not changed by the presence of 5% halothane (Fig. 3b).

Control experiments, using the same protocols, but in the absence of halothane revealed that the second 5-HT contraction evoked in 0 Ca solution or in presence of drugs was not significantly different from the first in NX and CH rings (data not shown).

In order to assess if the effect of halothane was because of a decrease of extracellular Ca\textsuperscript{2+} entry, experiments in the absence of external Ca\textsuperscript{2+} or in presence of verapamil or cadmium were performed. A typical example of result obtained using cadmium has been given in Figure 3a. In 14 NX rings (N = 4), and in the absence of external Ca\textsuperscript{2+}, 5-HT-induced contractions were not significantly different (P > 0.05) in the presence or absence of 5% halothane (Fig. 3b). Furthermore, verapamil (n = 8, N = 3) and cadmium (n = 11, N = 5) blocked the effect of halothane on NX rings and in the presence of these drugs, the 5-HT-induced contractions were not changed by the presence of 5% halothane (Fig. 3b).

Control experiments, using the same protocols, but in the absence of halothane revealed that the second 5-HT contraction evoked in 0 Ca solution or in presence of drugs was not significantly different from the first in NX and CH rings (data not shown).
Experiments carried out on CH rings

In order to test the influence of external Ca\(^{2+}\) entry on 5-HT-induced contractions in CH rings, experiments were performed in the absence of external Ca\(^{2+}\) or in the presence of verapamil or cadmium.

In CH rings, the removal of external Ca\(^{2+}\) and addition of verapamil or cadmium both significantly (\(P < 0.05\)) and reversibly decreased the amplitude of the 5-HT-induced contractions (Table 1). Using one-way ANOVA and unpaired \(t\)-test, it was found that verapamil had the same effect on 5-HT-induced contraction, whilst cadmium had less influence on decreasing the amplitude of contraction in CH rings compared with removal of external calcium. Furthermore, the remaining contractions, induced by 5-HT in 0 Ca solution or in the presence of verapamil or cadmium was significantly greater (\(P < 0.05\)) in CH rings than in NX rings (Table 1).

The influence of halothane on contractions evoked by 10\(^{-4}\) M 5-HT on CH rings was tested. On these CH rings, halothane (5%) applied 10 min before 5-HT, induced a decrease in the amplitude of 5-HT contractions (Fig. 4a). This effect was significant (\(P < 0.05\)) for concentrations of halothane >2% (Fig. 4b). A maximum effect of halothane was observed at 5% in the range of concentration used (1–5%). This maximum concentration was then used for all subsequent experiments.

In hypoxic tissue, wash out of halothane was accompanied by an increase of 5-HT contractions with values between 122 and 164% (an example has been given in Fig. 4a). Compared with NX rings, halothane was less effective than in CH rings and this was significant (\(P < 0.05\)) for concentrations of anaesthetic from 1 to 4% (Fig. 4b).

In order to study the effect of halothane upon receptor sensitivity to 5-HT dose–response curves in the presence and absence of halothane were produced (Fig. 5). The mean pD\(_{2}\) values for 5-HT in the presence and absence of halothane was not significantly different in these CH rings (4.7 ± 0.5 vs. 4.8 ± 0.9; \(P > 0.05\)).

In order to assess if the effect of halothane on CH rings was because of a decrease of extracellular Ca\(^{2+}\) entry experiments in the absence of external Ca\(^{2+}\) or in presence of verapamil or cadmium were performed. In 20 CH rings (\(N = 5\)), halothane had no significant effect on the contractions induced by 5-HT in the absence of external Ca\(^{2+}\) (Fig. 6b). Comparable results were observed in the presence of verapamil (\(n = 16, N = 5\)). In contrast, cadmium did not block the effect of halothane and 5-HT-induced contractions were significantly reduced (\(P < 0.05\)) on 12 CH rings (\(N = 4\)) in its presence (Fig. 6a, b). The result obtained in the presence of cadmium has been given in Figure 6a.

Control experiments for NX and CH rings, using same protocols but in the absence of halothane, revealed that the second 5-HT contraction evoked in 0 Ca solution or in presence of drugs was not significantly different from the first (data not shown).

**DISCUSSIONS**

The results showed that halothane decreased 5-HT-induced contractions in both NX and CH rings with a more pronounced effect on NX rings. Halothane acted through a cadmium-sensitive pathway in NX rings and another cadmium-insensitive pathway in CH rings.
The 5-HT is a potent vascular modulator existing in pulmonary circulation and thus plays an important role in regulating pulmonary vasoreactivity in physiological and pathological conditions (Egermayer et al. 1999, MacLean 1999). It constricts pulmonary artery (PA) mainly through the 5-HT2A receptors (Martin 1994, MacLean 1999) and by releasing Ca^{2+} from intracellular stores and promoting Ca^{2+} influx through Ca^{2+} channels in PA smooth muscle cells (Yuan et al. 1997).

This work has demonstrated that a larger part of the contraction-induced by 5-HT was because of an increase of intracellular Ca^{2+} through external Ca^{2+} entry in NX rings than in CH rings. The precise mechanisms involved in Ca^{2+} entry in response to 5-HT in PA are unknown and Ca^{2+} can enter the cell through both voltage-dependent Ca^{2+} channels and receptor-activated Ca^{2+} permeable channels.

Nevertheless, the amplitudes of 5-HT-induced contractions were found to be similar in the presence of 10^{-4} M cadmium and after the removal of external Ca^{2+} in NX rings. At such concentration cadmium totally blocked voltage-dependent Ca^{2+} channels (Clapp & Gurney 1991) in PA cells and higher concentrations were necessary to block receptor-activated Ca^{2+} permeable channels (Rüegg et al. 1989). From the results of the present work, it was speculated that 5-HT-induced contractions in NX rings involved Ca^{2+} entry through voltage-dependent Ca^{2+} channels and probably L-type Ca^{2+} channels which are positively regulated by 5-HT (Hirakawa et al. 1995).

To support the notion, the effect of another voltage-dependent Ca^{2+} channel blocker verapamil (10^{-5} M) was tested. This blocker also decreased the 5-HT-induced NX ring contractions but with more effect than the removal of external Ca^{2+} or cadmium. This can be explained by another non-specific action of verapamil, which can also act at the level of G proteins and phospholipase C (PLC) (Kobayashi et al. 1991).

Nevertheless, in CH rings, 5-HT-induced contractions were less sensitive to external Ca^{2+}, cadmium or verapamil, with remaining contractions around 50% for each condition. This showed that 5-HT-induced contractions mainly by the release of Ca^{2+} from internal stores in CH rings and mainly by activation of sarcoplasmic Ca^{2+} pathway in NX rings. Furthermore, in CH rings, this Ca^{2+} entry was totally sensitive to verapamil but not to cadmium. This suggested that voltage-dependent Ca^{2+} channels (cadmium and verapamil-sensitive) were activated in 5-HT-induced contractions.

In addition, a cadmium-insensitive, but verapamil-sensitive action of 5HT was demonstrated.
sensitive, pathway was activated for sarcosomal calcium entry.

Effects of halothane on 5-HT-induced contractions
Halothane, at the clinical concentration use, decreased the amplitude of 5-HT-induced contractions of NX rings and CH rings. This effect was not surprising as it was well known that the overall effect of halothane and other volatile anaesthetics was to decrease the mean arterial pressure associated with peripheral vasodilation (Stevens & Kingston 1992).

The effect can be explained by a direct action of halothane on smooth muscle cells as has been described for coronary arteries in which halothane decreased 5-HT-evoked contractions (Witzeling et al. 1990).

As halothane did not change the pD2 value for 5-HT this excluded a decrease of 5-HT receptor sensitivity. The mechanism of halothane on 5-HT-induced contractions is unknown and has not previously been described in PA, especially for the chronic hypertension model where we first demonstrated that CH rings were less sensitive to halothane than NX rings.

The work showed that the relaxing action of halothane in NX rings and CH rings was abolished by the removal of Ca2+ from the external solution and by the addition of verapamil. Whilst cadmium also abolished the effect of halothane on NX rings, this was not the case for CH rings. The work suggested that halothane decreased 5-HT-evoked contractions by an inhibitory action on voltage-dependent Ca2+ channels (cadmium- and verapamil-sensitive) and probably on L-type Ca2+ current in NX rings. This effect has previously been described for many cells including cardiac cells (Bosnjak et al. 1991, Eskinder et al. 1991) and systemic artery cells (Buljubasic et al. 1992a,b). Nevertheless, the effect has yet to be confirmed for isolated PA cells.

In CH rings, the removal of Ca2+ from the external solution and the presence of verapamil abolished the effect of halothane but the presence of cadmium did not. This suggested that in these rings halothane reduced a different sarcosomal calcium entry than in NX rings. It was demonstrated that this other pathway, cadmium-insensitive but verapamil-sensitive, was activated in CH rings. Verapamil also suppressed the action of halothane on both rings. This was not surprising in NX rings, assuming that halothane as well as 10−4 M cadmium decreased voltage-dependent Ca2+ channels. In CH rings, however, the effect was explained by a similar action of halothane and verapamil on a pathway other than voltage-dependent Ca2+ channels. This pathway may have been linked to intracellular biochemical events classically implied in this type of contraction. Indeed, both verapamil and halothane are known to suppress inositol phosphate formation probably at the level of G proteins and PLC (Kobayashi et al. 1991, Sill et al. 1991, Ozhan et al. 1994, Namba & Tsuchida 1996).

The effect of halothane on myofilament calcium sensitivity cannot be excluded. However, Akata & Boyle (1995) found that halothane only modestly decreased sustained force induced by free Ca2+ in skinned rat mesenteric artery.

In conclusion, halothane decreased 5-HT-induced contractions in isolated PA by a direct action on smooth muscle cell membrane without direct modification of the internal Ca2+ store in both NX and CH rings. This effect was more substantial in NX rings compared with CH rings and involved different mechanisms. Halothane decreased 5-HT contractions in normal rats by inhibition of cadmium-sensitive pathways and by a cadmium-insensitive pathway in chronic hypoxic rats. Therefore, in patients with chronic pulmonary hypertension, the PA vasomotoricity, regulated by agonist-receptor induced tone modulation, could be different during volatile-anaesthesia compared with patients without pulmonary hypertension.

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