

Effects of Isoflurane and Halothane on Contractility and the Cyclic 3', 5'-Adenosine Monophosphate System in the Rat Aorta

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The effects of isoflurane and halothane on the contractility and on the cyclic 3', 5'-adenosine monophosphate (cAMP) system of the vascular smooth muscle of rat aorta were studied *in vitro*. Isoflurane and halothane inhibited phenylephrine-induced aortic contraction in a dose-dependent manner. Halothane-induced relaxation was greater than that induced by isoflurane. One and 2 per cent halothane caused approximately the same degree of relaxation as 4 and 6 per cent isoflurane, respectively. Propranolol (10^{-8} M) failed to block this action. Isoflurane and halothane also stimulated the formation of cyclic AMP. In the aorta pre-labeled with ^{14}C -adenine, isoflurane in concentrations of 2, 4, 6, and 8 per cent increased the labeled cAMP/ATP ratio 10, 17, 41, and 72 per cent, respectively. Halothane in concentrations of 1, 2, and 5 per cent increased the labeled cyclic AMP/ATP ratio 19, 37, and 58 per cent, respectively. Only the increases with 6 and 8 per cent isoflurane and with 2 and 5 per cent halothane were statistically significant. The anesthetic-induced increase in the labeled cAMP/ATP ratio was not altered by propranolol (10^{-8} M). Isoflurane and halothane did not change the phosphodiesterase activity. These results support the hypothesis that the cardiovascular depression during isoflurane and halothane anesthesia results, at least in part, from their direct relaxing action on vascular smooth muscle. The stimulation of cAMP formation may explain the mechanism of the anesthetic-induced relaxation. This action does not appear to be mediated through the beta-adrenergic receptors. (Key words: Anesthetics, volatile; isoflurane; Anesthetics, volatile; halothane; Arteries; halothane; Arteries; isoflurane; Sympa-

thetic nervous system; beta-adrenergic receptors; Metabolism; adenosine monophosphate; Metabolism; phosphodiesterase.)

ISOFLURANE (Forane $\text{\textcircled{S}}$) and halothane produce cardiovascular depression which can interfere with the body's homeostatic mechanisms. The fall in arterial pressure in man during isoflurane anesthesia seems primarily related to a decrease in peripheral vascular resistance with minimal depression of the cardiac output,^{1,2} whereas halothane causes both a decrease in peripheral vascular resistance and myocardial depression.³ The decrease in peripheral vascular resistance during halothane anesthesia results from actions at many sites, among which is a direct relaxing action on the vascular smooth muscle. It has been reported that halothane relaxes the rabbit aorta *in vitro*, probably by affecting the contractile process through an unknown mechanism.⁴ A similar study of isoflurane's action on the vascular smooth muscle, which could explain the decrease in the peripheral vascular resistance during isoflurane anesthesia, has not been published.

A dearth of knowledge about smooth muscle tone and contractility has hindered our attempts to explain the mechanism behind the direct relaxing action of anesthetics on vascular smooth muscle. Recent studies showed the importance of the cyclic 3',5'-adenosine monophosphate (cAMP) system in smooth muscle function.⁵⁻⁷ An increase in intracellular cAMP brought about by adenylyl cyclase stimulation, phosphodiesterase inhibition, or addition of exogenous cAMP is associated with decreased tone and contractility of smooth muscle. Also, halothane has been shown to produce uterine relaxation, and to stimulate both adenylyl cyclase

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§ Forane, trademark of Ohio Medical Products, a Division of Airco, Inc.

and phosphodiesterase, resulting in a net increase in cAMP in the rat uterus.⁸ Therefore, we hypothesized that the direct relaxing action of anesthetics on vascular smooth muscle might be related to the cAMP system. In order to test this hypothesis, we investigated the effects of isoflurane and halothane on the contractility of rat aorta *in vitro*, and their effects on the biosynthesis of cAMP and on the phosphodiesterase activity in this tissue.

Methods

Male Sprague-Dawley rats weighing 125–150 g were used. The rat was decapitated and the aorta rapidly removed and dissected free of fatty and connective tissue. For the study of contractility, spirally cut aortic strips were suspended under 4-g tension in a tissue bath containing Krebs-Ringer bicarbonate (KRB) solution at pH 7.4 and 37 C. A gas mixture consisting of 5 per cent carbon dioxide and 95 per cent oxygen was bubbled continuously through the solution. Contraction of the strip in response to increasing concentrations of phenylephrine (10^9 – 10^4 M) was measured isometrically with a force-displacement transducer (Grass FT 03) and a Beckman recorder. The strip was then exposed to isoflurane or halothane in various concentrations for 30 minutes and the response to phenylephrine determined again. The anesthetic was discontinued and the procedure repeated 30 minutes later. The phenylephrine dose-response curves during the control and recovery periods were averaged and compared with that obtained during exposure to the anesthetic.

In another series of experiments, propranolol (10^{-6} M) was added to the KRB solution prior to the determination of phenylephrine dose-response curves.

Cyclic AMP formation was studied using an assay modified after Shimizu *et al.*⁹ The aorta was incubated in 10 ml of KRB solution (pH 7.4, 37 C) containing 50 μ Ci of ¹⁴C-adenine (specific activity, 50 mCi/mM, International Chemical and Nuclear Corp., Irving, California) for one hour and then removed and bisected along its axis. Half of the labeled aorta was placed in 10 ml of KRB solution (pH 7.4, 37 C) that contained 10 mM theophylline (used to inhibit phosphodi-

esterase) and was in equilibrium with 5 per cent carbon dioxide and 95 per cent oxygen. The other half of the aorta was placed in a similar solution equilibrated with the anesthetic mixture. After 10 minutes of exposure to the anesthetic or the carrying gas, the tissue was removed, immediately frozen in liquid nitrogen, and homogenized in 0.6 M perchloric acid under cold conditions. The homogenate was centrifuged at $6,000 \times g$ for 10 minutes at 4 C and the supernatant was neutralized with 4 N potassium hydroxide. Cyclic AMP and ATP were then separated on an ion-exchange column (Dowex 50W-X4, H⁺ form, 35 mm \times 20 mm²) according to the method of Krishna.¹⁰ The radioactivity in the cyclic AMP and ATP fractions was counted in Bray's solution¹¹ using an Intertechnique liquid scintillation spectrometer. The result was expressed as a ratio between labeled cAMP and ATP. An increase in the ratio indicated accelerated cAMP formation or turnover.

In order to test whether the effect of the anesthetic was related to stimulation of beta-adrenergic receptors, we performed additional experiments in which propranolol (10^{-6} M) was added to the KRB-theophylline solution during exposure of the aortic strips to the anesthetic. This concentration of propranolol has been found to inhibit completely the increase in the labeled cAMP/ATP ratio induced by isoproterenol.¹²

The phosphodiesterase activity was determined using the method of Triner *et al.*¹³ Briefly, test tubes containing a mixture of 10 μ mol cAMP, 10 mM MgSO₄, and 2 μ Ci ³H-cAMP (specific activity, 24 Ci/mM, New England Nuclear Corp., Boston, Mass.) were equilibrated with the anesthetic mixture or the carrying gas for 15 minutes. The addition of a portion of the pooled homogenate of six rat aortas started the reaction. After eight minutes of incubation at 37 C, the reaction was stopped by boiling. Cyclic AMP was isolated on an ion-exchange resin column and its radioactivity determined as described above. A measure of the phosphodiesterase activity was obtained by comparing the change in labeled cyclic AMP in the mixture exposed to the anesthetic with that exposed to the carrier gas.

Isoflurane and halothane were vaporized using an Ohio Vernitrol Vaporizer and diluted

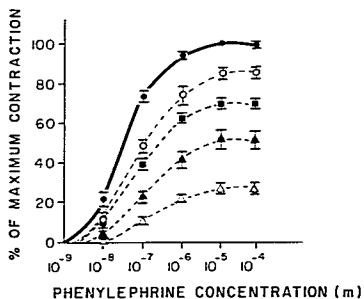


FIG. 1. Effects of 1, 2, 4, and 6 per cent halothane on phenylephrine-induced contractions of rat aorta *in vitro*. Each value represents the mean \pm SE of five experiments. Solid circles, control; open circles, 1 per cent halothane; squares, 2 per cent halothane; solid triangles, 4 per cent halothane; open triangles, 6 per cent halothane.

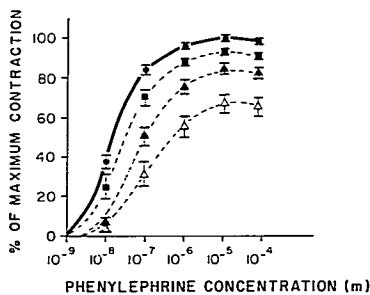


FIG. 2. Effects of 2, 4, and 6 per cent isoflurane on phenylephrine-induced contractions of rat aorta. Mean \pm SE of five experiments. Solid circles, control; solid squares, 2 per cent isoflurane; solid triangles, 4 per cent isoflurane; open triangles, 6 per cent isoflurane.

with a mixture of 5 per cent carbon dioxide and 95 per cent oxygen. The concentration of halothane was monitored by a Dräger Narkotest-M which had been calibrated by gas chromatography. The concentration of isoflurane was calculated using calibrated flowmeters and the vapor pressure curve of the anesthetic.[†]

Statistical comparisons between control and experimental data were performed using Student's *t* test and the *t* test for paired data. Values were held to be significant when $P < 0.05$. The data are presented as mean \pm SE.

Results

EFFECTS OF ISOFLURANE AND HALOTHANE ON THE CONTRACTILITY OF RAT AORTA

Isoflurane and halothane inhibited phenylephrine-induced aortic contraction in a dose-dependent manner. Figures 1 and 2 show the dose-response curves obtained during the control period and during the period of exposure to these anesthetics. Halothane-induced relaxation was greater than isoflurane-induced relaxation at the same concentration. One per cent halothane caused approximately the same degree of relaxation as 4 per cent

isoflurane, the maximum contraction produced by phenylephrine being decreased by 15 per cent. In the presence of 1 per cent halothane or 4 per cent isoflurane, the concentration of phenylephrine required to produce aortic contraction 50 per cent of maximum was approximately five times that needed during control and recovery periods.

Two per cent halothane further decreased the contractile response to phenylephrine, and this decrease was approximately the same as that produced by 6 per cent isoflurane. The maximum contraction during exposure to the anesthetic was reduced by 32 per cent (fig. 3), and the concentration of phenylephrine that caused a contraction 50 per cent of the control maximal contraction was 12 times greater during the exposure to the anesthetic than during the control period (2.5×10^{-7} M vs 2.0×10^{-8} M).

Propranolol (10^{-6} M) failed to reverse isoflurane- or halothane-induced relaxation of the rat aorta (figs. 4 and 5).

EFFECTS OF ISOFLURANE AND HALOTHANE ON CYCLIC AMP FORMATION AND PHOSPHODIESTERASE ACTIVITY IN RAT AORTA

Isoflurane and halothane stimulated the formation of cAMP in direct relation to concentration. Isoflurane in concentrations of 2, 4, 6, and 8 per cent increased the labeled

[†] Vitcha, J. F., personal communication.

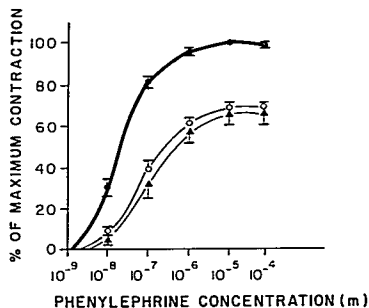


FIG. 3. Effects of 2 per cent halothane and 6 per cent isoflurane on phenylephrine-induced contractions in rat aorta. Data are derived from figures 1 and 2. Solid circles, control; open circles, 2 per cent halothane; solid triangles, 6 per cent isoflurane.

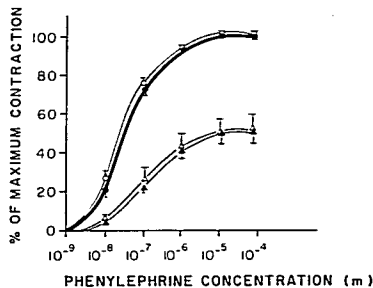


FIG. 4. Effect of propranolol on the halothane-induced inhibition of the contraction of rat aorta in response to phenylephrine. Mean \pm SE of five experiments. Open circles, propranolol (10^{-8} M); solid circles, control; open triangles, 4 per cent halothane plus propranolol (10^{-6} M); solid triangles, 4 per cent halothane.

cAMP/ATP ratio 10, 17, 41, and 72 per cent, respectively. Halothane in concentrations of 1, 2, and 5 per cent increased the cAMP/ATP ratio 19, 37, and 58 per cent, respectively. Only the increases with 6 and 8 per cent isoflurane and with 2 and 5 per cent halothane were statistically significant (tables 1 and 2).

The halothane-induced increase in the labeled cAMP/ATP ratio was greater than that produced by isoflurane at the same concentration. With 1 per cent halothane, the increase in the labeled cAMP/ATP ratio was approximately equal to that induced by 4 per cent isoflurane. Also, 2 per cent halothane and 6 per cent isoflurane increased the cAMP/ATP ratio to approximately the same extent.

Propranolol (10^{-6} M) had no effect on the anesthetic-induced increase in cAMP formation.

The basal activity of phosphodiesterase in the rat aortic homogenate (0.925 ± 0.03 μ mol of cAMP used/mg protein/minute) was not significantly affected by isoflurane (to 8 per cent) or halothane (to 6 per cent).

Discussion

The nature of the biochemical processes involved in smooth muscle contraction and relaxation are poorly understood. However,

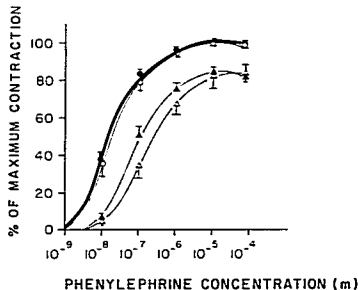


FIG. 5. Effect of propranolol on the isoflurane-induced inhibition of the contraction of rat aorta in response to phenylephrine. Mean \pm SE of five experiments. Solid circles, control; open circles, propranolol (10^{-8} M); solid triangles, 4 per cent isoflurane; open triangles, 4 per cent isoflurane plus propranolol (10^{-6} M).

recent evidence indicates that cAMP and the enzymes controlling its intracellular concentration (adenyl cyclase and phosphodiesterase) are intricately involved in the process of smooth muscle activity.^{5,6,7} The dibutyl derivative of cAMP produces striking decreases in the tone and contractility of vascular smooth muscle.⁵ Various drugs that induce vascular smooth muscle relaxation have been found to increase the concentration of cAMP,

TABLE 1. Effect of Halothane on cAMP Formation in Rat Aorta

	n	cAMP/ATP*	Per Cent Increase
Control	24	0.175 ± 0.010	18.8
Halothane, 1 per cent	24	0.208 ± 0.019	
Control	33	0.187 ± 0.011	37.4†
Halothane, 2 per cent	33	0.257 ± 0.014	
Control	21	0.202 ± 0.013	58.4†
Halothane, 5 per cent	21	0.320 ± 0.022	

* Mean ± SEM.

† P < 0.05.

TABLE 2. Effect of Isoflurane on cAMP Formation in Rat Aorta

	n	cAMP/ATP*	Per Cent Increase
Control	29	0.209 ± 0.010	10.5
Isoflurane, 2 per cent	29	0.231 ± 0.013	
Control	24	0.203 ± 0.017	16.7
Isoflurane, 4 per cent	24	0.237 ± 0.022	
Control	25	0.188 ± 0.013	41.5†
Isoflurane, 6 per cent	25	0.266 ± 0.018	
Control	11	0.184 ± 0.018	71.7†
Isoflurane, 8 per cent	11	0.316 ± 0.054	

* Mean ± SEM.

† P < 0.05.

TABLE 3. Effects of Isoflurane and Halothane on cAMP Formation at Equal Relaxing Concentrations in Rat Aorta

	Per Cent Inhibition of Phenylephrine-induced Contraction	Per Cent Increase in cAMP/ATP
Halothane, 2 per cent	30	37.4
Isoflurane, 6 per cent	32	41.5

either through inhibition of phosphodiesterase or through stimulation of adenylyl cyclase.⁵ Why an increased intracellular level of cAMP causes smooth muscle relaxation is unknown; however, Andersson has postulated a relationship between the concentrations of cAMP and calcium ions.⁷ According to Andersson, an increase in cAMP stimulates a calcium-accumulating process in the cell, which leads to a reduced calcium ion concentration in the vicinity of the contractile

elements. This reduced calcium ion concentration would then interfere with the interaction of the contractile elements and result in smooth muscle relaxation.

We have shown in this study that isoflurane and halothane have a direct relaxing action on the rat aorta *in vitro*, the results with halothane confirming the findings of Price and Price in the rabbit aorta⁴; also, isoflurane and halothane stimulate cAMP formation in a dose-dependent manner, while having no effect on the phosphodiesterase activity. The amount of anesthetic-induced relaxation by each agent is directly related to the extent of stimulation of intracellular cAMP formation; when isoflurane and halothane produce the same extent of relaxation of the aorta, the increases in cAMP formation induced by the corresponding concentrations of the anesthetics are comparable (table 3). These data, viewed with the foreknowledge of the proposed role of cAMP in smooth muscle contractility, support the hypothesis that the direct relaxing action of isoflurane and halothane is related to increased cAMP formation.

Propranolol, in a concentration shown to antagonize the action of isoproterenol, failed to block the relaxing action of isoflurane and halothane on the rat aorta, nor did it alter the anesthetic-induced stimulation of cAMP formation. Other investigators have presented evidence indicating that halothane may stimulate beta-adrenergic receptors.¹⁴⁻¹⁵ It has also been suggested that the cardiovascular changes seen with isoflurane anesthesia might be explained by beta-adrenergic receptor activation.¹ On the other hand, Yang *et al.* showed that the relaxing action of halothane on rat uterine smooth muscle is not related to beta-adrenergic stimulation.⁸ Our data with isoflurane and halothane in the rat aorta also indicate that the action of these anesthetics on the vascular smooth muscle is not mediated through beta-adrenergic receptors.

The question whether the increased cAMP formation is a result of adenylyl cyclase stimulation is not clearly answered. Since in this study we labeled the tissue with ¹⁴C-adenine (the immediate precursor of cAMP, ATP, is unable to diffuse across the cell membrane), we are unable to state categorically that the increased formation of cAMP was the result of stimulation of adenylyl cyclase.

However, it has been reported that halothane stimulates adenylyl cyclase in the rat uterus⁹ and adipose tissue,¹⁶ and that most drugs that cause increases in intracellular cAMP either stimulate adenylyl cyclase or inhibit phosphodiesterase. Since isoflurane and halothane have no effect on the activity of phosphodiesterase in the rat aorta, it is therefore likely that the increase in intracellular cAMP is the result of the stimulation of adenylyl cyclase.

Our biochemical and functional data offer an explanation for the direct smooth muscle-relaxing action of isoflurane and halothane, which contributes to the cardiovascular depression during administration of these agents in man. In addition, it is quite possible that anesthetic-induced changes in the cAMP system may explain the alterations in the responses to endogenously released and exogenously administered catecholamines and vasopressors. Other cardiovascular phenomena observed during anesthesia also may be related to changes in the cAMP system. However, until we gain more insight into the mechanism behind vascular smooth muscle tone and contractility, and the action of anesthetics on smooth muscle function, our understanding of these relationships will continue to be handicapped.

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