Halothane and isoflurane preferentially inhibit prostanoid-induced vasoconstriction of rat aorta

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In a previous study, we demonstrated that halothane and isoflurane inhibit binding of thromboxane A_2 to its receptors on human platelets and thus inhibit prostanoid-induced aggregation strongly. The aim of this study was to determine whether volatile anaesthetics inhibit prostanoid-induced vasoconstriction preferentially. Rat isolated aortic rings were mounted in organ baths and their isometric tension was measured. They were contracted with STA2 (a stable thromboxane A_2 analogue), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), phenylephrine, and 20 mM KCl, and then exposed to halothane (0.5–3%), isoflurane (0.5–3%), and sodium nitroprusside (SNP, 10^{-9}–3×10^{-7} M). Halothane (2–3%) and isoflurane (2–3%) induced greater relaxation of aortic rings precontracted with STA2 and $PGF_{2\alpha}$ than of those precontracted with phenylephrine ($P < 0.01$). Halothane induced greater relaxation of rings precontracted with KCl than phenylephrine only at 3%, whereas isoflurance relaxed rings precontracted with KCl more than those with phenylephrine at 0.5, 2 and 3% ($P < 0.05$). In contrast, SNP relaxed rings precontracted with $PGF_{2\alpha}$, KCl and phenylephrine equally, but induced smaller relaxations of those precontracted with STA2 ($P < 0.05$). We conclude that halothane and isoflurane inhibit prostanoid-induced vasoconstriction preferentially, possibly by interacting with prostanoid receptors.

Lors d'une étude antérieure, nous avons démontré que l'halothane et l'isoflurane inhibaient la liaison de la thomboxane A_2 avec ses récepteurs situés sur les plaquettes humaines et inhibaient fortement ainsi l'agrégation induite par les prostanoïdes. Cette étude vise à déterminer si les anesthésiques volatils inhibent la vasoconstriction induite par les prostanoïdes de façon préférentielle. Des anneaux isolés d'aorte de rat sont introduits dans des bains organiques et leur tension isométrique est mesurée. On les fait a'abord contracter avec du STA2 (un analogue stable de la thomboxane A_2), de la prostaglandine $F_{2\alpha}$ ($PGF_{2\alpha}$), de la phényléphrine, et 20 mM de KCl, et on les expose ensuite à l'halothane (0,5% à 3,0%), l'isoflurane (0,5%–0,3%) et au nitroprussiate de soude (SNP, 10^{-9}–3×10^{-7} M). L'halothane (2–3%) et l'isoflurane (2–3%) produisent une plus grande relaxation des anneaux aortiques précontractés avec la phényléphrine ($P < 0,01$). L'halothane produit, mais seulement à 3%, ie plus grande relaxation des anneaux précontractés avec .. KCL qu'avec la phényléphrine, alors que l'isoflurane aux concentrations de 0,5 2 et 3% relaxe les anneaux précontractés avec le KCl plus que ceux contractés avec la phényléphrine ($P < 0,05$). Par contre, le SNP relaxe également les anneaux précontractés avec la $PGF_{2\alpha}$, le KCl et la phényléphrine, mais produit une relaxation moins grande sur ceux qui ont été précontractés avec du STA2 ($P < 0,05$). Nous concluons que l'halothane et l'isoflurane inhibent la vasoconstriction induite par les prostanoïdes de façon préférentielle, possiblement par interaction avec les récepteurs prostanoïdes.

Key words
ANESTHETICS, VOLATILE: halothane, isoflurane;
ARTERIES: aorta;
HORMONES: prostaglandin.

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The magnitude of the relaxant action of volatile anaesthetics on isolated vascular smooth muscle varies depending upon the contractile agonist.[1,2] Halothane has been shown to interfere selectively with α_2-adrenoceptor-mediated vasoconstriction whilst having no effect on α_1-mediated responses of the rat aorta.[2] Halothane and isoflurane depress serotonin-evoked porcine coronary arterial contraction strongly, but have little effect upon that evoked by histamine.[1]

Recently, we demonstrated that halothane and isoflurane reduce receptor binding of thromboxane A_2 and abolish secondary aggregation of platelets, but have little

effect on epinephrine-induced primary aggregation.[3] The vascular smooth muscle of the rat aorta possesses both adrenoceptors and thromboxane A_2 receptors, stimulation of which induce vasoconstriction. However, the relative effects of volatile anaesthetics on the responses to these vasoconstrictors have yet to be elucidated.

The aim of this study was to determine whether volatile anaesthetics have a similar preferential inhibitory effect on thromboxane A_2 mediated responses in vascular smooth muscles to that observed in platelets, as the vascular smooth muscle and platelet thromboxane A_2 receptors have been suggested to be identical.[4] In this study, therefore, the relaxant effects of halothane and isoflurane on aortic rings precontracted with STA2, a thromboxane A_2 analogue, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), another vasoconstrictor prostaglandin, phenylephrine, and 20 mM KCl were compared. Also, the relaxant effects of sodium nitroprusside (SNP) on aortic rings precontracted with these agonists were compared.

Methods

The study was approved by the Kyoto University Animal Use Committee. Male Wistar rats weighing 250 to 615 g were anaesthetized with intraperitoneal injections of sodium pentobarbital (50 $mg \cdot kg^{-1}$) and killed by exsanguination. The chest of each rat was opened, the descending portion of the thoracic aorta was isolated, cut into rings approximately 3 mm long and the endothelium was removed mechanically by gently rubbing the luminal surface with a tungsten stick. Each ring was mounted on tungsten triangles (the lower was fixed and the other was attached to a force-displacement transducer (type 45196A, Nihondenki San-ei Co., Tokyo, Japan)) and suspended in 10 ml organ baths containing Krebs' bicarbonate solution of the following composition (mM): NaCl, 118.2: KCl, 4.6: $CaCl_2$, 2.5: KH_2PO_4, 1.2: $MgSO_4$, 1.2: $NaHCO_3$, 24.8 and glucose, 10. The bathing fluid was maintained at 37 ± 0.5° C and aerated with a mixture of 5% carbon dioxide and 95% oxygen to maintain the pH within the range of 7.35–7.45. The isometric tension of each aortic ring was displayed and recorded using a heat-writing oscillograph (Rectigraph 8K, Nihondenki San-ei Co., Japan) and the resting tension was adjusted to 1.0 g. Before starting the experiment, the rings were allowed to equilibrate for at least 60 min, during which the bath fluid was replaced with fresh fluid every 15 min. Halothane and isoflurane were introduced into the O_2-CO_2 mixture through agent specific vaporizers Fluotec3 and Fortec respectively (both Cyprane Keighley, England) and their concentrations were measured and adjusted using an Atom 303 anaesthetic agent monitor (Atom Co., Tokyo, Japan). In our previous study using the same methods of halothane and isoflurane administration, the concentration of halothane and isoflurane dissolved in the bathing fluid measured by gas chromatography reached equilibrium within 5–7 min.[5]

The rings were contracted with KCl 30 mM and then exposed to acetylcholine 10^{-6} M to confirm that the endothelium was absent. The rings were then washed with fresh Krebs' solution every ten minutes at least three times until they had reequilibrated, then constricted with STA2 ($1–3 \times 10^{-9}$ M), $PGF_{2\alpha}$ ($3–6 \times 10^{-6}$ M), phenylephrine ($1–3 \times 10^{-7}$ M), or KCl (20 mM). When the tension development reached maximal steady state, cumulative concentrations of halothane or isoflurane (both 0.5, 1.0, 2.0 and 3.0%) were introduced until a new steady state was reached (10–20 min). The reversibility of the anaesthetics' effects was tested by terminating anaesthetics administration. Sodium nitroprusside ($10^{-9}–3 \times 10^{-7}$ M) was added directly to the bath fluid cumulatively. When the responses to the anaesthetics or SNP had been determined, papaverine (10^{-4} M) was added to each tissue bath to attain maximal relaxation and the relaxations induced by the anaesthetics and SNP were expressed as percentages of this maximal relaxation.

Drugs used were halothane (Takeda Pharmaceutical Co., Osaka, Japan), isoflurane (Dainabot, Osaka, Japan), prostaglandin $F_{2\alpha}$ (Ono Pharmaceutical Co., Tokyo, Japan), phenylephrine (Sigma, St. Louis, Mo.), acetylcholine (Daiichi Pharmaceutical Co., Tokyo, Japan), sodium nitroprusside (Katayama Chemical Co., Osaka, Japan) and papaverine hydrochloride (Dainippon Pharmaceutical Co., Osaka, Japan). The STA2 was provided by Ono Pharmaceutical Co. The STA2 was dissolved in 1% methanol, the final concentration of which was <0.01%. All the other drugs, except halothane and isoflurane, were dissolved and diluted in distilled water as necessary and added directly to the bathing fluid; the volumes added were <150 μl (1.5% of the bath fluid).

All the results are expressed as means ± SEM. The data were analyzed statistically using one-way analysis of variance between groups and Scheffe's F test. Differences at $P < 0.05$ were considered significant. All statistical analyses were performed using StatviewII on an Apple Macintosh II computer.

Results

The absolute values of the mean precontraction induced by STA2 ($1–3 \times 10^{-9}$ M), $PGF_{2\alpha}$ ($3–6 \times 10^{-6}$ M), phenylephrine ($1–3 \times 10^{-7}$ M) and KCl (20 mM) were 1,799 ± 83 ($n = 23$), 1,423 ± 87 ($n = 23$), 1,488 ± 94 ($n = 21$), and 1,261 ± 92 mg ($n = 19$), respectively. The precontraction induced by STA2 was greater than those by $PGF_{2\alpha}$ and KCl ($P < 0.05$).

Halothane and isoflurane (0.5, 1.0, 2.0 and 3.0%) induced concentration-dependent relaxation of aortic rings

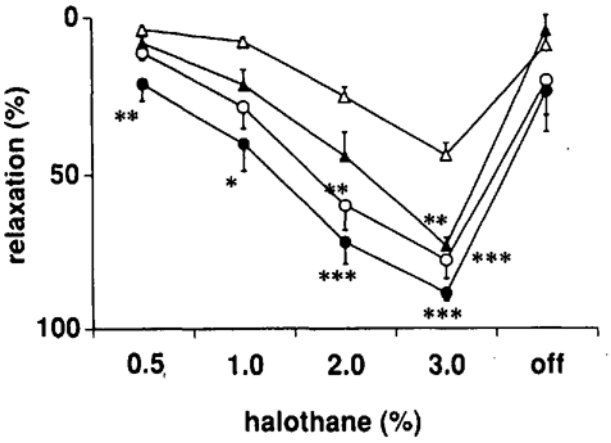

FIGURE 1 Effects of cumulative concentrations of halothane on aortic rings precontracted with STA2 (○), $PGF_{2\alpha}$ (●), phenylephrine (△), and KCl (▲). Relaxation induced by papaverine 10^{-4} M was taken as 100%. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus phenylephrine group.

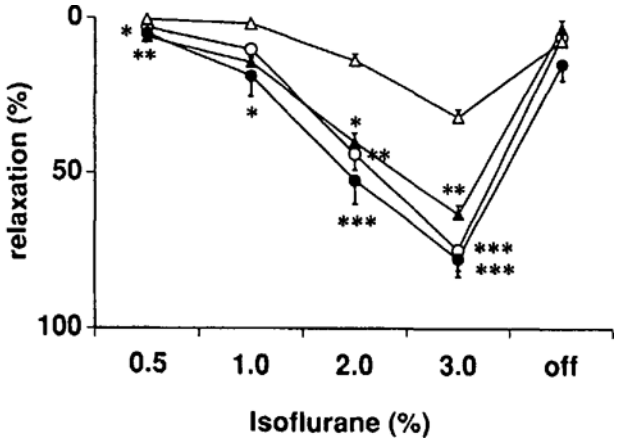

FIGURE 2 Effects of cumulative concentrations of isoflurane on aortic rings precontracted with STA2 (○), $PGF_{2\alpha}$ (●), phenylephrine (△), and KCl (▲). Relaxation induced by papaverine 10^{-4} M was taken as 100%. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus phenylephrine group.

precontracted with each agent. Halothane and isoflurane induced greater relaxation of aortic rings precontracted with STA2, $PGF_{2\alpha}$ and KCl than of those precontracted with phenylephrine (Figures 1 and 2). Although KCl-induced contraction appeared less affected by these anaesthetics than STA2- and $PGF_{2\alpha}$-induced contraction, their differences were not statistically significant. Within 20–40 min of terminating anaesthetic administration, the aortic rings regained the tension at a slightly lower level than that prior to anaeshetic administration; a typical recording is shown in Figure 3. The mean decrease in tension of aortic rings precontracted with STA2, $PGF_{2\alpha}$, phenylephrine and KCl relative to the maximal relaxation induced by papaverine after termination of 3% halothane administration were 20.0 ± 10.8%, 23.3 ± 12.6%, 8.8 ± 2.9%, and 4.2 ± 4.8% respectively (NS), and those after isoflurane were 5.8 ± 1.9, 14.8 ± 5.3, 7.1 ± 2.4 and 3.1 ± 2.6% (NS).

Sodium nitroprusside (10^{-9}–3×10^{-7} M) induced dose-dependent relaxation in aortic rings precontracted with each agonist. The mean maximal relaxation induced by SNP was lower in rings precontracted with STA2 than those with $PGF_{2\alpha}$, phenylephrine, and KCl (Figure 4). The mean maximal relaxant effect of SNP on rings precontracted with $PGF_{2\alpha}$, phenylephrine and KCl did not differ.

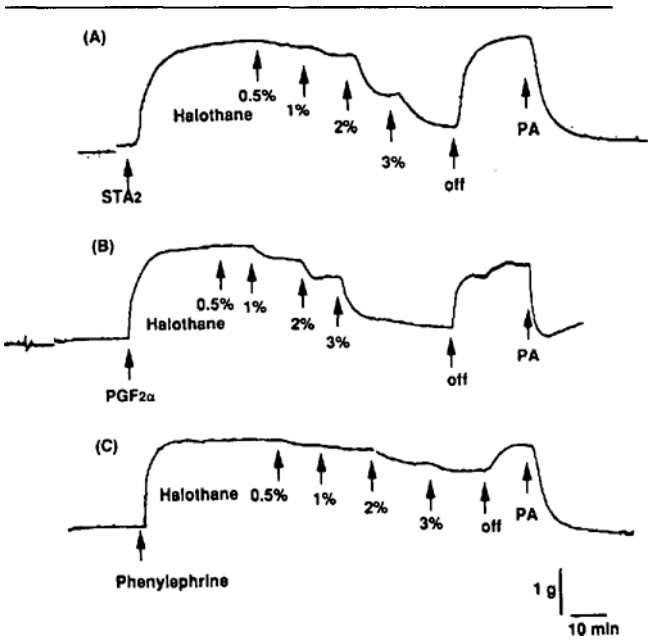

FIGURE 3 Actual recordings of changes in tension induced by cumulative concentrations of halothane (0.5, 1.0, 2.0, and 3.0%) of aortic rings precontracted with STA2 (A), $PGF_{2\alpha}$ (B), and phenylephrine (C). PA, 10^{-4} M papaverine.

Discussion

Contraction of vascular smooth muscle is induced by increased cytoplasmic Ca^{++} concentrations, which stimulate the actin-myosin contractile system by activating various regulatory proteins. The mechanism responsible for such increase in cytoplasmic Ca^{++} levels include Ca^{++} influx from the extracellular space and Ca^{++} release from its intracellular storage sites. Depolarization of vascular smooth muscle membranes by high concentrations of K^{+} induces vasoconstriction, primarily by Ca^{++} influx as a result of opening voltage-dependent Ca^{++} channels. Re-

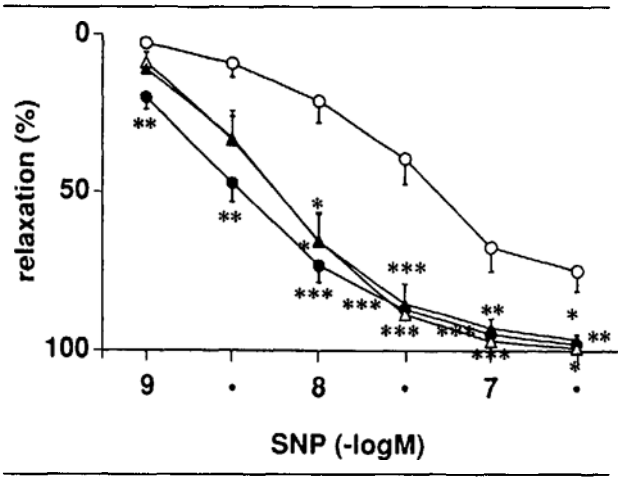

FIGURE 4 Effects of cumulative concentrations of SNP on aortic rings precontracted with STA2 (O), $PGF_{2\alpha}$ (●), phenylephrine (Δ), and KCl (▲). Relaxation induced by papaverine 10^{-4} M was taken as 100%. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ versus phenylephrine group.

ceptor stimulation by agonists such as norepinephrine, induces stimulation of phospholipase C, as well as Ca^{++} influx via receptor-operated Ca^{++} channels.[6] Phospholipase C stimulation causes hydrolysis of inositol phospholipid (PI turnover) which generates inositol 1,4,5-triphosphate and diacylglycerol; the former releases Ca^{++} from its intracellular Ca^{++} stores, such as the sacroplasmic reticulum, and the latter activates protein kinase C to sensitize the contractile proteins.[7,8]

Three types of vasoconstrictor agents were used in this study: (1) a high concentration of K^+, which induces depolarization that results in Ca^{++} influx from the extracellular fluid through voltage-dependent Ca^{++} channels, (2) phenylephrine, a selective α_1-adrenoceptor agonist and (3) prostanoids (STA2, a stable thromboxane A_2 analogue, and $PGF_{2\alpha}$). Prostanoids, including thromboxane A_2 and $PGF_{2\alpha}$, have been reported in common with phenylephrine, to exert their effects by binding to their specific receptors, resulting in Ca^{++} influx via receptor-operated Ca^{++} channels and release of Ca^{++} from its intracellular stores by stimulating PI turnover in vascular smooth muscle cells.[9]

In this study, we attempted to induce precontraction of similar magnitudes with STA2, $PGF_{2\alpha}$, phenylephrine and KCl. However, the contractions induced by $PGF_{2\alpha}$, phenylephrine, and KCl developed rapidly and leveled off within 10 min of administration, whereas those induced by STA2 developed and leveled off very slowly. Consequently, STA2 induced a greater maximal precontraction than the other agents.

We compared the relaxant effects of two volatile anaesthetics with those of SNP, which exerts its effects by increasing cyclic GMP in vascular smooth muscle cells directly without stimulating specific receptors.[10] Unlike halothane and isoflurane, SNP relaxed aortic rings precontracted with $PGF_{2\alpha}$, phenylephrine, and KCl equally (Figure 4). Similar results were obtained with dog isolated mesenteric arteries precontracted with $PGF_{2\alpha}$ and phenylephrine.[11] However, rat aortic rings precontracted with STA2 were affected less by SNP and this resistance may be due to the greater precontraction evoked by this agent. In fact, when aortic rings were precontracted with 40 mM KCl, which induced greater response than 20 mM, the SNP-induced relaxation was lower than that of rings precontracted with 20 mM KCl and comparable to that of rings precontracted with STA2 (data not shown).

We used two different types of receptor stimulator (1) phenylephrine, a selective α_1-adrenoceptor agonist, and (2) two vasoconstrictor prostanoids, STA2 abd $PGF_{2\alpha}$. Halothane and isoflurane both induced greater relaxation of aortic rings precontracted with STA2 and $PGF_{2\alpha}$ than with phenylephrine (Figures 2 and 3). This heterogeneity appeared to be a specific effect of the volatile anaesthetics, as SNP relaxed $PGF_{2\alpha}$- and phenylephrine-precontracted aortic rings to similar extents. Moreover, rings precontracted with STA2, which were rather resistant to the relaxant effect of SNP were as susceptible to the volatile anaesthetics as those precontracted with $PGF_{2\alpha}$.

The magnitude of the depressant action of volatile anaesthetics has been reported to vary depending upon the contractile agonists or contractile mechanisms involved. Bollen *et al.* demonstrated that prostanoid-induced contraction is more susceptible to halothane and isoflurane than K^+-induced contraction of the porcine coronary artery.[12] Larach *et al.* showed that halothane interfered with α_2-adrenoceptor-mediated vasoconstriction but had no effect on α_1-mediated vascular responses of the rat aorta.[2] Sill *et al.* reported that halothane and isoflurane depressed serotonin-evoked contractions strongly, but had little effect upon those evoked by histamine, in the porcine coronary artery.[1] The results of our study showed that halothane and isoflurane suppressed prostanoid-induced contractions strongly, but had less effect on those induced by phenylephrine, which also are receptor-mediated.

In the present study, KCl-induced contraction appeared to be less affected by halothane and isoflurane, but the trend was not statistically significant. The discrepancy between our results and those of Bollen *et al.* might be explained by differences in the species (rat aorta versus porcine coronary artery) or differences in the agonist used (20 mM KCl, STA2 and $PGF_{2\alpha}$ versus 30 mM KCl and U44069).[12]

The mechanisms whereby volatile anaesthetics exert differential effects on the responses to different agonists remain to be elucidated. The sites of action of halothane

and isoflurane appear to be receptors or cell membranes as the intracellular mechanisms involved in prostanoid- and phenylephrine-induced vascular smooth muscle contraction are similar. Sill *et al.* also observed differential effects of halothane and isoflurane on serotonin- and histamine-induced vascular contraction, which are both receptor-mediated and involve similar intracellular mechanisms.[9] Further support for this hypothesis is provided by our recent study in which halothane was found to reduce receptor binding of thromboxane A_2 and abolish secondary aggregation of human platelets, which is believed to be caused by thromboxane A_2, but had little effect on the primary aggregation induced by epinephrine, which is mediated by α_1- and α_2-adrenoceptors.[3]

Thromboxane A_2 is synthesized in various tissues and appears to induce some pathophysiological conditions, such as vasospasm, bronchoconstriction, and platelet aggregation,[13] and $PGF_{2\alpha}$ also contributes to vasoconstriction and bronchoconstriction as well. In addition, abundant $PGF_{2\alpha}$ is produced by the uterus, and it evokes uterine contractions.[14] Volatile anaesthetics possess bronchodilatory properties, depress uterine activity and prolong bleeding time.[15–17] Our *in vitro* finding that volatile anaesthetics preferentially inhibit prostanoid-induced response might in part explain the underlying mechanism of their characteristics in the clinical situation.

In summary, the relaxant effects of halothane and isoflurane on aortic rings precontracted with prostanoids and phenylephrine were compared; halothane and isoflurane depressed prostanoid-induced contractions more strongly than those induced by phenylephrine. Further investigations are required to elucidate the clinical importance of the inhibitory effects of volatile anaesthetics on prostanoid-evoked and/or mediated physiological and pharmacological responses.

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