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Interaction of Isoflurane and Sevoflurane with α- and β-adrenoceptor Stimulations in Rat Myocardium

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Background: **Halothane potentiates the positive inotropic effects of α- and β-adrenoceptor stimulations but impairs the positive lusitropic effect of β-adrenoceptor stimulations. However, the interactions of isoflurane and sevoflurane with α- and β-adrenoceptor stimulation have not been entirely defined.**

Methods: **The effects of 1 minimum alveolar concentration isoflurane and sevoflurane on the inotropic responses induced by phenylephrine (10^{-8} to 10^{-4} M) or isoproterenol (10^{-8} to 10^{-4} M) were studied in rat left ventricular papillary muscles *in vitro* (Krebs-Henseleit solution, 29°C; *p*H, 7.4; 0.5 mM calcium; stimulation frequency, 12 pulses/min). The positive lusitropic effects of α- and β-adrenoceptor stimulations were studied under isotonic and isometric conditions. Data are mean percentages of baseline ± SEM.**

Results: **In control groups, phenylephrine (134 ± 8%; $P < 0.05$) and isoproterenol (171 ± 7%; $P < 0.05$) induced a positive inotropic effect. Isoflurane enhanced the positive inotropic effects of phenylephrine (185 ± 10%; $P < 0.05$) and of isoproterenol (203 ± 11%; $P < 0.05$). Sevoflurane enhanced the positive inotropic effects of phenylephrine (187 ± 10%; $P < 0.05$) and of isoproterenol (228 ± 11%; $P < 0.05$). These potentiations were similar to those previously reported with halothane. Isoflurane and sevoflurane did not modify the positive lusitropic effects under low and high loads of isoproterenol.**

Conclusion: **Although isoflurane and sevoflurane have moderate negative inotropic effects, they potentiated the positive inotropic effects of α- and β-adrenoceptor stimulations but did not modify the positive lusitropic effects of β-adrenoceptor stimulation. (Key words: Contractility; heart; papillary muscle; relaxation; volatile anesthesia.)**

HALOTHANE enhances the positive inotropic effect of α- and β-adrenoceptor stimulations in isolated myocardium.[1,2] A growing body of evidence suggests that this potentiation is related to the interaction of halothane with G proteins coupled to the adrenoceptors.[3–5] Thus Schmidt *et al.*[6] have shown that halothane inhibits the function of the inhibitory G proteins probably by interfering with the effects of α- or βγ-subunits on the effector. Isoflurane is used more widely than halothane, and sevoflurane is a new volatile anesthetic that enables induction by mask in adults and children,[7] but their interactions with α- and β-adrenoceptors have not been entirely defined. Further, an *in vitro* study recently suggested that sevoflurane may disrupt the relation between the β-adrenoceptor and a stimulating G-protein, leading to a decrease in cyclic adenosine monophosphate synthesis.[8] In contrast, it has been reported that isoflurane potentiates the pulmonary vasodilator response to sympathetic β-adrenoceptor activation.[9]

Compared with α-adrenoceptor stimulation, β-adrenoceptor stimulation markedly enhances myocardial relaxation.[10] We have shown that halothane impairs the positive lusitropic effects of β-adrenoceptor stimulation in rat myocardium, probably by interfering with sarcoplasmic reticulum function.[2] Many studies have indi-

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cated that isoflurane and sevoflurane impair sarcoplasmic reticulum less than halothane.[2,11,12] However, the interactions of isoflurane and sevoflurane with the positive lusitropic effects of β-adrenoceptor stimulation have not been determined.

Therefore, we conducted an *in vitro* study to determine the interaction of isoflurane and sevoflurane with the inotropic and lusitropic responses of isolated rat myocardium to α- and β-adrenoceptor stimulations.

Materials and Methods

The animals were cared for according to the recommendations of the Helsinski Declaration, and the study was performed according to the regulations of the official edict of the French Ministry of Agriculture.

Experimental Protocol

After brief anesthesia with ether, the hearts were quickly removed from adult male Wistar rats weighing 250–300 g. Left ventricular papillary muscles were carefully excised and suspended vertically in a 200-ml jacketed reservoir with Krebs-Henseleit bicarbonate buffer solution containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.1 mM KH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and 4.5 mM glucose. The Krebs-Henseleit solution was prepared daily with highly purified water. The jacketed reservoir was maintained at 29°C with a thermostatic water circulator with continuous monitoring of the solution temperature using a temperature probe. Preparations were field stimulated at 12 pulses/min by two platinum electrodes with rectangular wave pulses lasting 5 ms just above threshold. The bathing solution was bubbled with 95% oxygen and 5% carbon dioxide, resulting in a *p*H of 7.4. After a 60-min stabilization period at the initial muscle length at the apex of the length-active isometric tension curve (L_{max}), papillary muscles recovered their optimal mechanical performance, which remained stable for several hours.

Suitable preparations were selected as previously described.[2,13] The control values of each mechanical parameter were recorded. Then the extracellular concentration of calcium ($[Ca^{2+}]o$) was decreased from 2.5 to 0.5 mM because rat myocardial contractility is nearly maximum at 2.5 mM, and thus it is difficult to quantify a positive inotropic response without decreasing $[Ca^{2+}]o$. Thereafter the inotropic response to either α- or β-adrenoceptor stimulations were studied in separate groups of papillary muscles in the absence or in the presence of isoflurane or sevoflurane at 1 minimum alveolar concentration (MAC). Because control values differ from one muscle to another, inotropic responses were expressed as a percentage of baseline values, as previously reported.[2]

In control groups, α-adrenoceptor stimulation was induced with cumulative concentrations of phenylephrine (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M) with propranolol (10^{-6} M). To study β-adrenoceptor stimulation, cumulative concentrations of isoproterenol (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M) with phentolamine (10^{-6} M) were used. The volume of drugs did not exceed 2% of the bath volume. All drugs were purchased from Sigma-Aldrich Chimie (L'isle d'Abeau Chesnes, France). Propranolol and phentolamine were added 15 min before phenylephrine or isoproterenol were introduced, respectively.

Because halogenated anesthetics are negative inotropic agents and thus could modify the inotropic reserve, we also studied the effects of α-adrenoceptor (n = 8) and β-adrenoceptor (n = 8) stimulations at a lower calcium (0.4 mM) concentration in separate groups of papillary muscles. Decreasing calcium concentration from 0.5 to 0.4 mM induced a negative inotropic effect that was of the same magnitude as those induced by 1 MAC isoflurane or sevoflurane. These experiments allowed us to verify that decreasing contractility *per se* does not induce a potentiation of of α- and β-adrenoceptor stimulations.

Administration of Halogenated Anesthetics

Isoflurane (Fortec 3; Cyprane Ltd., Keighley, UK) and sevoflurane (Sevotec 3; Ohmeda, West Yorkshire, UK) were added to the carbon dioxide and oxygen mixture with a calibrated vaporizer. The gas mixture bubbled continuously in the bathing solution. To minimize evaporation of anesthetics vapors, the jacketed reservoir was covered with a thin paraffin sheet. Anesthetic concentrations in the gas phase were monitored continuously using an infrared calibrated analyzer (Artema MM206; Taema, Antony, France). Isoflurane and sevoflurane concentrations used were 0.8 and 1.4 vol%, corresponding to 1 MAC in the adult rat at 29°C.[14–16] After a 30-min period of equilibration with halogenated anesthetics, the inotropic responses to either α- or β-adrenoceptor stimulations were studied in the same cumulative manner as in the control groups.

Electromagnetic Lever System and Recording

The electromagnetic lever system has been described previously.[17] Briefly, the load applied to the muscle was

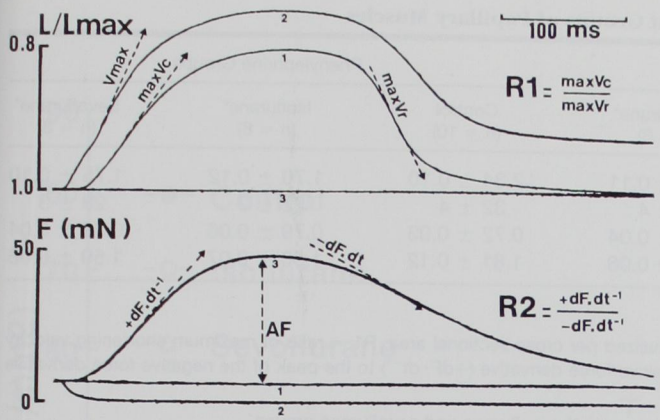

Fig. 1. Mechanical parameters of contraction and relaxation. (*Upper*) Muscle shortening length (L/L_{max}) plotted against time. (*Lower*) Force (F) plotted against time. Twitch 1 was loaded with preload only at L_{max}. Twitch 2 was loaded with the same preload as twitch 1 but was abruptly clamped to zero load with critical damping just after electrical stimulus; maximum unloaded shortening velocity (V_{max}) was determined from this twitch. Twitch 3 was fully isometric; isometric active force (AF) was determined from this twitch. Coefficient R1, the ratio of maximum shortening velocity ($_{max}Vc$) to maximum lengthening velocity ($_{max}Vr$), tests lusitropy under low load; coefficient R2, the ratio of the peak of the positive force derivative (+dF/dt) to the peak of the negative force derivative (−dF/dt), tests lusitropy under high load.

determined using a servomechanism-controlled current through the coil of an electromagnet. Muscular shortening induced a displacement of the lever, which modulated the light intensity of a photoelectric transducer. All analyses were made from digital records of force and length obtained with a computer, as previously described.[13]

Mechanical Parameters

Conventional mechanical parameters at L_{max} were calculated from three twitches (fig. 1). The first twitch was isotonic and was loaded with the preload corresponding to L_{max}. The second twitch was abruptly clamped to zero load just after the electrical stimulus. The muscle was released from preload to zero load with a critical damping to slow the first and rapid shortening overshoot resulting from the recoil of series passive elastic components, as previously reported.[18] The maximum unloaded shortening velocity (V_{max}) was determined from this twitch. The third twitch was fully isometric at L_{max}.

The mechanical parameters characterizing the contraction and relaxation phases and the coupling between contraction and relaxation are defined as follows (fig. 1).

Contraction Phase. We determined V_{max} using the zero-load clamp technique, maximum shortening velocity ($_{max}Vc$) of the twitch with preload only, maximum isometric active force normalized per cross-sectional area (AF); and the peak of the positive force derivative normalized per cross-sectional area (+dF/dt). V_{max} and AF tested the inotropic state under low (isotonic) and high (isometric) loads, respectively.

Relaxation Phase. We determined maximum lengthening velocity of the twitch with preload only ($_{max}Vr$) and the peak of the negative force derivative at L_{max} normalized per cross-sectional area (−dF/dt). These two parameters studied relaxation under low- and high-loading conditions, respectively. Because changes in the contraction phase induce coordinated changes in the relaxation phase, $_{max}Vr$ and −dF/dt cannot assess lusitropy,[2] and thus variations in contraction and relaxation must be considered simultaneously to quantify drug-induced changes in lusitropy. Therefore indexes of contraction–relaxation coupling have been developed.[19]

Contraction–Relaxation Coupling. Coefficient R1 = $_{max}Vc/_{max}Vr$ evaluated the coupling between contraction and relaxation under low load, and thus the lusitropy in a manner that is independent of inotropic changes.[2] Under isotonic conditions, the amplitude of sarcomere shortening is greater than that observed under isometric conditions.[20] Because of the lower sensitivity of myofilaments for calcium when cardiac muscle is markedly shortened under low load, relaxation proceeds more rapidly than contraction, apparently as a result of the rapid uptake of calcium by the sarcoplasmic reticulum. Thus, in rat myocardium, R1 tests sarcoplasmic reticulum uptake function. Coefficient R2 = (+dF/dt)/(−dF/dt) evaluated the coupling between contraction and relaxation under high load in a manner that less depends on inotropic changes.[2] When the muscle contracts isometrically, sarcomeres shorten very little.[20] Because of the higher sensitivity of myofilaments for calcium,[21] the time course of relaxation is determined by calcium unbinding from troponin C rather than by calcium sequestration by the sarcoplasmic reticulum. Thus R2 indirectly reflects myofilament calcium sensitivity.[10]

At the end of the study, the muscle cross-sectional area was calculated from the length and weight of papillary muscle, assuming a density of 1.

Statistical Analysis

Data are expressed as means ± SEM. Control values between groups were compared by analysis of variance.

Table 1. Baseline Values of Mechanical Parameters in the Different Groups of Papillary Muscles

	Isoproterenol Groups			Phenylephrine Groups		
	Control (n = 10)	Isoflurane* (n = 8)	Sevoflurane* (n = 8)	Control (n = 10)	Isoflurane* (n = 8)	Sevoflurane* (n = 8)
V_{max} ($L_{max} \cdot s^{-1}$)	2.20 ± 0.15	2.29 ± 0.30	1.79 ± 0.11	2.34 ± 0.10	1.70 ± 0.12	1.75 ± 0.10
AF ($mN \cdot mm^{-2}$)	35 ± 5	29 ± 6	31 ± 4	32 ± 4	22 ± 3	28 ± 3
R1	0.68 ± 0.03	0.75 ± 0.05	0.80 ± 0.04	0.72 ± 0.03	0.79 ± 0.06	0.79 ± 0.04
R2	1.66 ± 0.06	1.51 ± 0.08	1.68 ± 0.06	1.81 ± 0.12	1.50 ± 0.07	1.59 ± 0.06

Data are mean ± SEM.

V_{max} = maximum unloaded shortening velocity; AF = isometric active force normalized per cross-sectional area; R1 = ratio of maximum shortening velocity ($_{max}Vc$) to maximum lengthening velocity ($_{max}Vr$); R2 = ratio of the peak of the positive force derivative ($+dF \cdot dt^{-1}$) to the peak of the negative force derivative ($-dF \cdot dt^{-1}$).

* Baseline values correspond to those obtained after halogenated anesthetics exposure in the isoflurane and sevoflurane groups.

Concentration-response curves were determined by fitting the data to Hill's sigmoid pharmacologic model, according to the following equation:

$$Eff_0 = Eff_{max} \cdot C \cdot (C_{50} + C)^{-1}$$

in which Eff_0 is the observed effect at the C concentration, Eff_{max} the maximum effect, and C_{50} the concentration that results in 50% of Eff_{max}. Iterative nonlinear least squares regression curve fitting was used to obtain the best fit (Matlab 1.2c software; The MathWorks, South Natick, MA). Comparison of several means was performed using analysis of variance and the Newman-Keuls test. Comparison of the relations between R1 or R2 (lusitropic effect) and AF (inotropic effect) between groups was performed using a multivariate analysis of variance, as previously reported.[2] All probability values were two tailed, and a probability value <0.05 was required to reject the null hypothesis. Statistical analysis was performed on a computer using NCSS 6.0 software (Statistical Solutions Ltd., Cork, Ireland).

Results

Sixty-nine left ventricular papillary muscles were studied. The mean L_{max} was 5.9 ± 0.2 mm; the mean cross-sectional area was 0.59 ± 0.03 mm^2; the mean ratio of resting force to total force was 0.08 ± 0.01; and the mean R1 was 0.71 ± 0.01. A decrease in contractility was observed as $[Ca^{2+}]o$ was decreased from 2.5 to 0.5 mM. The decrease in V_{max} (64 ± 2% of the value at $[Ca^{2+}]o$ of 2.5 mM) and AF (48 ± 2% of the value at $[Ca^{2+}]o$ of 2.5 mM) were consistent with previous reports.[2,13] No significant differences in baseline values were observed between groups (table 1).

In the control groups, phenylephrine and isoproterenol induced a positive inotropic effect as shown by the significant increase in V_{max} and AF (figs. 2 and 3; table 2). The positive inotropic effect of isoproterenol was greater than that observed with phenylephrine. These results correspond with those previously reported in rat myocardium.[2]

Figure 2 shows the absolute values of AF recorded in response to increasing concentrations of phenylephrine and isoproterenol, under control conditions, and in the presence of 1 MAC isoflurane and sevoflurane. Despite the moderate negative inotropic effects induced by isoflurane (AF, 81 ± 2% of baseline; n = 16) and sevoflurane (AF, 92 ± 8% of baseline; n = 16), absolute baseline values of inotropic parameters were not significantly different between the control groups and the isoflurane or sevoflurane groups (table 1). However, because the baseline value of AF differs markedly from one papillary muscle to another, it is difficult to show a pharmacologic effect when using absolute values (fig. 2). When measured as a percentage of baseline halogenated anesthetic response, the positive inotropic effects of phenylephrine (fig. 3A) and isoproterenol (fig. 3B) were enhanced in the presence of 1 MAC isoflurane or sevoflurane compared with the control groups (table 2). Median effective concentration values were not significantly different between groups, indicating no significant shift between the concentration-response curves (table 2). In contrast, decreasing contractility by decreasing the calcium concentration (from 0.5 to 0.4 mM) did not induce any significant potentiation of α- and β-adrenoceptor stimulations (fig. 4). The decrease in AF obtained by the decrease in calcium concentration (78 ± 4% of baseline) was not significantly different

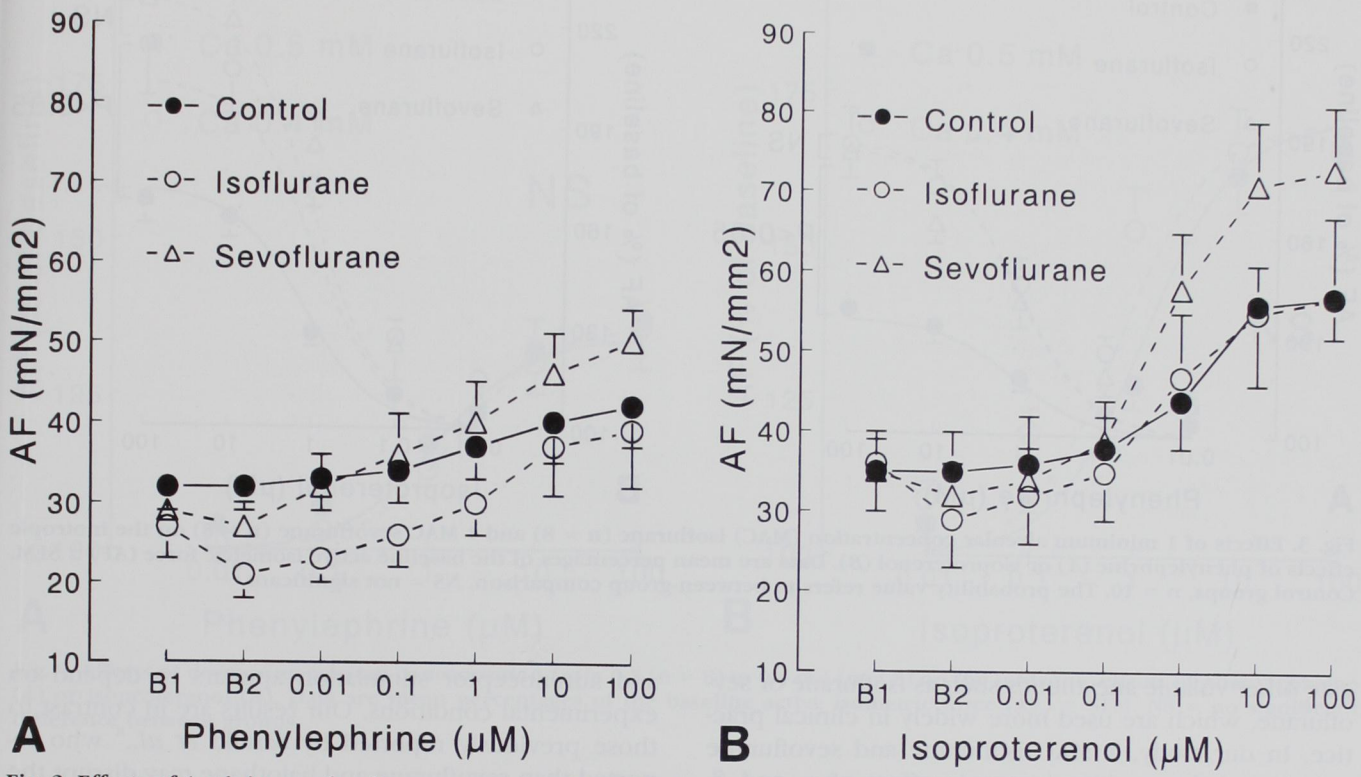

Fig. 2. Effects of 1 minimum alveolar concentration (MAC) isoflurane (n = 8) and 1 MAC sevoflurane (n = 8) on the inotropic effects of phenylephrine (*A*) or isoproterenol (*B*). Data are absolute mean values of the active isometric force (AF) ± SEM. Control groups, n = 10. B1 corresponds to baseline values at a calcium concentration of 0.5 mM. B2 corresponds to baseline values after halogenated anesthetic exposure in the isoflurane and sevoflurane groups. Because the baseline value of AF differs markedly among papillary muscles, it is difficult to show a pharmacologic effect when using absolute values.

from that induced by 1 MAC isoflurane (81 ± 2%) but was significantly greater than that induced by 1 MAC sevoflurane (92 ± 8%; $P < 0.05$).

Isoproterenol induced a marked positive lusitropic effect under low and high loads, as shown by the significant decreases in R1 and R2 (table 3). This result was consistent with those previously reported in the rat myocardium.[2,10] In the presence of 1 MAC of isoflurane or sevoflurane, isoproterenol still induced significant decreases in R1 and R2 (table 3), and the magnitude of these effects were similar to those observed in the control group (table 3). Nevertheless, the lusitropic effects of β-adrenoceptor stimulation are highly correlated to its inotropic effect, and, as we stated before, isoflurane and sevoflurane potentiated the inotropic effect of β-adrenoceptor stimulation. Consequently, we studied the relations between AF (inotropic) and R1 or R2 (lusitropy) to assess precisely the possible interaction of halogenated anesthetics with the lusitropic effects of β-adrenoceptor stimulation, as previously reported.[2] As figure 5 shows, isoflurane and sevoflurane did not significantly modify these relations.

Discussion

The main finding of this study is that isoflurane and sevoflurane enhanced the positive inotropic effects of α- and β-adrenoceptor stimulations. Further, in contrast to halothane,[2] isoflurane and sevoflurane did not modify the positive lusitropic effects of β-adrenoceptor stimulation.

Halothane has been shown to potentiate the positive inotropic effect of α- and β-adrenoceptors in rat ventricular myocardium and in human atrial and ventricular myocardium.[1,2] However, these studies did not investi-

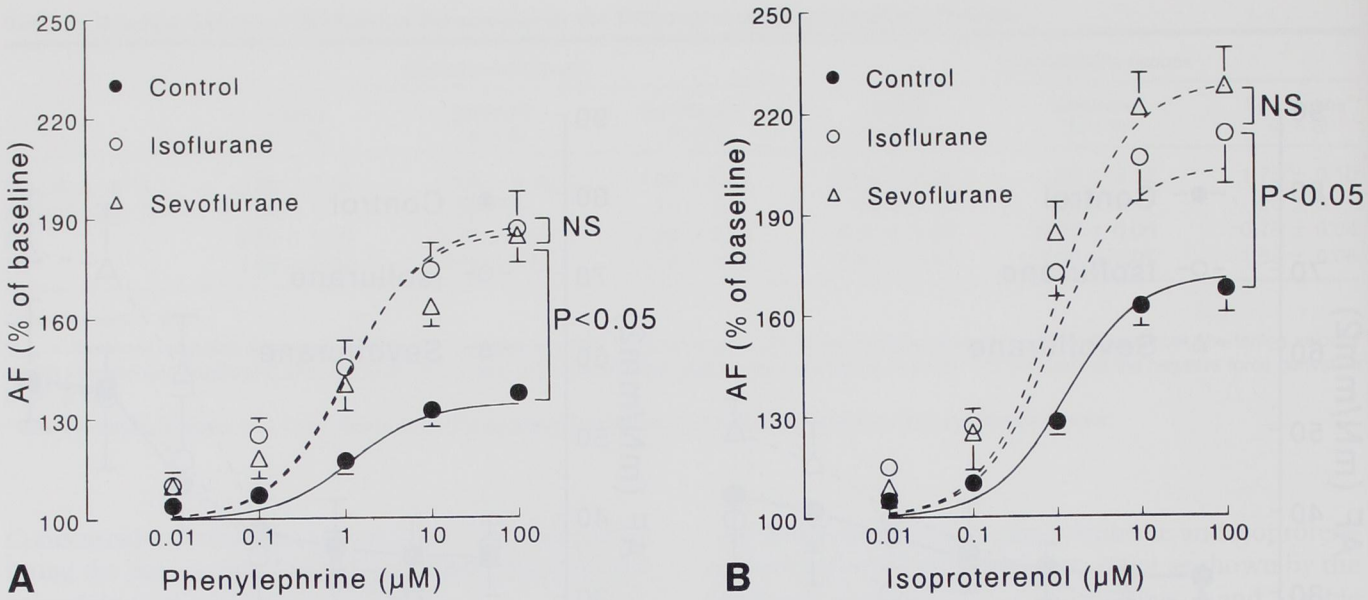

Fig. 3. Effects of 1 minimum alveolar concentration (MAC) isoflurane (n = 8) and 1 MAC sevoflurane (n = 8) on the inotropic effects of phenylephrine (*A*) or isoproterenol (*B*). Data are mean percentages of the baseline active isometric force (AF) ± SEM. Control groups, n = 10. The probability value refers to between-group comparison. NS = not significant.

gate other volatile anesthetics such as isoflurane or sevoflurane, which are used more widely in clinical practice. In our study, 1 MAC isoflurane and sevoflurane potentiated the positive inotropic effect of α- and β-adrenoceptor stimulations, and the magnitude of these potentiations were comparable to those previously observed in the presence of 1 MAC halothane.[2] Nevertheless, the interaction between halogenated anesthetics and adrenoceptor stimulation appears to depend on experimental conditions. Our results are in contrast to those previously reported by Sanuki *et al.*,[8] who reported than sevoflurane and halothane may disrupt the relation between the β-adrenoceptor and the G_s protein, leading to a decrease in cyclic adenosine monophosphate synthesis in the rat myocardial membrane. In contrast, more recent results suggest that halothane

Table 2. Effects of Isoflurane (1 MAC) and Sevoflurane (1 MAC) on Inotropic Response to α- or β-Adrenoceptor Stimulation

Mechanical Parameters	Concentration Effect Curve Parameters	Control Groups	Isoflurane Groups	Sevoflurane Groups
Phenylephrine groups		(n = 10)	(n = 8)	(n = 8)
V_{max}	Eff_{max} (%)	126 ± 3*	142 ± 8*†	146 ± 6*†
	EC_{50} (μM)	1.29 ± 0.70	0.92 ± 0.41	1.08 ± 0.56
AF	Eff_{max} (%)	134 ± 8*	185 ± 10*†	187 ± 10*†
	EC_{50} (μM)	1.17 ± 0.70	1.03 ± 0.31	1.13 ± 0.68
Isoproterenol groups		(n = 10)	(n = 8)	(n = 8)
V_{max}	Eff_{max} (%)	169 ± 5*	196 ± 8*†	205 ± 7*†
	EC_{50} (μM)	1.20 ± 0.21	0.99 ± 0.17	0.93 ± 0.16
AF	Eff_{max} (%)	171 ± 7*	203 ± 11*†	228 ± 11*†
	EC_{50} (μM)	1.26 ± 0.09	0.92 ± 0.14	1.01 ± 0.09

Data are mean ± SEM.

V_{max} = maximum unloaded shortening velocity; AF = isometric active force normalized per cross-sectional area; Eff_{max} = maximum effect; EC_{50} = concentration that results in 50% of Eff_{max}.

* $P < 0.05$ *versus* baseline.

† $P < 0.05$ *versus* control group.

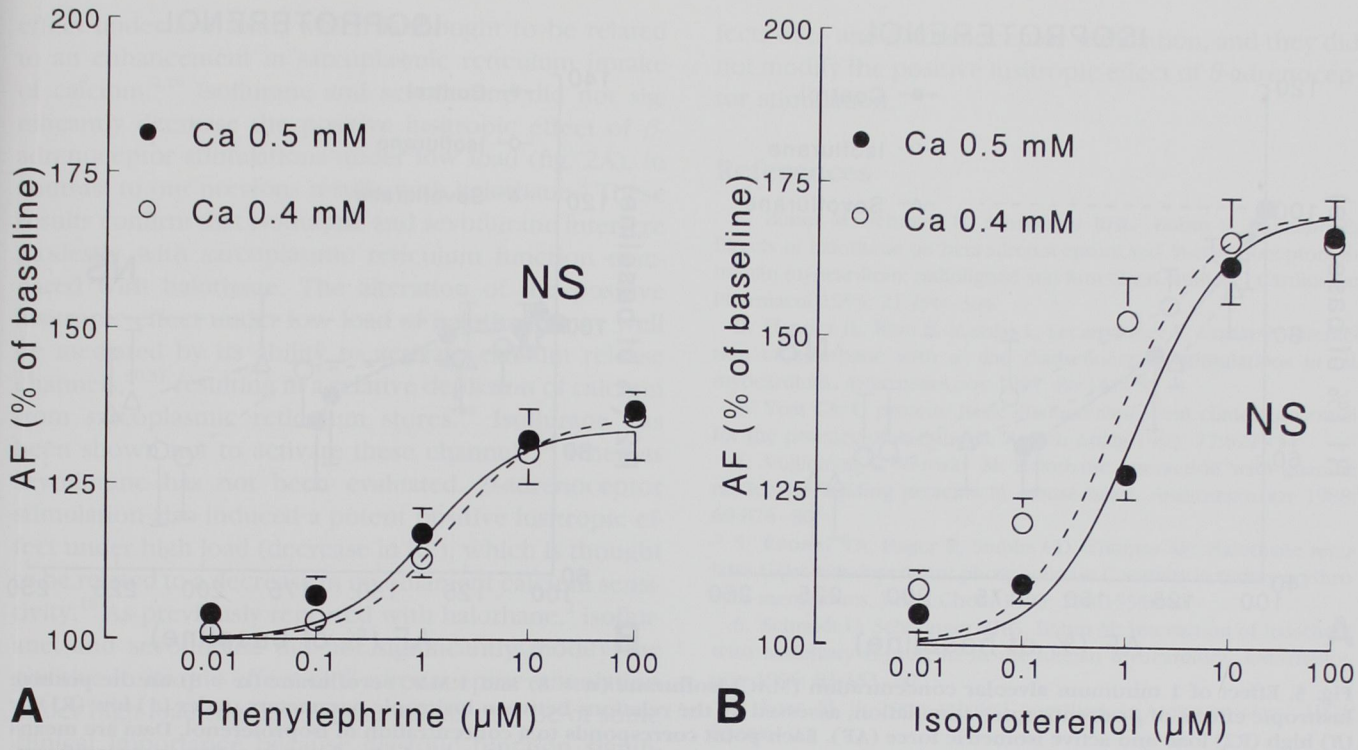

Fig. 4. Effects of decreasing calcium concentration from 0.5 (n = 8) to 0.4 mM (n = 8) on the inotropic effects of phenylephrine (*A*) or isoproterenol (*B*). Data are mean percentages of the baseline active isometric force (AF) ± SEM. NS = no significant difference between groups.

actually potentiates adrenoceptor stimulation.[1,2,6,22] Species differences are not likely to explain this discrepancy because potentiation of adrenoceptor stimulation was observed in rat[2] and in human myocardium.[1,22] The interaction between halogenated anesthetics and adrenoceptor stimulation may also depend on the tissue studied. In dogs fitted with long-term monitoring instruments, Lennon and Murray[9] observed that the pulmonary vasodilator response to β-adrenoceptor stimulation is potentiated during isoflurane anesthesia, whereas Park *et al.*[23] reported that isoflurane attenuates β-adrenergic vasodilation in rat coronary arteries *in vitro*. Further, although halothane facilitates catecholamines-induced arrhythmias[24] and potentiates the positive inotropic effect of isoproterenol,[22] Stowe *et al.*[25] reported that halothane and isoflurane attenuate the effect of adrenergic stimulation on sinoatrial nodal pacemaker cells.

Table 3. Effects of Isoflurane (1 MAC) and Sevoflurane (1 MAC) on Lusitropic Responses to β-Adrenoceptor Stimulation

Mechanical Parameters	Concentration Effect Curve Parameters	Control Group (n = 10)	Isoflurane Group (n = 8)	Sevoflurane Group (n = 8)
R1	Eff_{max} (%)	60 ± 2*	61 ± 4*	56 ± 4*
	EC_{50} (μM)	0.28 ± 0.08	0.17 ± 0.15	0.29 ± 0.08
R2	Eff_{max} (%)	83 ± 4*	79 ± 4*	84 ± 3*
	EC_{50} (μM)	0.63 ± 0.12	0.62 ± 0.14	0.82 ± 0.22

Data are mean ± SEM.

R1 = ratio of maximum shortening velocity ($_{max}Vc$) to maximum lengthening velocity ($_{max}Vr$); R2 = ratio of the peak of the positive force derivative ($+dF \cdot dt^{-1}$) to the peak of the negative force derivative ($-dF \cdot dt^{-1}$); Eff_{max} = maximum effect; EC_{50} = concentration that results in 50% of Eff_{max}.

* $P < 0.05$ *versus* baseline; no significant differences between groups.

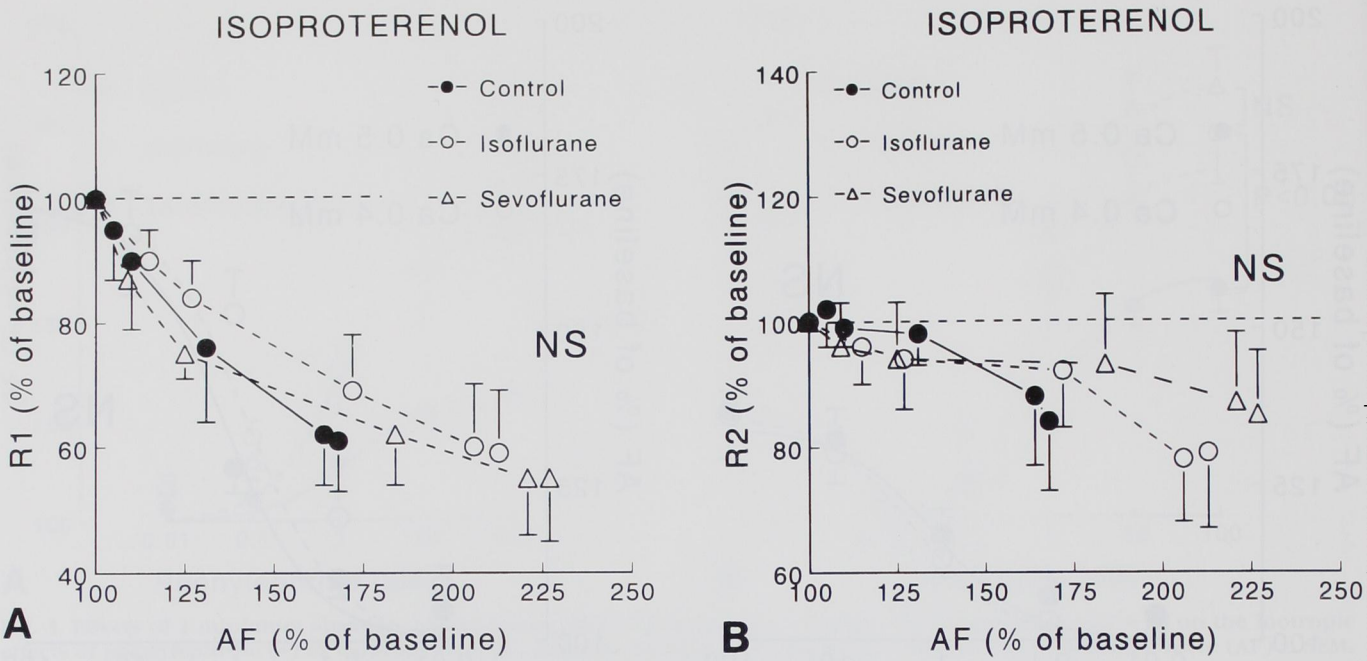

Fig. 5. Effect of 1 minimum alveolar concentration (MAC) isoflurane (n = 8) and 1 MAC sevoflurane (n = 8) on the positive lusitropic effects of β-adrenoceptor stimulation, assessed by the relations between lusitropic parameters under (*A*) low (R1) or (*B*) high (R2) load and active isometric force (AF). Each point corresponds to a concentration of isoproterenol. Data are means ± SEM. The probability value refers to between-group comparison using multivariate analysis of variance. NS = not significant.

Our study did not allow us to determine the precise mechanism(s) involved in the potentiation of the positive inotropic effect of α- and β-adrenoceptor stimulations. We did not observe any shift in the concentration-response curves as shown by the lack of difference between the median effective concentrations (table 2), suggesting that isoflurane and sevoflurane did not modify the affinity or number of receptors. A growing body of evidence suggested that volatile anesthetics may interfere with the signal transduction pathway involving G-protein-coupled receptors. G proteins have a pivotal regulatory role as membrane-associated signal transducers, and they may be involved in various cardiac pathophysiologic states, such as aging or heart failure.[26,27] α_1-Adrenoceptors are coupled with a G_q protein, leading to activation of phospholipase C and then production of inositol triphosphate and 1,2 diacylglycerol, which increases intracellular calcium concentration and activates protein kinase C, respectively.[3] β-adrenoceptors are associated with G_s and G_i proteins, which modulate adenylyl cyclase and thus cyclic adenosine monophosphate production, which in turn activates cyclic adenosine monophosphate-dependent protein kinase A.[3] It has been suggested that halothane may decrease the inhibitory effect of G_i protein on adenyl cyclase activity.[6,22] This effect may be involved in the facilitation of catecholamine-induced arrhythmias observed in patients anesthetized with halothane[28] and in the potentiation of the inotropic effects of adrenoceptor stimulations observed in *in vitro* experiments.[1,2] In addition, it has been shown that halothane stimulates G-protein-dependent phospholipase C activity and then increases inositol triphosphate formation.[5] Few studies have examined the interactions of other volatile anesthetics with G proteins. Nevertheless, in rat brain stem membrane, interference with muscarinic receptor–G-protein coupling appears to be a common property of volatile anesthetics.[29] Our results suggest that the interaction of halothane, isoflurane, and sevoflurane with myocardial G protein produces similar effects.

In the rat myocardium, R1 quantitates lusitropic effects under low load, which reflects mainly the calcium uptake by the sarcoplasmic reticulum.[2,19] We have shown that isoflurane and sevoflurane induced no major lusitropic effect under low load.[2,12] These results are in accordance with experimental *in vitro* studies showing that isoflurane and sevoflurane exert modest inhibitory effects on cardiac sarcoplasmic reticulum function compared with those induced by halothane.[11,16] β-adrenoceptor stimulation induces a potent positive lusitropic

effect under low load, which is thought to be related to an enhancement in sarcoplasmic reticulum uptake of calcium.[2,10] Isoflurane and sevoflurane did not significantly decrease the positive lusitropic effect of β-adrenoceptor stimulations under low load (fig. 2A), in contrast to our previous results with halothane.[2] These results confirm that isoflurane and sevoflurane interfere modestly with sarcoplasmic reticulum function compared with halothane. The alteration of the positive lusitropic effect under low load of halothane may well be mediated by its ability to activate calcium release channels,[30,31] resulting in a relative depletion of calcium from sarcoplasmic reticulum stores.[11] Isoflurane has been shown not to activate these channels,[11] whereas sevoflurane has not been evaluated. β-adrenoceptor stimulation also induced a potent positive lusitropic effect under high load (decrease in R2), which is thought to be related to a decrease in myofilament calcium sensitivity.[10] As previously reported with halothane,[2] isoflurane, and sevoflurane did not significantly modify the positive lusitropic effect of β-adrenoceptor stimulation under high load (fig. 2B). These results may be of some clinical importance because diastolic function significantly influences overall cardiac performance and because diastolic dysfunction may precede, or substantially contribute to, abnormalities of systolic function in various diseases.[32] Further, catecholamines play a major role in the modulation of cardiac relaxation under physiologic and pathologic conditions.[10,33]

The following points must be considered when assessing the clinical relevance of our results. First, α- and β-adrenoceptor stimulation were performed in the presence of β and α blockade, respectively. However, it was shown recently that α-adrenoceptor stimulation may modulate β-adrenoceptor response.[33] Second, this study was conducted at 29°C and at a low-stimulation frequency. Papillary muscles must be studied at this temperature because stability of mechanical parameters is not sufficient at 37°C. The low frequency was necessary because high-stimulation frequency induces core hypoxia.[34] Third, the study was performed in rat myocardium, which differs from human myocardium. The α-adrenoceptor density and consequently the positive inotropic effect induced by their stimulation is greater in rats than in humans.[35] Nevertheless, the relative importance of α-adrenoceptors in cardiac contractility can be increased in the presence of disease.[36]

In conclusion, in isolated rat myocardium, although isoflurane and sevoflurane have moderate negative inotropic effects, they enhance the positive inotropic effects of α- and β-adrenoceptor stimulation, and they did not modify the positive lusitropic effect of β-adrenoceptor stimulation.

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