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In Vitro *Effects of Desflurane, Sevoflurane, Isoflurane, and Halothane in Isolated Human Right Atria*

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Background: **Direct myocardial effects of volatile anesthetics have been studied in various animal species *in vitro*. This study evaluated the effects of equianesthetic concentrations of desflurane, sevoflurane, isoflurane, and halothane on contractile parameters of isolated human atria *in vitro*.**

Methods: **Human right atrial trabeculae, obtained from patients undergoing coronary bypass surgery, were studied in an oxygenated (95% O_2–5% CO_2) Tyrode's modified solution ($[Ca^{2+}]_o$ = 2.0 mM, 30°C, stimulation frequency 0.5 Hz). The effects of equianesthetic concentrations (0.5, 1, 1.5, 2, and 2.5 minimum alveolar concentration [MAC]) of desflurane, sevoflurane, isoflurane, and halothane on inotropic and lusitropic parameters of isometric twitches were measured.**

Results: **Isoflurane, sevoflurane, and desflurane induced a moderate concentration-dependent decrease in active isometric force, which was significantly lower than that induced by halothane. In the presence of adrenoceptor blockade, the desflurane-induced decrease in peak of the positive force derivative and time to peak force became comparable to those induced by isoflurane. Halothane induced a concentration-dependent decrease in time to half-relaxation and a contraction–relaxation coupling parameter significantly greater than those induced by isoflurane, sevoflurane and desflurane.**

Conclusions: **In isolated human atrial myocardium, desflurane, sevoflurane, and isoflurane induced a moderate concentration-dependent negative inotropic effect. The effect of desflurane on time to peak force and peak of the positive force derivative could be related to intramyocardial catecholamine release. At clinically relevant concentrations, desflurane, sevoflurane, and isoflurane did not modify isometric relaxation. (Key words: Adrenoceptor; catecholamine; myocardial relaxation; volatile anesthetics.)**

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DIRECT myocardial effects of volatile anesthetics have been widely studied *in vitro* in various mammalian species, such as the guinea pig,[1] ferret,[2,3] rat,[4,5] rabbit,[6] dog,[7] and cat.[8] However, structural differences between animal myocardium and human myocardium[9] induce important functional differences.[10,11] Although species differences may be useful to assess the effects of drugs on some subcellular components of the cardiac muscle, it is generally admitted that these data cannot be extrapolated directly to the human cardiac muscle. In the past decade, isolated human right atrial tissue obtained during cardiac surgery has been used to assess the inotropic effects of various drugs.[12] Recently, Gelissen *et al.*[13] studied the direct, negative inotropic effects of intravenous anesthetics in isolated human right atrial muscles. Only two studies investigated the direct myocardial effects of halothane and isoflurane on human myocardium; mainly, their action on potentials using conventional microelectrode techniques.[14,15] Moreover, the direct ef-

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Table 1. Patient Demographic Data, Preoperative Drug Treatment, Preoperative Left Ventricular Ejection Fraction

Groups	Age (yr)	Preoperative Drug Treatment	EF (%)
Halothane (n = 10)	64 ± 9	ACEI, βAB, CA, FUR, MOL, TNT, CBZ, PHE	57 ± 13
Isoflurane (n = 10)	67 ± 6	ACEI, AMIO, βAB, CA, FUR, MOL, TNT	62 ± 9
Sevoflurane (n = 10)	70 ± 6	ACEI, AMIO, βAB, CA	56 ± 9
Desflurane (n = 10)	72 ± 6	ACEI, βAB, CA, K^+A, MOL, TNT	59 ± 9
Tyramine (n = 6)	61 ± 9	ACEI, βAB, CA, FUR, MOL, TNT	64 ± 19
Desflurane AB (n = 6)	68 ± 6	βAB, CA, K^+A, MOL, TNT	61 ± 15

Data are mean ± SD.

Desflurane AB = desflurane in the presence of α- and β-adrenoceptor blockade; EF = left ventricular ejection fraction; ACEI = angiotensin-converting enzyme inhibitors; TNT = nitroglycerin; βAB = β-adrenergic blocking drugs; AMIO = amiodarone; CA = calcium channel antagonists; FUR = furosemide; MOL = molsidomine; K^+A = potassium channel agonists; CBZ = carbamazepine; PHE = phenobarbital.

fects of sevoflurane and desflurane have never been studied in isolated human myocardium. The purpose of this study was to compare the direct inotropic effects of a wide range of equianesthetic concentrations of halothane, isoflurane, sevoflurane, and desflurane in isolated human right atrial muscles.

Materials and Methods

Experimental Conditions

After the approval of the local medical ethics committee and informed consent were obtained, 52 right atrial trabeculae from humans were obtained from 52 patients scheduled for routine coronary artery bypass surgery. Patient characteristics, preoperative drug treatment, and preoperative left ventricular ejection fraction are reported in table 1. All patients received midazolam, sufentanil, etomidate, pancuronium, and isoflurane, except four who received flunitrazepam instead of midazolam. The right atrial appendage was removed during surgery for the purpose of cannulation before initiation of cardiopulmonary bypass. Immediately after removal by the surgeon, the appendage was placed in a hermetic bottle containing 100 ml cold (4°C), preoxygenated (95% O_2 and 5% CO_2) Tyrode's modified solution containing 120 mM NaCl, 3.5 mM KCl, 1.1 mM $MgCl_2$, 1.8 mM NaH_2PO_4, 25.7 mM $NaHCO_3$, 2.0 mM $CaCl_2$, and 11 mM glucose.

A long, thin, rectilinear trabecula was dissected carefully in oxygenated Tyrode's modified solution. During this dissection, great care was taken not to damage the tissue by stretching or clamping it with forceps or scissors. The trabecula was suspended vertically between stainless steel clips in a 200-ml jacketed reservoir containing the Tyrode's modified solution as described previously. The average time between removal by the surgeon and immersion in the jacketed reservoir was 10 min. The jacketed reservoir was maintained at 30°C using a thermostatic water circulator. The bathing solution was bubbled with carbogen (95% O_2–5% CO_2), resulting in a pH of 7.40 and a partial pressure of oxygen (P_{O_2}) of 600 mmHg. Isolated muscles were field stimulated at 0.5 Hz using two platinum electrodes with rectangular wave pulses of 5-ms duration that were 20% above threshold (CMS 95107; Bionic Instrument, Paris, France).

At the end of each experiment, the length and the weight of the muscle were measured. The cross-sectional muscle area was calculated from its weight and length assuming a cylindric shape and a density of 1. To avoid core hypoxia, trabeculae in the study should have a cross-sectional area less than 1.2 mm^2, an active force (AF) > 5.0 mN/mm^2, and a resting force/total force less than 0.45.

At the beginning of the study, six trabeculae were fixed in formol and later embedded in paraffin. Histologic study was performed on 3-μm thick longitudinal sections stained with hematoxylin–eosin, Safran, and Mallory's azan. This histologic study showed that myocytes inside the trabeculae were parallel to the longitudinal axis of the trabeculae and thus aligned with the axis of force measurement.

Mechanical Parameters Measured

Right atrial trabeculae were mounted between an isometric force transducer (UC3; Gould, Cleveland, OH) and a stationary clip in the jacketed reservoir. After a 60- to 90-min stabilization period at the initial muscle length at the apex of the length-active isometric tension curve (L_{max}), atrial trabeculae recovered optimal mechanical performance, which remained stable for many hours. The force developed by the muscle and its positive derivative were measured continuously, digitized at a sampling frequency of 200 Hz, and stored on the hard

disk of a microcomputer for analysis (MacLab; AD Instrument, Sydney, Australia).

Contraction Phase. Total force and resting force were measured. The inotropic state in isometric conditions was tested by the maximum isometric active force normalized per cross-sectional area, the peak of the positive force derivative normalized per cross-sectional area (+dF/dt), and the time to peak force (TPF).

Relaxation Phase. The relaxation phase of the isometric twitch was tested by the time to half-relaxation ($t_{1/2}$), which has been shown to be a good index of isometric relaxation in mammalian myocardium.[16] This parameter has been shown to be insensitive to increases in contractility induced by increasing extracellular calcium concentration. Conversely, isoproterenol as low as 10^{-10} M significantly decreased $t_{1/2}$.[16] Because it has been well-established that changes in the contraction phase induce coordinated changes in the relaxation phase,[17,18] the peak of the negative force derivative normalized per cross-sectional area (−dF/dt) could not assess isometric relaxation independently of the contraction phase. Thus, variations in contraction and relaxation must be considered simultaneously to quantify drug-induced changes in myocardial lusitropy. Therefore, indexes of contraction–relaxation coupling have been developed.[18,19]

Contraction–Relaxation Coupling. The coefficient R2 = (+dF/dt)/(−dF/dt) tests the coupling between contraction and relaxation during high load, and thus the lusitropic state during high load in a manner that is less dependent on inotropic changes. When the muscle contracts isometrically, sarcomeres shorten very little.[20] Because of a higher sensitivity of myofilament for calcium,[21] the relaxation time course is mainly determined by calcium unbinding from troponin C rather than by Ca^{2+} sequestration by the sarcoplasmic reticulum or Ca^{2+} extrusion *via* the Na^{+}-Ca^{2+} exchange. Thus, R2 (contraction–relaxation coupling during high load) indirectly reflects myofilament calcium sensitivity.[18,19,22] The contraction–relaxation coupling parameter R2 has been widely used[4,5,18,19,22] as an index of myocardial lusitropy.[23]

Experimental Protocol

We studied the effects of increasing equianesthetic concentrations (0.5, 1, 1.5, 2, and 2.5 minimum alveolar concentration [MAC]) of halothane (n = 10), isoflurane (n = 10), sevoflurane (n = 10), and desflurane (n = 10) on mechanical parameters of human right atrial trabeculae. Halothane (Fluotec 3; Cyprane LTD, Keighley, UK), isoflurane (Fortec 3; Cyprane LTD, Keighley, UK), desflurane (Devapor; Dräger, Lübeck, Germany), and sevoflurane (Sevotec 3; Ohmeda, West Yorkshire, UK) were added with specific vaporizers to the carbogen. The gas mixture was bubbling continuously in the bathing solution. To minimize evaporation of anesthetic vapors, the jacketed reservoir was almost hermetically sealed with a thin paraffin. Anesthetic concentrations in the gas phase were continuously measured using an infrared calibrated analyzer (Capnomac; Datex, Helsinki, Finland). One MAC of volatile anesthetics in the gas phase were 0.75 vol% for halothane, 1.15 vol% for isoflurane, 2 vol% for sevoflurane, and 6 vol% for desflurane. A 10-min period of equilibration was allowed at each anesthetic concentration. At the end of each experiment, the muscle was allowed to recover for 30 min.

Because desflurane induced the smallest decrease in AF and +dF/dt, and because it was the only volatile anesthetic that induced a significant decrease in TPF, we tested the hypothesis that desflurane may release catecholamines stored in isolated human right atrial muscles, as previously reported in rat myocardium.[5] To verify that isolated human right atrial trabeculae contained catecholamines, six additional muscles were exposed to 10^{-3} M tyramine, as previously described.[5] Then, six additional muscles were exposed to increasing concentrations of desflurane after α- and β-adrenoceptor blockade with 10^{-6} M phentolamine and 10^{-6} M propranolol, respectively. Tyramine, phentolamine, and propranolol were purchase from Sigma-Aldrich Chimie (L'isle d'Abeau, Chesnes, France).

Statistical Analysis

Data are expressed as mean ± SD. Control values between groups were compared using analysis of variance. Comparison of several means was performed using a repeated-measures analysis of variance and the Newman-Keuls test. All *P* values were two-tailed, and a *P* value of less than 0.05 was necessary to reject the null hypothesis. Statistical analysis was performed on a computer using Statview 4.5 software (SAS Institute Inc, Cary, NC).

Results

Fifty two human right atrial trabeculae were studied. No significant differences were seen in control values for L_{max}, cross-sectional area (CSA), ratio of resting force to total force (RF/TF), and main mechanical parameters between all groups (table 2).

Table 2. Control Values of Main Mechanical Parameters of Human Right Atrial Trabeculae

	Group					
	Halothane (n = 10)	Isoflurane (n = 10)	Sevoflurane (n = 10)	Desflurane (n = 10)	Desflurane AB (n = 6)	Tyramine (n = 6)
L_{max} (mm)	8.4 ± 1.2	7.2 ± 1.6	8.7 ± 2.1	8.5 ± 1.7	7.0 ± 1.3	6.4 ± 1.9
CSA (mm^2)	0.90 ± 0.38	0.72 ± 0.21	0.90 ± 0.29	0.94 ± 0.30	0.75 ± 0.22	0.75 ± 0.22
AF (mN/mm^2)	27 ± 11	27 ± 14	27 ± 12	30 ± 17	26 ± 13	26 ± 10
RF (mN/mm^2)	10 ± 4	12 ± 3	10 ± 4	10 ± 5	10 ± 2	11 ± 3
RF/TF	0.28 ± 0.08	0.33 ± 0.10	0.28 ± 0.05	0.30 ± 0.07	0.39 ± 0.08	0.42 ± 0.10
R2	3.01 ± 1.16	2.71 ± 0.77	2.59 ± 0.68	2.16 ± 0.38	2.74 ± 0.56	2.97 ± 0.88
$T_{1/2}$ (ms)	232 ± 54	185 ± 58	191 ± 40	174 ± 24	193 ± 36	227 ± 35
TPF (ms)	156 ± 31	160 ± 36	150 ± 21	159 ± 19	162 ± 13	158 ± 10

Data are mean SD.

Desflurane AB = desflurane in the presence of α- and β-adrenoceptor blockade; L_{max} = maximal length at the apex of the length-active force curve; CSA = cross-sectional area; AF = acting isometric force normalized per cross-sectional area; RF = resting force normalized per cross-sectional area; RF/TF = ratio of resting force on total force; R2 = contraction–relaxation coupling parameter under high load; $T_{1/2}$ = time to half relaxation; TPF = time to peak force.

Direct Negative Inotropic Effects of Volatile Anesthetics

Figure 1 shows that the moderate concentration-dependent decrease in AF induced by isoflurane, sevoflurane, and desflurane was comparable with and significantly lower than that induced by halothane. The concentration-dependent decrease in +dF/dt induced by halothane was significantly greater than that induced by isoflurane, sevoflurane, and desflurane. However, the desflurane-induced decrease in +dF/dt was significantly lower than that induced by isoflurane (fig. 1). In the presence of α- and β-adrenoceptor blockade, the decrease in +dF/dt induced by desflurane was significantly greater than that induced by desflurane alone, but was comparable to that induced by isoflurane and sevoflurane (fig. 1).

The decrease in TPF induced by desflurane was significantly greater than that induced by halothane, isoflu-

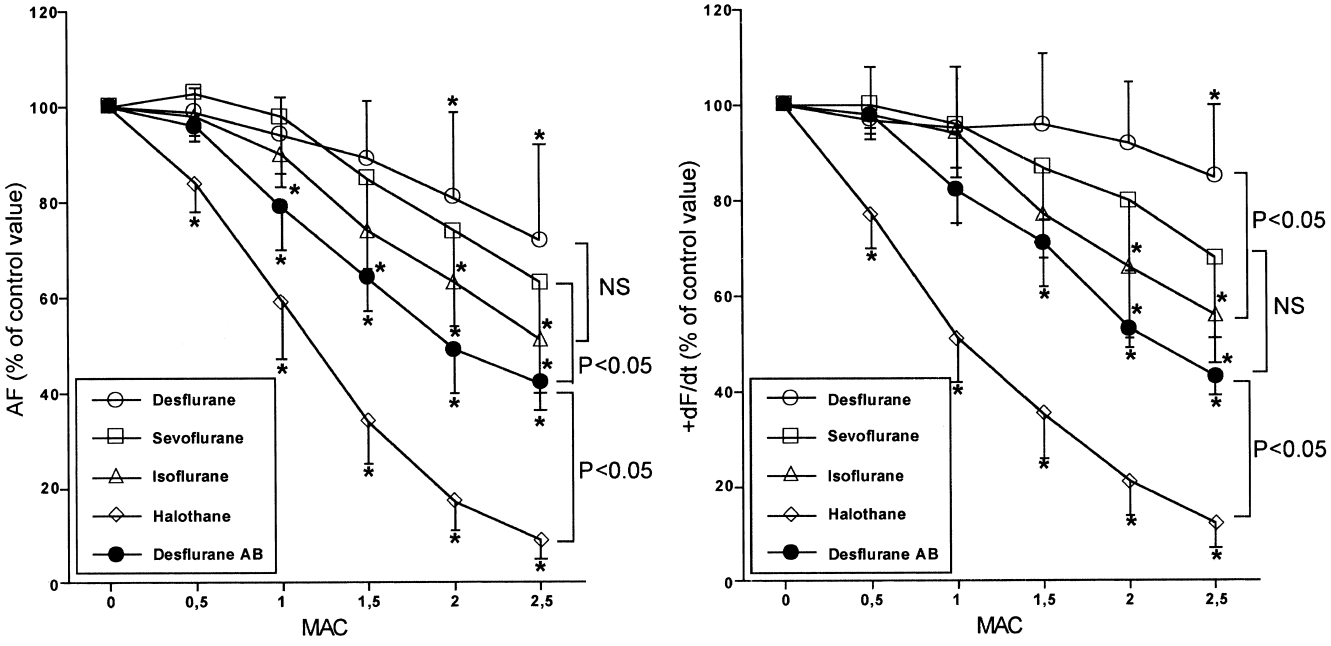

Fig. 1. Concentration-dependent effects of halothane (n = 10), isoflurane (n = 10), sevoflurane (n = 10), and desflurane (n = 10) in the presence of α- and β-adrenoceptor blockade (Desflurane AB; n = 6) on maximum active force normalized per cross-sectional area (AF, *left*) and the peak of the positive force derivative (+dF/dt, *right*). Data are the mean ± SD. *$P < 0.05$ *versus* control values. AB = adrenoceptor blockade.

rane, and sevoflurane, which were comparable. In the presence of α- and β-adrenoceptor blockade, the decrease in TPF induced by desflurane was comparable to that induced by halothane, isoflurane, and sevoflurane (fig. 2).

Recovery of AF and +dF/dt was observed in all muscles within 20 min after administration of volatile anesthetics were discontinued. Values of AF measured 20 min after exposure of halothane (AF: 95 ± 6% of control value), isoflurane (AF: 99 ± 8% of control value), sevoflurane (AF: 101 ± 10% of control value), and desflurane (AF: 100 ± 6% of control value) did not significantly differ from control values.

Direct Effects of Volatile Anesthetics on Isometric Relaxation

Halothane, isoflurane, sevoflurane, and desflurane decreased $t_{1/2}$ in a concentration-dependent manner. The decreases in $t_{1/2}$ induced by isoflurane, sevoflurane, and desflurane were comparable and significantly lower than those induced by halothane.

Halothane induced a significantly greater decrease in R2 than that induced by isoflurane, sevoflurane, and desflurane, which were comparable. The decrease in R2 induced by halothane became significant and maximal at 1 MAC, and the decrease induced by isoflurane was significant at 2 MAC and 2.5 MAC. Sevoflurane and desflurane did not significantly modify R2 even at high concentrations.

Direct Effects of Tyramine on Isolated Human Right Atria

Tyramine, 10^{-3} M, induced a significant increase in AF (145 ± 26% of the control value; $P < 0.05$) and +dF/ dt (154 ± 31% of the control value; $P < 0.05$) and a significant decrease in TPF (80 ± 8% of the control value; $P < 0.05$).

Discussion

The main results of this study are as follows: (1) in isolated human atrial myocardium, the concentration-dependent negative inotropic effects of desflurane, sevoflurane, and isoflurane were comparable with and significantly lower than that induced by halothane; (2) at clinically relevant concentrations isoflurane, sevoflurane, and desflurane did not modify the contraction–relaxation coupling parameter during high load; (3) the small effect on +dF/dt and the decrease in TPF induced by desflurane appears to be related to intramyocardial catecholamine release.

Inotropic Effects

It has been well-established that halothane is a more potent negative inotropic agent than isoflurane, sevoflurane, and desflurane.[1–5] More recently, it has been shown, in rat left ventricular papillary muscles, that desflurane may induce intramyocardial catecholamine release.[5] However, these results could not be extrapolated directly to human myocardium because of interspecies differences.[9–11] In human clinical studies, hemodynamic and echographic variables used to assess myocardial contractility were not completely independent of heart rate, preload, and afterload. Moreover, the effects of pathophysiologic state, treatments, age, and autonomic nervous system activity could not be excluded completely. Therefore, studying human myocardial tissue *in vitro* is the only way to determine the direct myocardial effects of drugs on human myocardium. Luk *et al.*[15] studied the electrophysiologic and mechanical effects of halothane and isoflurane on isolated human atrial fibers obtained from various acquired and congenital heart diseases. Although these authors showed that halothane induced a greater negative inotropic effect than did isoflurane, the magnitude of the negative inotropic effect reported in their study (72% and 54% decrease in contractile force in the presence of 1 MAC halothane and isoflurane, respectively) is not in agreement with that reported in our study. Moreover, in the study by Luk *et al.,*[15] the contractile force did not return to control values during the recovery phase, suggesting that preparations were not stable during the time of experiments. Indeed, experimental conditions used by Luk *et al.*[15] (temperature, 37°C; stimulation frequency, 1 Hz; pH, 7.30) have been shown to induce core hypoxia in isolated muscles.[24] In our study, the negative inotropic effect of volatile anesthetics was totally reversible, suggesting that our preparations did not undergo the mounting procedure or core hypoxia. Thus, the magnitude of the negative inotropic effect of volatile anesthetics we report herein is lower than that reported by Luk *et al.*[15] and in accordance with clinical studies dealing with cardiovascular effects of volatile anesthetics on human volunteers.[25,26]

In the presence of α- and β-adrenoceptor blockade, (1) the decrease in TPF induced by desflurane was no longer different than those induced by sevoflurane, isoflurane, and halothane; (2) the decrease in AF and +dF/dt was more pronounced than with desflurane alone but still

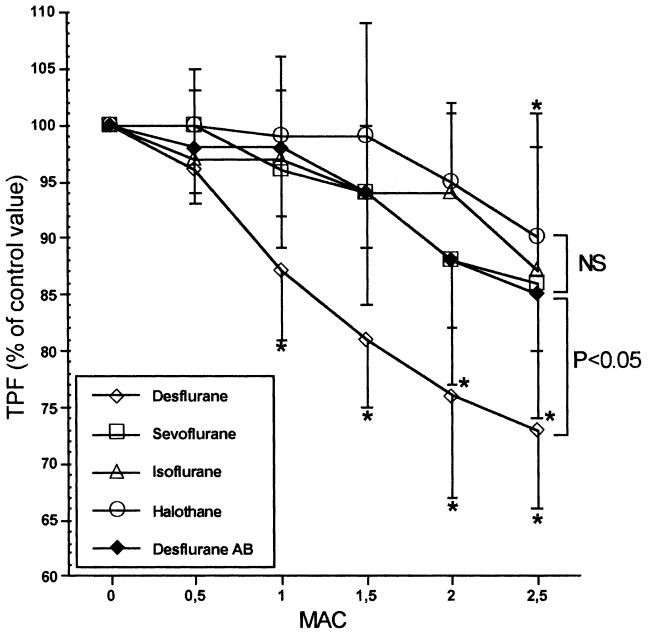

Fig. 2. Concentration-dependent effects of halothane (n = 10), isoflurane (n = 10), sevoflurane (n = 10), and desflurane (n = 10) in the presence of α- and β-adrenoceptor blockade (Desflurane AB; n = 6) on time to peak force. Data are the mean ± SD. *$P < 0.05$ *versus* control values. TPF = time to peak force.

comparable to that induced by isoflurane. These results suggested that desflurane may release catecholamines stored in isolated human atrial trabeculae, as previously reported in isolated rat papillary muscles.[5] Indeed, we demonstrated that tyramine induced a positive inotropic effect, suggesting that isolated human right atrial trabeculae contain catecholamines. Our results should be considered with clinical studies that show that desflurane may induce sympathetic activation from various mechanisms.[27] However, our study did not allow us to determine the precise origins of intramyocardial catecholamine release induced by desflurane: nerve endings of extracardiac neurons, intrinsic cardiac neurons, nonneuronal cardiac adrenergic cells. Further studies are needed to elucidate this point, and the pathophysiologic relevance of such release remains to be determined.

The main mechanisms by which volatile anesthetics induce myocardial depression are profound alterations in the cellular components involved in intracellular calcium homeostasis.[28] The differences in myocardial depressant effects of volatile anesthetics may be explained by the differential effects on the calcium inward currents (I_{Ca}), sarcoplasmic reticulum (SR), and myofilaments. Thus, it has been shown (1) that halothane induced a more profound depression of I_{Ca}, presumably *via* L-type Ca^{2+} channels, than did isoflurane[29] and (2) that halothane, but not isoflurane, depleted the SR of Ca^{2+}[30] and gated the cardiac SR Ca^{2+} release channel into the open state.[31] The effects of sevoflurane and desflurane on SR function remain unknown. However, it has been suggested, based on the lack of effect on postrest potentiation and the equivalent inotropic and lusitropic effects,[4,5] that the effects on SR functions may be similar to those of isoflurane. Halothane and isoflurane have been shown to decrease myofilament calcium responsiveness in human, skinned cardiac fibers.[32] The decrease in myofilament calcium responsiveness has been involved in the negative inotropic effect induced by low concentrations of halothane in rat myocardium.[33] Further experiments are needed to determine the effects of sevoflurane and desflurane on myofilament calcium responsiveness. Moreover, the effects of volatile anesthetics on cross-bridge kinetic and force generation remain unknown.

Lusitropic Effects during High Load

Our study showed that the decrease in $t_{1/2}$ induced by isoflurane, sevoflurane, and desflurane was comparable with and significantly lower than that induced by halothane (fig. 3). These results are different from those reported by Housmans *et al.*[3] for isolated ferret ventricular myocardium. Although these authors also reported a concentration-dependent decrease in $t_{1/2}$ with halothane, enflurane, and isoflurane, they did not observe any differences in the magnitude of this effect between volatile anesthetics. This could be explained by species differences and lower concentration of volatile anesthetics tested.

At clinically relevant concentrations, isoflurane, sevoflurane and desflurane did not significantly modify the contraction–relaxation coupling parameter R2 (fig. 3), suggesting that they did not induce any lusitropic effect during high load. These results are in agreement with those previously reported in rat ventricular myocardium.[4,5] In our study, halothane decreased R2 with a maximal and significant effect at 1 MAC, suggesting that it decreased myofilament calcium responsiveness. This is in accordance with the results from Jiang and Julian,[33] who demonstrated that the halothane-induced decrease in myofilament calcium responsiveness is mainly observed at low concentrations. However, a decrease in contractility may induce a slight decrease in R2,[4,5] according to the myofilament cooperativity concept.[17] Nevertheless, this effect could be attributed to the decrease in contractility because AF and +dF/dt decreased in a concentration-dependent manner, whereas R2 did

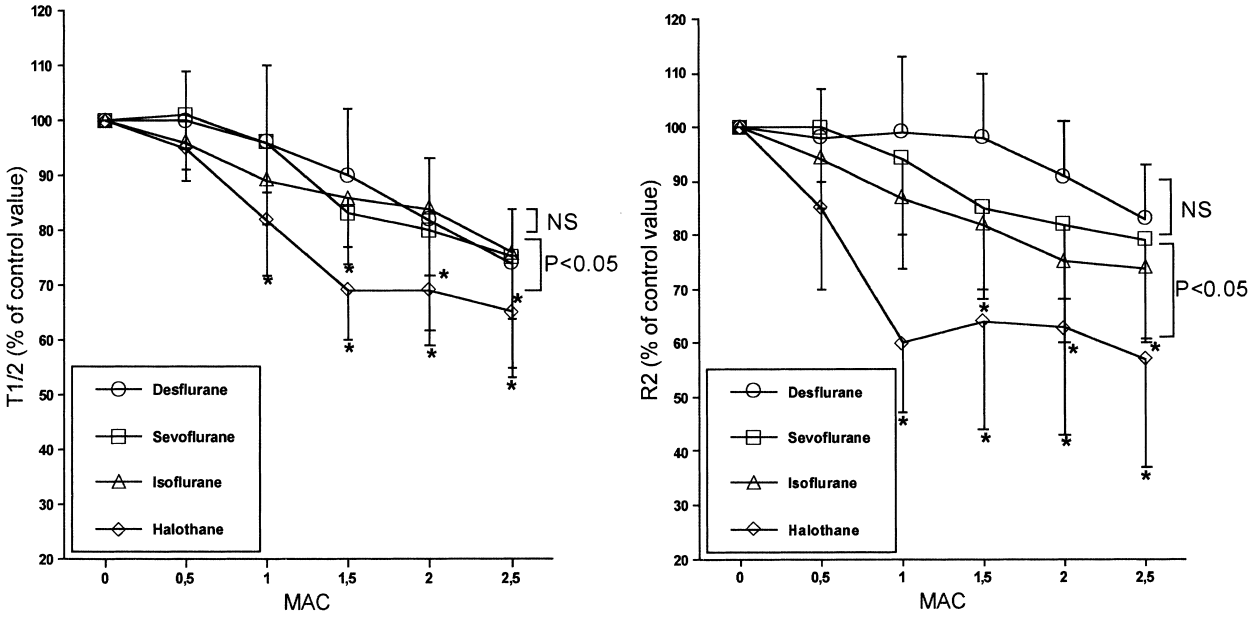

Fig. 3. Concentration-dependent effects of halothane (n = 10), isoflurane (n = 10), sevoflurane (n = 10), and desflurane (n = 10) on time to half-relaxation ($T_{1/2}$, *left*) and contraction–relaxation coupling parameter during high load (R2, *right*). Data are the mean ± SD. **P* < 0.05 *versus* control values.

not. These results should be interpreted cautiously because we studied only isometric relaxation; further experiments are necessary because shortening occurs *in vivo* and because physiologic relaxation is auxotonic.[17] However, these results may have some clinical importance because diastolic function is difficult to assess precisely in clinical studies because it significantly influences overall cardiac performance and because diastolic dysfunction may precede, or substantially contribute to, abnormalities of systolic function in various pathologic conditions.

Force decline during isometric twitch results from complex interactions among (1) the decrease in intracellular Ca^{2+} concentration (*i.e.*, sarcoplasmic reticulum Ca^{2+} uptake, sarcolemmal Na^{+}–Ca^{2+} exchange): (2) the changes in affinity of troponin C for Ca^{2+}; and (3) the dynamic of myosin cross-bridges detachment from actin after Ca^{2+} is lost from troponin C. Halothane may inhibit sarcolemmal Ca^{2+} adenosine triphosphatase activity,[34] which is a low-capacity Ca^{2+} transport mechanism that appears to play a minor role in mammalian relaxation.[10] In isolated rat heart cells, it has been shown that halothane inhibits Ca^{2+} channel more than Na^{+}–Ca^{2+} exchange, suggesting that the negative inotropic and lusitropic effects of halothane were related mainly to its effects on I_{Ca} and SR function.[35] Although the effects of halothane on cardiac SR Ca^{2+} adenosine triphosphatase remains controversial, it seems that they remain modest.[36]

Limitation of the Study

The following points must be considered in the assessment of the clinical relevance of our results. First, because this study was conducted *in vitro*, it dealt only with intrinsic myocardial contractility and did not account for vasodilator effects of volatile anesthetics nor their influences on sympathetic nervous system tone *in vivo*. Observed changes in cardiac function after anesthetic administration also depend on modifications in heart rate, venous return, afterload, sympathetic nervous system activity, and compensatory mechanisms. Second, we studied only isometric twitches whereas, *in vivo*, cardiac contraction faces various loading conditions. Third, this study was conducted at 30°C and at a low-stimulation frequency; however, isolated muscles must be studied at this temperature because 37°C and high-stimulation frequency may induce core hypoxia.[24] Moreover, low temperature modify Ca^{2+} homeostasis in myocardial cells and, then, may influence our results. Fourth, the study was performed on atrial myocardium, which differs from ventricular myocardium. In atrial myocardium, isometric twitch is shorter and force generation is lower than in ventricular myocardium. This is partly

because of a smaller releasable amount of Ca^{2+} and a faster uptake rate by SR.[37] Fifth, atrial trabeculae were obtained from patients with coronary artery disease who were treated long-term with numerous drugs. It could not be totally excluded that these drugs may have influenced our results. Nevertheless, we did not observe any differences between the effects of volatile anesthetics on atrial trabeculae in regard to drugs taken by patients.

In conclusion, in isolated human right atrial muscles, the concentration-dependent negative inotropic effects of desflurane, sevoflurane, and isoflurane were comparable with and significantly lower than those induced by halothane. Moreover, the moderate, negative inotropic effect of desflurane may be related to intramyocardial catecholamine release. In contrast to halothane, desflurane, sevoflurane, and isoflurane had no significant lusitropic effect during high load.

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