# Effects of Desflurane in Rat Myocardium

## Comparison with Isoflurane and Halothane

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*Background*: The cardiovascular effects of desflurane have been investigated in several *in vivo* animal and human studies. To determine the possible contributions of myocardial depression, the effects of desflurane on various contractile parameters in isolated cardiac papillary muscles were compared with those of isoflurane and halothane.

*Methods*: The effects of desflurane, isoflurane, and halothane (0.5–2.5 minimum alveolar concentration [MAC]) were studied in rat left ventricular papillary muscles (29°C; pH 7.40; stimulation frequency, 12 pulses/min). The inotropic effects were compared under low (isotony) and high (isometry) loads, using the maximum unloaded shortening velocity ($V_{max}$) and maximum isometric active force (AF). The lusitropic effects were compared in isotonic and isometric conditions.

*Results*: Desflurane has no significant inotropic effect (AF at 2.5 MAC: 95 ± 11% of control values; NS) in contrast with halothane and isoflurane (AF at 2.5 MAC: 37 ± 14 vs. 65 ± 10%, respectively; $P < 0.05$). After α- and β-adrenoceptor blockade or pretreatment with reserpine, desflurane induced a negative inotropic effect (AF at 2.5 MAC: 83 ± 11 vs. 89 ± 8%, respectively) that was not significantly different from that of isoflurane (AF at 2.5 MAC: 80 ± 12%). Halothane induced a negative lusitropic effect under low load, which was significantly greater than those of isoflurane and desflurane. In contrast to halothane, isoflurane and desflurane induced no significant lusitropic effect under high load and did not modify postrest potentiation. These results suggest that desflurane did not impair sarcoplasmic reticulum function.

*Conclusions:* When compared with isoflurane, desflurane induced a moderate positive inotropic effect related to intramyocardial catecholamine release. After adrenoceptor blockade, desflurane induced a negative inotropic effect comparable with that induced by isoflurane. (Key words: Anesthetics, volatile: desflurane; isoflurane; halothane. Heart, papillary muscle: contraction; relaxation; postrest potentiation. Sympathetic nervous system: intramyocardial catecholamines.)

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DESFLURANE is a recently introduced inhalational anesthetic agent that is identical in structure to isoflurane except for the substitution of fluorine for chlorine. Desflurane has several advantages compared with other volatile anesthetics: low blood and tissues solubilities, resulting in rapid induction and emergence; *in vitro* stability; and lack of substantial metabolism or toxicity. Desflurane and isoflurane induce a similar level of cardiac depression,[1] which appears less extensive than that of halothane.[2] Desflurane decreases systemic vascular resistance, which is less pronounced than that of isoflurane and may contribute to its maintenance of higher mean arterial pressure.[3,4] Recent investigations have shown that rapid increases in desflurane concentrations can induce increases in heart rate and blood pressure due to sympathetic activation.[5–7] The precise mechanisms of this sympathetic activation have not been completely understood and may involve airway irritation by this pungent agent, transient disinhibition of centers modulating sympathetic efferent outflow, and peripheral actions on sympathetic nerve endings.[8,9]

Because of concomitant changes in preload, systemic

resistance, and central nervous system activity, the precise effects of anesthetic agents on intrinsic myocardial contractility are difficult to assess *in vivo*. This is particularly true with desflurane, which induces sympathetic activation.[8,9] Further, although halogenated anesthetic agents can alter myocardial relaxation, the lusitropic effects of desflurane have not been studied *in vitro*. Thus we conducted an *in vitro* study to compare the inotropic effects of equianesthetic concentrations of desflurane, isoflurane, and halothane on rat cardiac papillary muscle. In addition, we studied the action of these volatile anesthetics on lusitropy and postrest potentiation, which allowed us to analyze the effects of desflurane on sarcoplasmic reticulum (SR) functions in a biochemically unaltered preparation.[10,11]

## Materials and Methods

We used adult Wistar rats (Iffa Credo, L'Arbresle, France) weighing 250–300 g. Care of the animals conformed to the recommendations of the Helsinski Declaration, and the study was performed in accordance with the regulations of the official edict of the French Ministry of Agriculture.

### Experimental Protocol

After brief anesthesia with ether, the hearts were quickly removed, and left ventricular papillary muscles were carefully excised and suspended vertically in a 200-ml jacketed reservoir with Krebs-Henseleit bicarbonate buffer solution that contained 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.1 mM KH2PO4, 25 mM NaHCO3, 2.5 mM CaCl2, and 4.5 mM glucose. The Krebs-Henseleit solution was prepared daily with highly purified water (Ecopure; Barnstead/Thermolyne Corp., Dubuque, IA). The jacketed reservoir was maintained at 29°C with a thermostatic water circulator (Polystat 5HP; Bioblock, Illkirch, France) and continuous monitoring of the solution temperature with a temperature probe (Pt100; Bioblock). 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 $pH$ 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 many hours. Suitable preparations were selected as previously reported.[10]

Control values of each mechanical parameter were recorded. The extracellular calcium concentration ($[Ca^{++}]_o$) was decreased from 2.5 to 0.5 mM because rat myocardial contractility is nearly maximum at 2.5 mM,[12] and hence it is difficult to quantify inotropic changes without first decreasing $[Ca^{++}]_o$. In addition, in rat myocardium, a postrest potentiation study is more sensitive at low $[Ca^{++}]_o$,[10] and a high $[Ca^{++}]_o$ may mask a negative inotropic effect of drugs that interfere with transsarcolemmal calcium entry. Thereafter we studied the effects of equianesthetic concentrations (0.5, 1.0, 1.5, 2.0, 2.5 minimum alveolar concentration [MAC]) of desflurane (n = 10), isoflurane (n = 10), and halothane (n = 10) on mechanical parameters. Because increasing $[Ca^{++}]_o$ may reverse the inotropic effects of volatile anesthetics,[13] we compared the inotropic effects of desflurane, isoflurane, and halothane at a $[Ca^{++}]_o$ of 0.5 and 1.0 mM. Thus, in three additional groups of papillary muscles, $[Ca^{++}]_o$ was decreased from 2.5 to 1.0 mM, and we studied the inotropic effects of desflurane (n = 10), isoflurane (n = 10), and halothane (n = 10) at the same concentrations stated above from 0.5–2.5 MAC.

Because we noted that desflurane induced a positive inotropic effect, we investigated the mechanism of this effect in additional experiments. $\alpha$- and $\beta$-adrenoceptors were blocked with phentolamine (1 $\mu$M) and propranolol (1 $\mu$M) (Sigma-Aldrich Chimie, L'Isle d'Abeau Chesnes, France) and added to the bathing solution at the end of the stabilization period at 1.0 mM $[Ca^{++}]_o$ (the volume of each drug represented 0.5 ml; *i.e.*, 0.25% of the bath volume). Then desflurane (n = 10), isoflurane (n = 10), and halothane (n = 10) were studied at the same concentrations stated above from 0.5–2.5 MAC. The inotropic effect of desflurane (n = 10) was also studied in rats pretreated with reserpine (4 mg/kg injected intraperitonally 24 h before killing; Sigma-Aldrich Chimie). At this concentration, reserpine completely depletes the intramyocardial catecholamine stores in the rat.[14] To verify this point, catecholamine release from the papillary muscles was induced by tyramine (1 mM; n = 6), which was added to the bathing solution. Pretreatment of rats with reserpine (n = 6) or $\alpha$- and $\beta$-adrenoceptor blockade (n = 6) with phentolamine (1 $\mu$M) and propranolol (1 $\mu$M) completely abolished the positive inotropic effect of tyramine (fig. 1). Finally, in another group of papillary muscles (n = 8), we studied the effects of a rapid increase in desflurane concentration (from 0–2.5 MAC), which was compared

## Tyramine (1 mM)


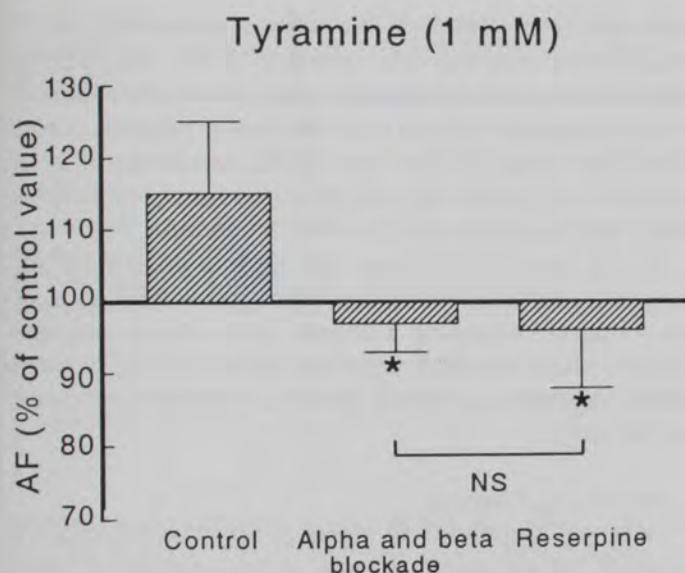
Fig. 1. Effects of tyramine (1 mM) on intramyocardial catecholamine store release assessed by an increase in isometric active force (AF). $\alpha$- and $\beta$-adrenoceptor blockade with propranolol and phentolamine or pretreatment with reserpine completely abolished the positive inotropic effect of tyramine. Data are mean $\pm$ SD (n = 6 in each group). *P < 0.05 versus the control group (tyramine alone). NS = not significant.

with progressive increases (0, 0.5, 1, 1.5, 2, and 2.5 MAC).

### Volatile Anesthetic Agent Administration

Desflurane (Devapor; Dräger, Lübeck, Germany), isoflurane (Fortec 3; Cyprane Ltd., Keighley, UK), and halothane (Fluotec 3; Cyprane Ltd.) were added with specific vaporizers to the carbogen. The gas mixture continuously bubbled in the bathing solution. To minimize evaporation of halogenated anesthetics, the jacketed reservoir was nearly completely sealed with a thin paraffin sheet (Parafilm M; American National Can, Greenwich, CT), as previously reported.[11] Anesthetic concentrations in the gas phase were continuously measured with an infrared calibrated analyzer (Capnomac; Datex, Helsinki, Finland). Halothane concentrations used were 0.3, 0.6, 0.9, 1.2, and 1.5 vol%; isoflurane concentrations used were 0.4, 0.8, 1.2, 1.6, and 2 vol%; and desflurane concentrations used were 1.8, 3.7, 5.6, 7.5, and 9.4 vol%. These concentrations are equivalent to 0.5, 1, 1.5, 2, and 2.5 MAC in the adult rat at 29°C, respectively.[15,16] A 20-min equilibration period was allowed between each anesthetic concentration and mechanical parameter recording.

### Electromagnetic Lever System and Recording

The electromagnetic lever system was described previously.[17] Briefly, the load applied to the muscle was 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.[10]

### Mechanical Parameters

Conventional mechanical parameters at $L_{max}$ were calculated from three twitches. 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.

**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 (isotony) and high (isometry) loads, respectively.

**Relaxation Phase.** We determined maximum lengthening velocity ($_{max}Vr$) of the twitch with preload only and the peak of the negative force derivative at $L_{max}$ normalized per cross-sectional area (-dF/dt). Examination of these two parameters allowed us to study the relaxation phase under low- and high-loading conditions, respectively. Because changes in the contraction phase induce coordinated changes in the relaxation phase,[11,19] $_{max}Vr$ and -dF/dt cannot assess lusitropy, and thus variations in contraction and relaxation must be considered simultaneously to quantify drug-induced changes in lusitropy. Indexes of contraction–relaxation coupling have been developed to study lusitropy.[19]

**Contraction–Relaxation Coupling.** The coefficient R1 = $_{max}Vc/_{max}Vr$ evaluated coupling between

contraction and relaxation under low load, and thus lusitropy in a manner that is independent of inotropic changes.[11] Under isotonic conditions, the amplitude of sarcomere shortening is greater than that observed under isometric conditions.[20] Because of the lower sensitivity of myofilament for calcium when cardiac muscle is markedly shortened under low load, relaxation proceeds more rapidly than contraction, apparently due to the rapid uptake of calcium by the SR. Thus, in rat myocardium, Rl tests SR uptake function. In contrast to $_{max}$Vr and -dF/dt, R1 is not significantly modified by major inotropic changes induced by decreasing $[Ca^{++}]_o$.[11] This is consistent with the fact that calcium uptake and release are precisely regulated by the SR.[21]

The coefficient R2 = $(+dF \cdot dt^{-1}/-dF \cdot dt^{-1})$ evaluated the coupling between contraction and relaxation under high load, and thus the lusitropy under high load in a manner that is less dependent on inotropic changes. When the muscle contracts isometrically, sarcomeres shorten very little.[20] Because of the higher sensitivity of myofilament for calcium,[22] the time course of relaxation is determined by calcium unbinding from troponin C rather than by calcium sequestration by the SR. Thus R2 indirectly reflects myofilament calcium sensitivity.[11,23] R2 is less modified by major inotropic changes induced by decreasing $[Ca^{++}]_o$ than +dF/dt and -dF/dt.[11] Because +dF/dt is depressed more than -dF/dt, the resulting decrease in R2 reflects a positive lusitropic effect. The slight decrease in R2, as $[Ca^{++}]_o$ is decreased, is consistent with the fact that calcium *per se* modulates myofilament calcium sensitivity, according to the cooperativity concept.[24] The parameters R1 and R2 have been validated as indexes of myocardial lusitropy in a mathematical model.[25]

**Postrest Potentiation.** Recovery of stable, reproducible isometric contraction after a rest interval (1 min) was studied to identify the effects of volatile anesthetic agents on SR functions. During rest in the rat myocardium, SR accumulates calcium above and beyond that accumulated with regular stimulation, and the first beat after the rest interval (B1) is more forceful than the last beat before the rest interval (B0).[10,26,27] During postrest recovery (B1, B2, B3 . . .), the SR-dependent part of activator calcium decreases somewhat toward a steady state, which is reached in a few beats. Thus the effects of volatile anesthetics on the postrest-potentiated contraction may provide insight into their effects on SR function in a biochemically intact preparation. The maximal isometric active force (AF) during postrest recovery was studied at a $[Ca^{++}]_o$ of 0.5 mм, after a 1-min

rest duration, and at a stimulation frequency of 12 pulses/min, and the rate constant of the exponential decay of AF was determined, as previously described.[10] $\tau$ is the number of beats required for the postrest potentiation to decay to one tenth of its maximum; it is assumed to represent the time required for the SR to reset itself and thus was used to test SR function.[27]

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. Shortening and lengthening velocities were expressed in $L_{max}$/s, with force expressed in millinewtons per square millimeter $(mN/mm^2)$.

### Statistical Analysis

Data are expressed as mean ± SD. Comparisons of control values among groups were performed using analysis of variance (ANOVA). The effects of anesthetic agents were compared using a repeated-measures ANOVA and the Newman-Keuls test. The beat-to-beat decay of active isometric force during postrest recovery was plotted against the number of beats and fitted to an exponential curve, and regression was performed using the least-squares method, as previously described.[10] All probability values were two-tailed, and values < 0.05 were required to reject the null hypothesis. Statistical analysis was performed on a computer using NCSS 6.0 software (Statistical Solutions Ltd., Cork, Ireland.

### Results

One hundred twenty-six left ventricular papillary muscles were studied. The mean $L_{max}$ was 5.1 ± 1.8 mm (range, 3–8 mm); the mean cross-sectional area was 0.70 ± 0.17 mm$^2$ (range, 0.30–0.80 mm$^2$); the mean ratio of resting force to total force was 0.14 ± 0.07 (range, 0.07–0.20); the mean contraction–relaxation coupling under low load (R1) was 0.67 ± 0.11 (range, 0.50–0.85), at a $[Ca^{++}]_o$ of 2.5 mм, and no significant differences were noted among the groups. A decrease in contractility was observed as $[Ca^{++}]_o$ was decreased from 2.5 to 0.5 mм (n = 30): Decreases in $V_{max}$ (72 ± 9% of the value at $[Ca^{++}]_o$ of 2.5 mм) and AF (59 ± 12% of the value at $[Ca^{++}]_o$ of 2.5 м) were consistent with those of previous reports.[10,11]

### Inotropic Effects

Table 1 depicts control values of the main mechanical parameters at a $[Ca^{++}]_o$ of 0.5 mм. No significant differ-

**Table 1. Comparison of Control Values of Main Mechanical Parameters of Left Ventricular Papillary Muscles at a $[Ca^{++}]_o$ of 0.5 mM**

| | Desflurane Group (n = 10) | Isoflurane Group (n = 10) | Halothane Group (n = 10) |
|---|---|---|---|
| $V_{max}$ ($L_{max}/s$) | 2.45 ± 0.35 | 2.21 ± 0.23 | 2.29 ± 0.43 |
| AF (mN/mm²) | 35 ± 12 | 38 ± 17 | 40 ± 9 |
| R1 (low load) | 0.69 ± 0.12 | 0.70 ± 0.07 | 0.61 ± 0.11 |
| R2 (high load) | 1.68 ± 0.22 | 1.70 ± 0.24 | 1.76 ± 0.19 |

Data are mean ± SD. No significant differences between groups.

$V_{max}$ = maximum unloaded shortening velocity; AF = isometric active force normalized per CSA; R1 = $_{max}Vc/_{max}Vr$; R2 = $+dF \cdot dt^{-1}/-dF \cdot dt^{-1}$.

ences among the groups were noted. At a $[Ca^{++}]_o$ of 0.5 mM, in isotonic ($V_{max}$) and isometric (AF) conditions, isoflurane and halothane induced a negative inotropic effect. The negative inotropic effect of halothane was more pronounced than that of isoflurane (fig. 2). In contrast, desflurane did not induce any significant negative inotropic effect (fig. 2).

Because $[Ca^{++}]_o$ modifies the inotropic effects of volatile anesthetics, we compared the inotropic effects of desflurane, isoflurane, and halothane at 0.5 and 1.0 mM $[Ca^{++}]_o$. At a $[Ca^{++}]_o$ of 1 mM, control values of AF did

not differ among the groups (49 ± 18 mN/mm² in the halothane group, 42 ± 15 mN/mm² in the isoflurane group, and 46 ± 20 mN/mm² in the desflurane group, NS). The maximal decrease in AF observed at 2.5 MAC of halothane was significantly more pronounced at a $[Ca^{++}]_o$ of 0.5 mM than at a $[Ca^{++}]_o$ of 1.0 mM (fig. 3). In contrast, the fractional contractile depression caused by 2.5 MAC isoflurane or desflurane did not differ between 1 and 0. 5 mM $[Ca^{++}]_o$ (fig. 3). At a $[Ca^{++}]_o$ of 1 mM, halothane induced a negative inotropic effect that was significantly greater than that induced by isoflurane (fig. 4). In contrast, desflurane induced no significant inotropic effect (fig. 4).

### Lusitropic Effects

At a $[Ca^{++}]_o$ of 0.5 mM, halothane induced a marked negative lusitropic effect under low load (increase in R1), suggesting a decrease in calcium uptake by the SR, whereas isoflurane and desflurane induced a very moderate negative lusitropic effect under low load (fig. 5); no significant differences were noted between desflurane and isoflurane. The lusitropic effects under low load of isoflurane and desflurane were significantly lower than that of halothane (fig. 5).

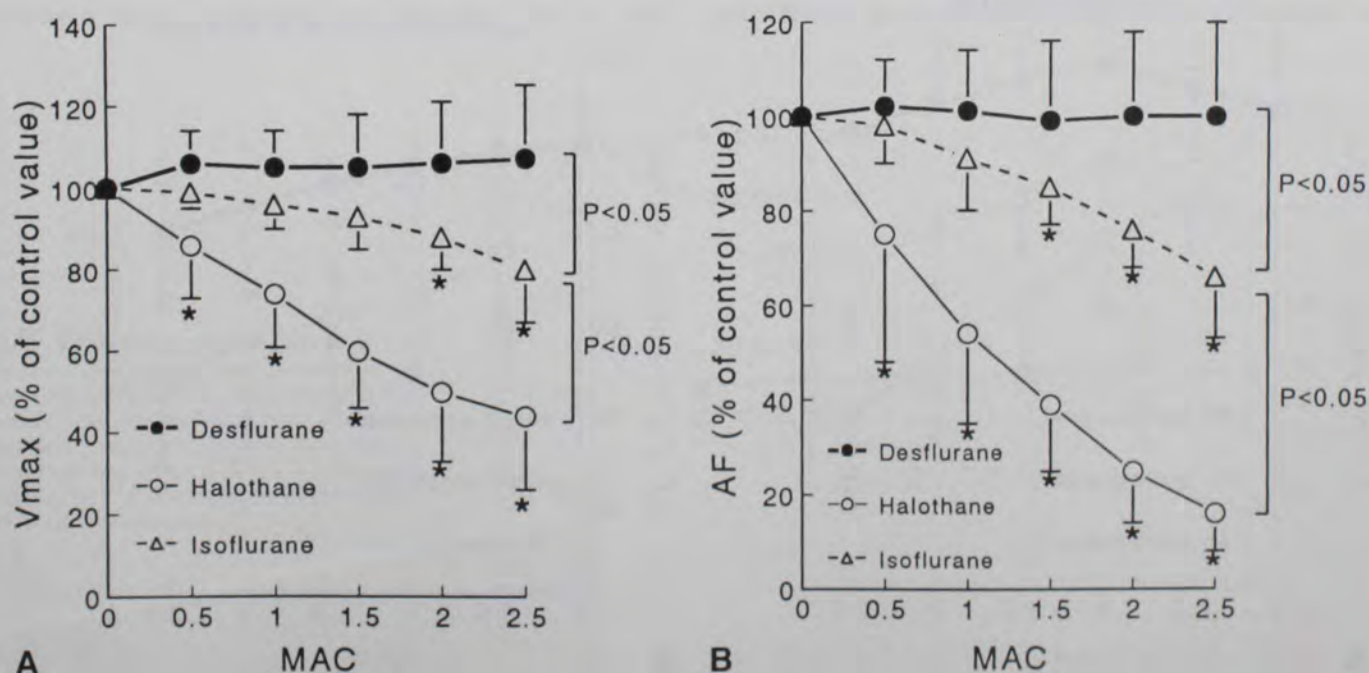Halothane induced a moderate positive lusitropic ef-



Fig. 2. Comparison of the effects of desflurane, isoflurane, and halothane at a $[Ca^{++}]_o$ of 0.5 mM on (A) maximum unloaded shortening velocity ($V_{max}$) and (B) isometric active force normalized per cross-sectional area (AF). Data are mean ± SD (n = 10 in each group). *P < 0.05 versus control values. Probability values refer to between-group comparisons.
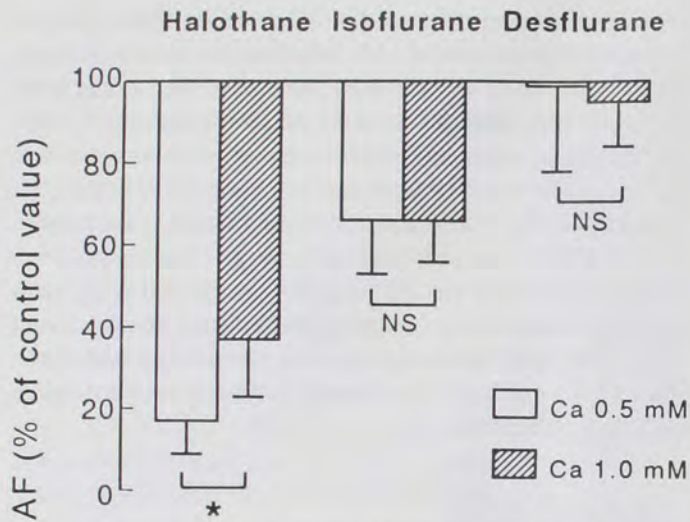
**Halothane Isoflurane Desflurane**



Fig. 3. Comparison of the maximal decrease in isometric active force (AF) observed at 2.5 MAC of desflurane, isoflurane, and halothane, at a $[Ca^{++}]_o$ of 0.5 mM or 1 mM. Data are mean ± SD (n = 10 in each group). *$P < 0.05$ between $[Ca^{++}]_o$ of 0.5 and 1.0 mM. NS = not significant.

of halothane. Previously we showed that the lusitropic effect of halothane under high load is not significantly different from that obtained by decreasing $[Ca^{++}]_o$, indicating that halothane *per se* does not modify the myofilament calcium sensitivity.[11] In contrast, whatever the concentration studied, desflurane and isoflurane induced no significant lusitropic effect under high load (fig. 5).

*Effects on Postrest Potentiation and Recovery*

The baseline postrest potentiation (B1/B0) of AF did not significantly differ among the study groups (138 ± 9% for halothane, 135 ± 5% for isoflurane, and 141 ± 13% for desflurane; NS). Halothane induced a significant decrease in postrest potentiation (fig. 6). In contrast, isoflurane and desflurane, regardless of the concentration studied, did not significantly modify the postrest potentiation of AF (fig. 6). The decay of mean active isometric force fitted well to an exponential curve ($0.96 < R < 0.99$), and the control values were not significantly different among the groups (table 2). Whatever the concentration studied, halothane, isoflurane, and desflurane did not significantly modify $\tau$ (table 2).

fect under high load (decrease in R2) (fig. 5), as previously reported.[11] Nevertheless, such a decrease in R2 must account for the marked negative inotropic effect
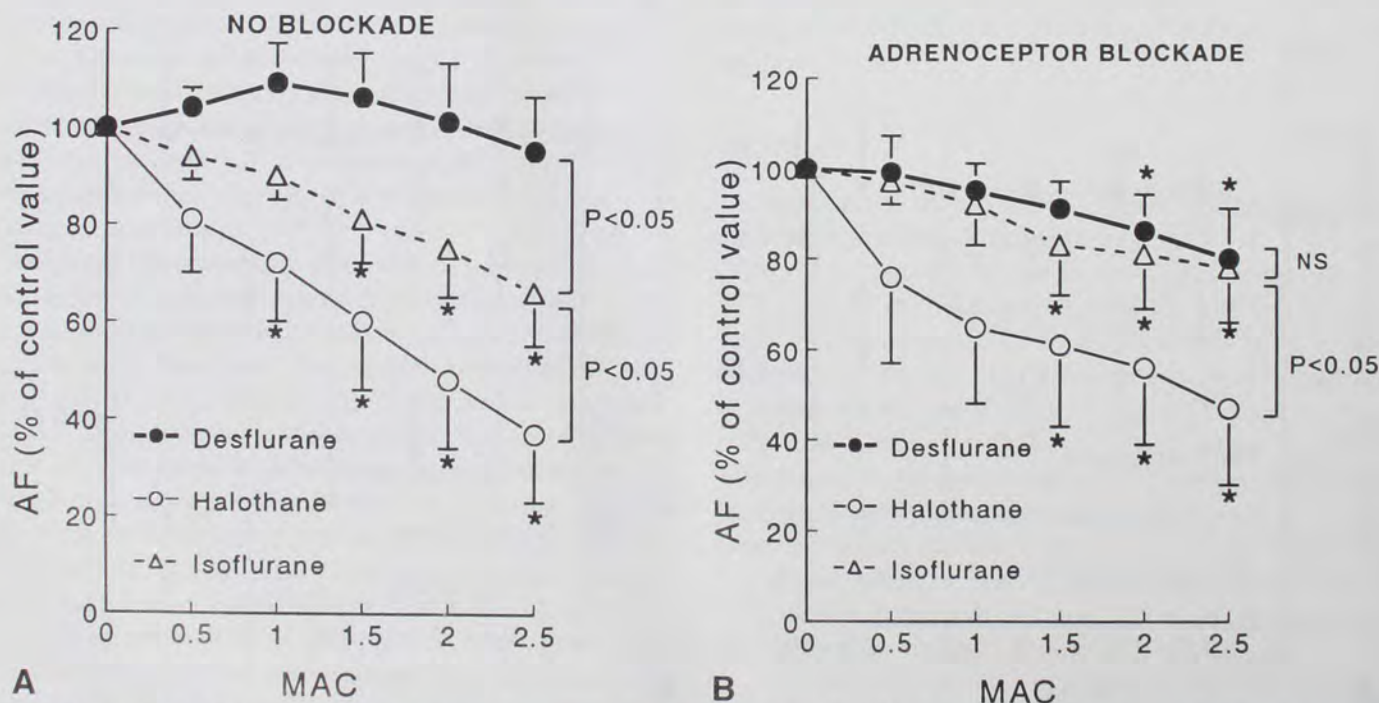


Fig. 4. Comparison of the effects of desflurane, isoflurane, and halothane at a $[Ca^{++}]_o$ of 1 mM without (*A*) or with (*B*) adrenoceptor blockade with propanolol and phentolamine on isometric active force normalized per cross-sectional area (AF). Data are mean ± SD (n = 10 in each group). *$P < 0.05$ *versus* control values. Probabilitly values refer to between-group comparisons. NS = not significant.
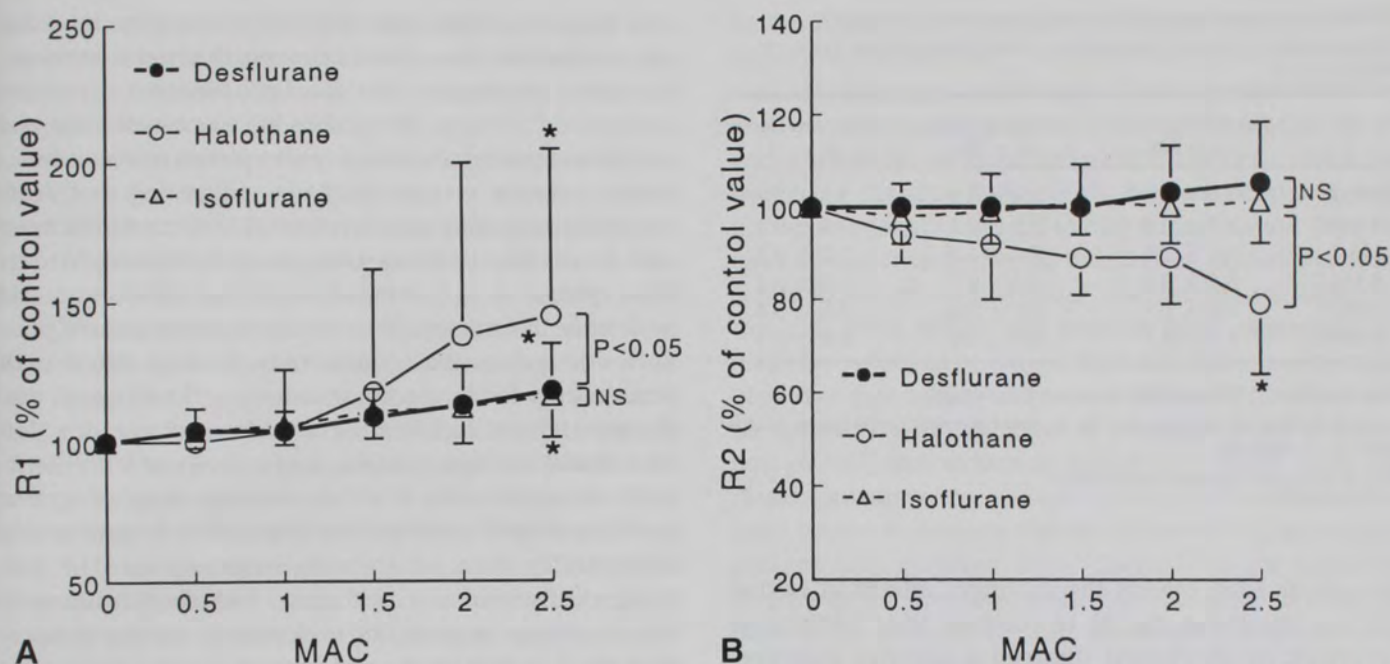
MYOCARDIAL EFFECTS OF DESFLURANE

Fig. 5. Comparison of the effects of desflurane, isoflurane, and halothane on lusitropy under (*A*) low (R1) and (*B*) high (R2) loads. Data are mean $\pm$ SD (n = 10 in each group). *$P < 0.05$ *versus* control values. Probability values refer to between-group comparisons. NS = not significant.

### Mechanism of the Positive Inotropic Effect of Desflurane

Because we noted that desflurane induced a positive inotropic effect, compared with isoflurane (fig. 4), we investigated the mechanism of this effect. The inotropic effects of desflurane, isoflurane, and halothane were studied after $\alpha$- and $\beta$-adrenoceptor blockade with propranolol and phentolamine. Adrenoceptor blockade did
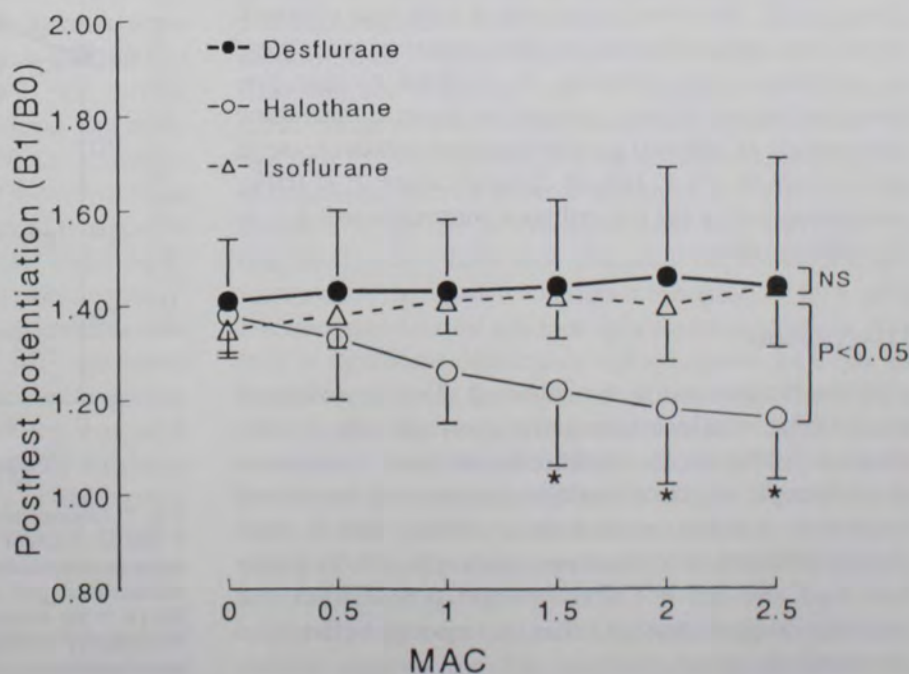
Fig. 6. Comparison of the effects of desflurane, isoflurane, and halothane on postrest potentiation. B1 is the first isometric contraction after rest, and B0 is the last isometric contraction before rest. Data are mean $\pm$ SD (n = 10 in each group). *$P < 0.05$ *versus* control values. NS = not significant.

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**Table 2. Comparison of the Rate Constant (τ) of the Exponential Decay of Isometric Active Force after Postrest Potentiation**

|  | Desflurane Group (n = 10) | isoflurane Group (n = 10) | Halothane Group (n = 10) |
|---|---|---|---|
| Control | 4.0 ± 0.8 | 3.4 ± 0.5 | 3.3 ± 0.7 |
| 0.5 MAC | 3.8 ± 0.8 | 3.2 ± 0.7 | 3.1 ± 0.3 |
| 1 MAC | 3.7 ± 0.5 | 2.9 ± 1.0 | 3.0 ± 0.4 |
| 1.5 MAC | 3.8 ± 0.6 | 3.8 ± 0.6 | 3.6 ± 0.7 |
| 2 MAC | 3.9 ± 0.7 | 3.8 ± 0.5 | 3.5 ± 0.4 |
| 2.5 MAC | 4.0 ± 0.7 | 3.7 ± 0.5 | 3.6 ± 1.4 |

Data are mean ± SD. No differences were significant.

τ is the number of beat required for postrest potentiation to decay to one tenth of its maximum.

MAC = minimum alveolar concentration.

not significantly modify the inotropic effects of isoflurane and halothane (fig. 4). In contrast, after adrenoceptor blockade, desflurane induced a negative inotropic effect that was not significantly different from that of isoflurane (fig. 4). After treatment with reserpine to deplete catecholamine stores (fig. 1), desflurane induced a negative inotropic effect that was not significantly different from that observed with adrenoceptor blockade (fig. 7).

Because sympathomimetic effects of desflurane *in vivo* have been reported to occur during rapid increases in desflurane concentration,[6] we studied the inotropic effect of a rapid increase in desflurane concentration (from 0–2.5 MAC) and compared it with that obtained at the same concentration but after progressive increase in desflurane concentration. In contrast to that previously observed *in vivo*, a rapid increase in desflurane concentration induced a more pronounced decrease in AF ($77 \pm 9$ *vs.* $95 \pm 11\%$ of control values; $P < 0.05$), suggesting that it did not enhance intramyocardial catecholamine release.

## Discussion

In the present study, we showed that (1) isoflurane and halothane induced a negative inotropic effect (halothane > isoflurane) but desflurane did not; (2) the positive inotropic effect of desflurane appears to be related to intramyocardial catecholamine release; and (3) desflurane induced only moderate lusitropic effects under low load and did not alter postrest potentiation and recovery, suggesting that it has no important effects on SR function.

In dogs fitted with instruments for long-term monitoring, desflurane caused less decrease in arterial pressure, systemic resistance, and cardiac function than did isoflurane.[2,28] These differences between isoflurane and desflurane were abolished with pharmacologic autonomic nervous system blockade, suggesting that desflurane may produce less depression in sympathetic tone and autonomic reflexes than does isoflurane. In contrast, Merin et al.[3] demonstrated that desflurane and isoflurane induce similar decreases in mean arterial pressure and myocardial contractility in dogs fitted with instruments for long-term monitoring. In humans, desflurane did not significantly modify cardiac index, left ventricular ejection fraction, and velocity of left ventricular circumferential fiber shortening, suggesting that desflurane may produce less depression in myocardial contractility than other halogenated agents.[4] In contrast, desflurane and isoflurane had similar hemodynamic effects in patients undergoing coronary artery surgery.[29] However, several recent investigations have shown that a rapid increase in desflurane concentration induces sympathetic activation,[5-9] which may be related to (1) airway irritation, because of the pungency of desflurane,[5,9] (2) transient disinhibition of centers
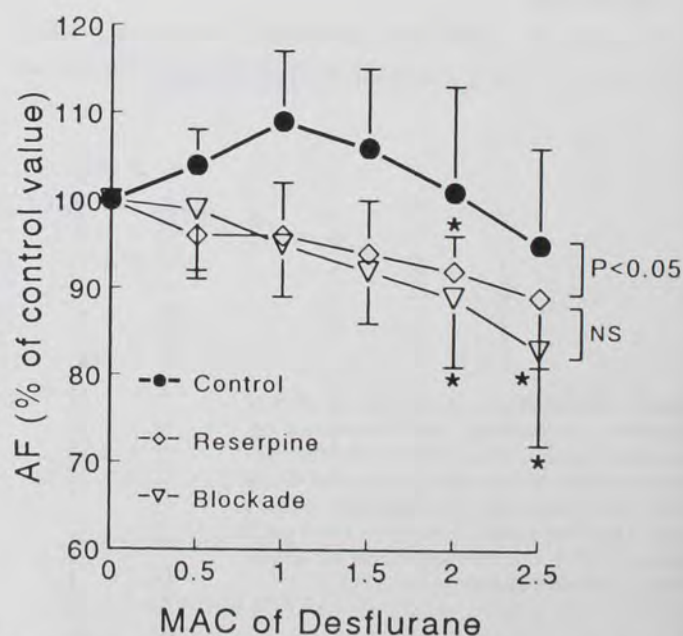
Fig. 7. Comparison of the effects of desflurane at a $[Ca^{++}]_o$ of 1 mM in control conditions, after adrenoceptor blockade, or after pretreatment with reserpine, on isometric active force normalized per cross-sectional area (AF). Data are mean ± SD (n = 10 in each group). *$P < 0.05$ *versus* control values. Probability values refer to between-groups comparisons. NS = not significant.

modulating sympathetic efferent outflow because of a rapid increase in the concentration of desflurane in the central nervous system,[6,8] and (3) peripheral actions on the sympathetic nerve endings.[8,9]

In our study, halothane induced a greater negative inotropic effect than isoflurane (figs. 2 and 4), as previously reported.[30] Once the MAC values are corrected according to the temperature,[16] the negative inotropic effects of halothane and isoflurane that we observed here corresponded with those previously reported.[31] In contrast, desflurane did not induce a negative inotropic effect (figs. 3 and 4). We found that the positive inotropic effect of desflurane was related to intramyocardial catecholamine release. Indeed, after adrenoceptor blockade or pretreatment with reserpine, desflurane induced a negative inotropic effect that was not significantly different from that of isoflurane (fig. 7). In contrast to previous studies *in vivo*,[6-8] the rapid increase in desflurane concentration caused a decrease in AF that was not consistent with enhanced catecholamine release, suggesting that this phenomenon is distinct from the increase in sympathetic activity.[5-9] Further, the increase in sympathetic activity occurs at high concentrations, in contrast to that observed in our study (fig. 2). Our results did not correspond with those of Boban *et al.*,[32] who did not find any significant difference in the inotropic effects of desflurane and isoflurane in isolated guinea-pig hearts. Several hypothesis could explain this discrepancy: species differences, temperature differences, and differences in the experimental model used. These authors used a Langerdorff preparation with Krebs-Ringer solution, with which oxygenation remains low and could modify the pharmacologic response to anesthetic agents.[33] Desflurane has been shown potentially to induce sympathetic activation, the mechanisms of which remain incompletely understood. Our study provides some evidence that this sympathetic activation can be related, at least partly, to a release of intramyocardial catecholamine stores. It should be emphasized that several studies have suggested that a peripheral site could partly mediate the sympathetic activation of desflurane.[5-9] Whereas a rapid increase in desflurane concentrations induces only a transient increase in heart rate and blood pressure, it also induces long-lasting increases in norepinephrine concentration.[6] Further, propofol, fentanyl, or lidocaine only partially attenuated the sympathetic activation of desflurane, suggesting that stimulation of the sympathetic efferent from the central nervous system or irritation of the airway are not the only mechanisms of this sympathetic activation.[8,9,34,35]

Our study did not allow us to determine the precise origin(s) of intramyocardial catecholamine release induced by desflurane: nerve endings of extracardiac neurons, intrinsic cardiac neurons,[36] or newly described non-neuronal intrinsic adrenergic cardiac cells.[37] Our study also did not enable us to determine whether the catecholamine release was exocytotic or nonexocytotic.[38] Further studies are needed to elucidate these points. Because peripheral autonomic neurons are believed to play important and complex roles in maintaining cardiac function in cardiac diseases[36] and ischemic preconditioning,[39] the pathophysiologic relevance of such release also remains to be determined. It could be beneficial because it facilitates better maintenance of cardiac contractility and arterial pressure than other volatile anesthetics do. Release of intramyocardial catecholamines may also induce increased myocardial work and cause coronary artery vasoconstriction, and desflurane has been shown to increase the risk of myocardial ischemia in patients with coronary artery disease.[40] In our study, the magnitude of intramyocardial catecholamine release appears to be moderate because the positive inotropic effect of desflurane remained modest. Further, halothane potentiates the inotropic effect of $\alpha$- and $\beta$-adrenoceptor stimulation.[11] Although such potentiation has not been yet reported with desflurane, it could have enhanced the myocardial effects of limited intramyocardial catecholamine release.

The main mechanisms by which volatile anesthetics induce myocardial depression are a profound alteration in the main cellular components involved in intracellular calcium homeostasis. Indeed, at clinically relevant concentrations, the decrease in myofilament calcium sensitivity induced by volatile anesthetics remains modest.[11,41] The differences in myocardial depressant effects of volatile anesthetics, mainly halothane and isoflurane, may be explained by their differential effects on the calcium inward currents ($I_{Ca}$) and SR function.[42,43] We observed that the negative inotropic effect of halothane, but not those of isoflurane or desflurane, was significantly greater at low compared with high $[Ca^{++}]_o$ (fig. 3). Because halothane markedly depressed $I_{Ca}$, the increase in $[Ca^{++}]_o$ may counteract this effect by increasing the transsarcolemmal calcium concentration gradient. The lack of significant differences in the negative inotropic effect induced by isoflurane or desflurane regardless of the $[Ca^{++}]_o$ may be attributed to the fact that these volatile anesthetics had lower inhibitory effects of $I_{Ca}$ than did halothane. However, the differential effects of halothane in high or low $[Ca^{++}]_o$ need further studies to determine which of the cellular components ($I_{Ca}$ and/or SR) is mainly affected by the change in $[Ca^{++}]_o$.

In the rat myocardium, the SR accounts for nearly total calcium removal from the cytoplasm during the relaxation

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phase. R1 tested the lusitropic state under low load and reflects the rapid uptake of calcium by the SR. We showed that halothane caused a larger increase in R1 than did desflurane and isoflurane (fig. 5). Thus our results suggest that desflurane did not markedly modify calcium uptake by the SR. Halothane decreased postrest potentiation, whereas isoflurane and desflurane did not (fig. 6). This result corresponds with our knowledge of the effects of halothane on SR functions. Indeed, it has been shown that halothane, but not isoflurane, depletes the SR of $Ca^{++}$.[43] This effect may be related to the fact that halothane, but not isoflurane, gates the cardiac SR $Ca^{++}$ release channel into the open state.[44] Because desflurane did not modify postrest potentiation, we suggest that it does not modify calcium release by the SR, SR calcium content, or the recirculation fraction of calcium within the SR. R2 tested the lusitropic state under high load and thus reflects myofilament calcium sensitivity. Our results showed that desflurane and isoflurane did not significantly modify R2 (fig. 5), suggesting that they did not modify myofilament calcium sensitivity. Halothane induced a slight decrease in R2, but this effect can be fully explained by its negative inotropic effect and not by a direct effect on myofilament calcium sensitivity.[11] The fact that desflurane did not modify myocardial relaxation may be clinically important because diastolic function significantly influences overall cardiac performance and because diastolic dysfunction may precede or substantially contribute to abnormalities of systolic function in various pathologic conditions. Nevertheless, it should be emphasized that relaxation is only one component of diastolic function and that extrinsic factors may influence cardiac relaxation *in vivo*. To date, conflicting results have been reported concerning the lusitropic effects of anesthetics agents *in vivo*.[45,46]

The following points must be considered in assessing the clinical relevance of our results. First, because this study was conducted *in vitro*, it addressed only intrinsic myocardial contractility. 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. This is important with desflurane, which also induces central nervous system sympathetic activation. Second, this study was carried out at 29°C and at a low-stimulation frequency; however, papillary muscles must be studied at this temperature because the stability of mechanical parameters is not sufficient at 37°C and at a low frequency because high-stimulation frequency may induce core hypoxia.[47] Third, it was performed on rat myocardium, which differs from human myocardium, but the effects of volatile

anesthetics on the myocardium appear to be very similar among species.[11,30,31] However, very few data are available concerning species differences in intracardiac autonomic neurons.[36]

In conclusion, in isolated rat myocardium, desflurane induced a moderate positive inotropic effect compared with isoflurane. This effect was related to intramyocardial catecholamine release, which could therefore participate in the sympathetic activation observed with desflurane *in vivo*. In contrast to halothane, desflurane and isoflurane had no important lusitropic effects and did not modify postrest potentiation and recovery, suggesting that they did not markedly modify SR functions.

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