

Myocardial Effects of Halothane and Sevoflurane in Diabetic Rats

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Background: Diabetes induces significant myocardial abnormalities, but the effects of halogenated anesthetics on this diseased myocardium remain a matter of debate.

Methods: Left ventricular papillary muscles and triton-skinned cardiac fibers were provided from control and streptozotocin-induced diabetic rats. The effects of halothane and sevoflurane were studied on inotropic and lusitropic responses, under low (isotony) and high (isometry) loads in papillary muscles and then on isometric tension–Ca²⁺ concentration (pCa) relations obtained in triton-skinned cardiac fibers. Data are presented as mean ± SD.

Results: Sevoflurane and halothane induced a negative inotropic effect that was more important in diabetic rats (active force: 1.5% halothane, 19 ± 6 vs. 24 ± 6% of baseline, *P* < 0.05; 3.6% sevoflurane, 47 ± 14 vs. 69 ± 17% of baseline, *P* < 0.05). However, when differences in minimum alveolar concentration were considered, no significant difference was observed between groups for halothane. The effects of halothane and sevoflurane on isotonic relaxation and postrest potentiation were not significantly different between groups. In contrast, the decrease in Ca²⁺ myofilament sensitivity produced by each anesthetic agent was greater in diabetic rats than in control rats (0.65% halothane, −0.15 ± 0.07 vs. −0.05 ± 0.04 pCa unit, *P* < 0.05; 1.8% sevoflurane, −0.12 ± 0.06 vs. −0.06 ± 0.04 pCa unit, *P* < 0.05).

Conclusions: The negative inotropic effect of halothane and sevoflurane was greater in diabetic rats, mainly because of a significant decrease in myofilament Ca²⁺ sensitivity.

SEVERAL complications may occur during the development of diabetes mellitus, related to hypertension, coronary artery disease, or a specific cardiomyopathy.¹ Moreover, during anesthesia and surgical procedures, patients with diabetes have an increased incidence of intraoperative hypotension.² To study the functional

changes of diabetic cardiomyopathy, the streptozotocin-induced diabetic rat has been widely used as an animal model of diabetes.^{3–7} The most significant functional changes encountered in the diabetic myocardium are represented by a slowing of contraction and relaxation velocities without a change in peak developed tension.⁵ These alterations can be related to a variety of anomalies involving the sarcoplasmic reticulum (SR), mitochondria, sodium–calcium exchange, calcium channel currents, and intracellular calcium metabolism, together with a decrease in cross-bridge cycling.^{4,6,8–11} Previous studies have demonstrated that pathologic states such as myocardial ischemia, cardiac insufficiency, or cardiac hypertrophy may accentuate the negative inotropic response to halogenated anesthetics.¹² On the other hand, Brian *et al.*¹³ have shown that the minimum alveolar concentration (MAC) values of halothane, isoflurane, and enflurane were diminished in diabetic rats compared with control rats. However, Hattori *et al.*⁷ have suggested that the myocardium of diabetic rats could be less sensitive to halogenated agents (halothane, enflurane, and isoflurane) than myocardium from healthy rats. Therefore, the effect of halogenated agents on myocardium from diabetic rats is still a matter of debate. In diabetic rats, the effect of halogenated anesthetics on myofilament calcium responsiveness is only known for halothane.¹⁴ This effect might be important because modification in myofilament calcium responsiveness has been described in diabetic myocardium,¹⁵ and halogenated anesthetics have been shown to act on myofilament Ca²⁺ sensitivity.¹⁶

Therefore, this experimental study was designed to compare the myocardial effects of halothane and sevoflurane in myocardium from diabetics and control rats. The experimental models used enabled us to investigate the effects on contraction (inotropy) and relaxation (lusitropy) at different loading conditions (isotony vs. isometry) and the direct effects on myofilament calcium sensitivity.

Materials and Methods

Care of the animals conformed to the recommendations of the Helsinki Declaration, and the study was performed in accordance with the regulations of the official edict of the French Ministry of Agriculture.

Animals

Six-week-old male Wistar rats (Iffa Credo, L'Arbresles, France) were each assigned to one of two groups, a

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control group and a diabetes mellitus group. In the diabetic group, streptozotocin was injected intravenously (65 mg/kg; Sigma Chemical, L'Isle d'Abeau Chesnes, France), and rats were studied 3–4 weeks later, as previously reported.^{4,17} All animals had continuous access to rat chow and were given water *ad libitum*. Transcutaneous determination of glucose blood concentration (Glucotrend®; Boehringer, Mannheim, Germany) was performed to ensure that the animals experienced diabetes (*i.e.*, blood glucose concentration > 25 mM/l). At the moment of killing, blood samples were withdrawn from diabetic (n = 12) and control rats (n = 6) and were centrifuged at 5,000g for 15 min; then, plasma fractions were collected and stored at –20°C for further determination of glucose and bicarbonate concentrations (Cobas integra 400; Roche Diagnostic, Mannheim, Germany).

Mechanical Study of Papillary Muscles

After brief anesthesia with pentobarbital sodium, the hearts were quickly removed, and then the left ventricular papillary muscles were carefully excised and suspended vertically in a 200-ml jacketed reservoir with Krebs-Henseleit bicarbonate buffer solution (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) maintained at 29°C with a thermostatic water circulator.¹⁸ Preparations were field stimulated at 10 pulses/min 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 7.40. 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. The extracellular calcium concentration ($[Ca^{2+}]_o$) was decreased from 2.5 mM to 0.5 mM because rat myocardial contractility is nearly maximum at 2.5 mM.¹⁹ Halothane (Fluotec 3; Cyprane Ltd., Keighley, United Kingdom) and sevoflurane (Sevotec 3; Ohmeda, West Yorkshire, United Kingdom) were added to the carbon dioxide–oxygen mixture using calibrated vaporizers, as previously described.^{18,20} Anesthetic concentrations in the gas phase were measured continuously using an infrared calibrated analyzer (Artema MM206; Taema, Antony, France). Halothane concentrations ranged from 0.3 to 1.5 vol%, and those of sevoflurane ranged from 0.7 to 3.6 vol%, equivalent to 0.5–2.5 MAC in the healthy rat.^{18–20} A correction factor of –23% was applied to estimate real MAC values in diabetic rats.¹³ The correction factor for isoflurane (–17%) was also applied for sevoflurane.¹³ A 20-min equilibration period was allowed between each anesthetic concentration.

The electromagnetic lever system has been described previously.¹⁹ All analyses were made from digital records of force and length obtained with a computer. Conven-

tional mechanical variables 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 with a critical damping. The third twitch was fully isometric at L_{\max} . We determined the maximum unloaded shortening velocity (V_{\max}) using the zero-load technique, and we determined maximum shortening ($_{\max}Vc$) and lengthening ($_{\max}Vr$) velocities and time to peak shortening of the twitch with preload only. In addition, the maximum isometric active force normalized per cross-sectional area (AF), the peaks of the positive ($+dF \cdot dt^{-1}$) and the negative ($-dF \cdot dt^{-1}$) force derivatives at L_{\max} normalized per cross-sectional area, and the time to peak force and time to half relaxation from the isometric twitch were recorded. Because changes in the contraction phase induce coordinated changes in the relaxation phase, indexes of contraction–relaxation coupling have therefore been developed to study lusitropy.²¹ Therefore, the R1 coefficient ($=_{\max}Vc/_{\max}Vr$) studies the coupling between contraction and relaxation under low load and thus lusitropy, in a manner that is independent of inotropic changes. R1 tests SR uptake function.^{18,21} The R2 coefficient ($= +dF \cdot dt^{-1}/-dF \cdot dt^{-1}$) studies the coupling between contraction and relaxation under high load and thus lusitropy, in a manner that is less dependent on inotropic changes.¹⁸

During rest in the rat myocardium, SR accumulates calcium, and the first beat after the rest interval is more forceful than the last beat before the rest interval.¹⁸ AF during postrest recovery was studied at a $[Ca^{2+}]_o$ of 0.5 mM and after a 1-min rest duration. 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.

Myofilament Calcium Sensitivity

Ventricular fiber bundles (approximately 200 μ m in diameter) were dissected from papillary muscles of the left ventricle in a zero- Ca^{2+} Krebs solution (pH 7.40) and were incubated for 1 h in a relaxing solution (see next paragraph) containing 1% Triton X-100 to dissolve the membranes without affecting the contractile proteins. After the skinning procedure, one bundle was immersed in a 0.8-ml chamber filled with the relaxing solution and mounted between a fixed end and a force transducer (Grass Model FT-03C; Quincy, MA), adjusted to slack length, stretched by 20%, and subjected to an activation–relaxation cycle. The muscle contracture was amplified on a differential amplifier (Biologic Amplifier 120; BioScience, Washington, DC) and was recorded on a Gould TA240 recorder (Gould Instruments, Valley View, OH). The length and diameter of the muscles were measured by use of a graticule in the dissecting microscope. The sarcomere length in our setup was verified by a cali-

Table 1. Characteristics of Control and Diabetic Rats

Variable	Control (n = 17)	Diabetes (n = 19)
Body weight, g	347 ± 11	202 ± 29*
Heart weight, mg	855 ± 37	542 ± 86*
Heart weight/body weight, mg/g	2.5 ± 0.1	2.7 ± 0.2
Blood glucose, mM	10 ± 1	50 ± 10*
Blood bicarbonates, mM	30 ± 1	30 ± 1

Values are presented as mean ± SD.

* $P < 0.05$ vs. control rats.

brated micrometer for several bundles in each experimental group under a $\times 400$ Zeiss lens (Carl Zeiss, Oberkochen, Germany). Values ranged between 2.2 and 2.4 μm for all bundles tested. For all experiments described below, the length of the fibers was kept constant to avoid sarcomere length-dependence changes in Ca^{2+} sensitivity. All experiments were performed at constant temperature (22°C).

The relaxing solution contained 10 mM MOPS (3-[N-morpholino]propanesulfonic acid), 170 mM K-propionate, 2.5 mM Mg-acetate, and 5 mM $\text{K}_2\text{-EGTA}$. Activating solutions had the same composition as the relaxing solution except that $\text{Ca}^{2+}\text{-EGTA}$ was substituted for $\text{K}_2\text{-EGTA}$ at various ratios. The concentrations of the different components in the solutions were calculated using program 3 of Fabiato and Fabiato to keep the ionic strength at 200 mM. The following stability constants were used in the calculations: $K_{\text{CaEGTA}} 1.919 \times 10^6 \cdot \text{M}^{-1}$, $K_{\text{CaATP}} 5.0 \times 10^3 \cdot \text{M}^{-1}$, $K_{\text{MgEGTA}} 40 \text{ M}^{-1}$, and $K_{\text{MgATP}} 1.0 \times 10^4 \cdot \text{M}^{-1}$.²² Solution also contained adenosine triphosphate (ATP; 2.5 mM) and phosphocreatine (10 mM), and pH was 7.00 ± 0.01 . Free Ca^{2+} concentrations of activating solutions ranged from pCa 6.2 ($[\text{Ca}^{2+}] = 0.63 \mu\text{M}$) to pCa 4.6 ($[\text{Ca}^{2+}] = 25.0 \mu\text{M}$, maximally activating solution), where $\text{pCa} = -\log_{10} [\text{Ca}^{2+}]$. All chemicals were obtained from Sigma Chemical Co.

To assess the effects of halothane and sevoflurane, the test solutions were equilibrated by continuous bubbling for 20 min with the chosen anesthetic agent. Halothane and sevoflurane were mixed with 100% nitrogen by means of calibrated vaporizers. The anesthetic concentrations in the gas phase were monitored with an infrared calibrated analyzer. The anesthetic concentrations used were 0.65 vol% and 1.80 vol% for halothane and sevoflurane, respectively. These concentrations were chosen to obtain anesthetic concentrations in the experimental solutions (22°C) roughly equivalent to those obtained using 0.90 vol% halothane and 2.20 vol% sevoflurane at 29°C (the temperature used in the papillary muscle experiments).

For each skinned cardiac bundle, a pCa-tension curve was obtained in baseline conditions by stepwise exposure of the fibers to solutions with increasing Ca^{2+} concentrations and measurements of developed tension. Ca^{2+} concentrations ranged from pCa 6.2 to pCa 4.6.

Intermediate tensions were expressed as a percentage of the maximal tension obtained at pCa 4.6. Data were fitted using nonlinear fit of the Hill equation (Enzfitter 1.05; Biosoft, Cambridge, United Kingdom). The slope coefficient (nH) as well as the pCa for half maximal tension (pCa_{50}) were calculated for each bundle.

Both anesthetics were then tested on the same fiber. Hence, pCa-tension curves were obtained for each anesthetic in a random order in each fiber. A final pCa-tension curve was obtained with solutions free of anesthetics. Because maximal activated tension decreased regularly in some fibers during the study and represented 75–95% of the initial developed force at the end of the overall experiment, tension values were normalized to their maximal value for each pCa-tension curve, and the mean value of the two control curves was used to assess the effects of each anesthetic studied.

In a second series of experiments, changes of tension at maximal Ca^{2+} -activated force were examined using a pCa 4.6 solution. Each fiber was exposed in random order to test solutions equilibrated with halothane or sevoflurane. Each test was immediately preceded and followed by determination of maximal Ca^{2+} -activated tension with the control test (*i.e.*, free of anesthetic) so that no significant differences between controls were observed. Isometric tension development from baseline to steady state was compared between test solutions and the mean of the two control measurements. Results

Table 2. Baseline Mechanical Variables of Papillary Muscles in Control and Diabetic Rats

Variable	Control (n = 16)	Diabetes (n = 16)
Characteristics		
L_{max} , mm	7.2 ± 1.1	6.4 ± 1.5
CSA, mm^2	0.62 ± 0.14	0.57 ± 0.08
RF/TF	0.10 ± 0.04	0.12 ± 0.04
Contraction		
$V_{\text{max}}, L_{\text{max}}/\text{s}$	3.10 ± 0.38	2.58 ± 0.15*
$_{\text{max}}Vc, L_{\text{max}}/\text{s}$	2.32 ± 0.34	1.80 ± 0.19*
TPS, ms	182 ± 12	228 ± 13*
AF, mN/mm^2	99 ± 43	86 ± 29
$+dF \cdot dt^{-1}$, $\text{mN} \cdot \text{s}^{-1} \cdot \text{mm}^{-2}$	1,374 ± 639	1,018 ± 394
TPF, ms	157 ± 12	198 ± 17*
Relaxation		
$_{\text{max}}Vr, L_{\text{max}}/\text{s}$	3.22 ± 0.53	3.31 ± 0.53
$-dF \cdot dt^{-1}$, $\text{mN} \cdot \text{s}^{-1} \cdot \text{mm}^{-2}$	505 ± 251	286 ± 93*
THR, ms	167 ± 40	215 ± 38*
Contraction-relaxation coupling		
R1, low load	0.73 ± 0.08	0.55 ± 0.07*
R2, high load	3.04 ± 0.90	3.61 ± 1.19

Values are presented as mean ± SD.

* $P < 0.05$ vs. control rats.

AF = isometric active force normalized per CSA; CSA = cross-sectional area; $+dF \cdot dt^{-1}$ = peak of the positive force derivative normalized per CSA; $-dF \cdot dt^{-1}$ = peak of the negative force derivative normalized per CSA; L_{max} = initial length; $_{\text{max}}Vc$ = maximum shortening velocity; $_{\text{max}}Vr$ = maximum lengthening velocity; R1 = $_{\text{max}}Vc/_{\text{max}}Vr$; R2 = $(+dF \cdot dt_{\text{max}}^{-1})/ -dF \cdot dt_{\text{max}}^{-1}$; RF/TF = ratio of resting force to total force; THR = time to half isometric relaxation; TPF = time to peak force; TPS = time to peak shortening; V_{max} = maximum unloaded shortening velocity.

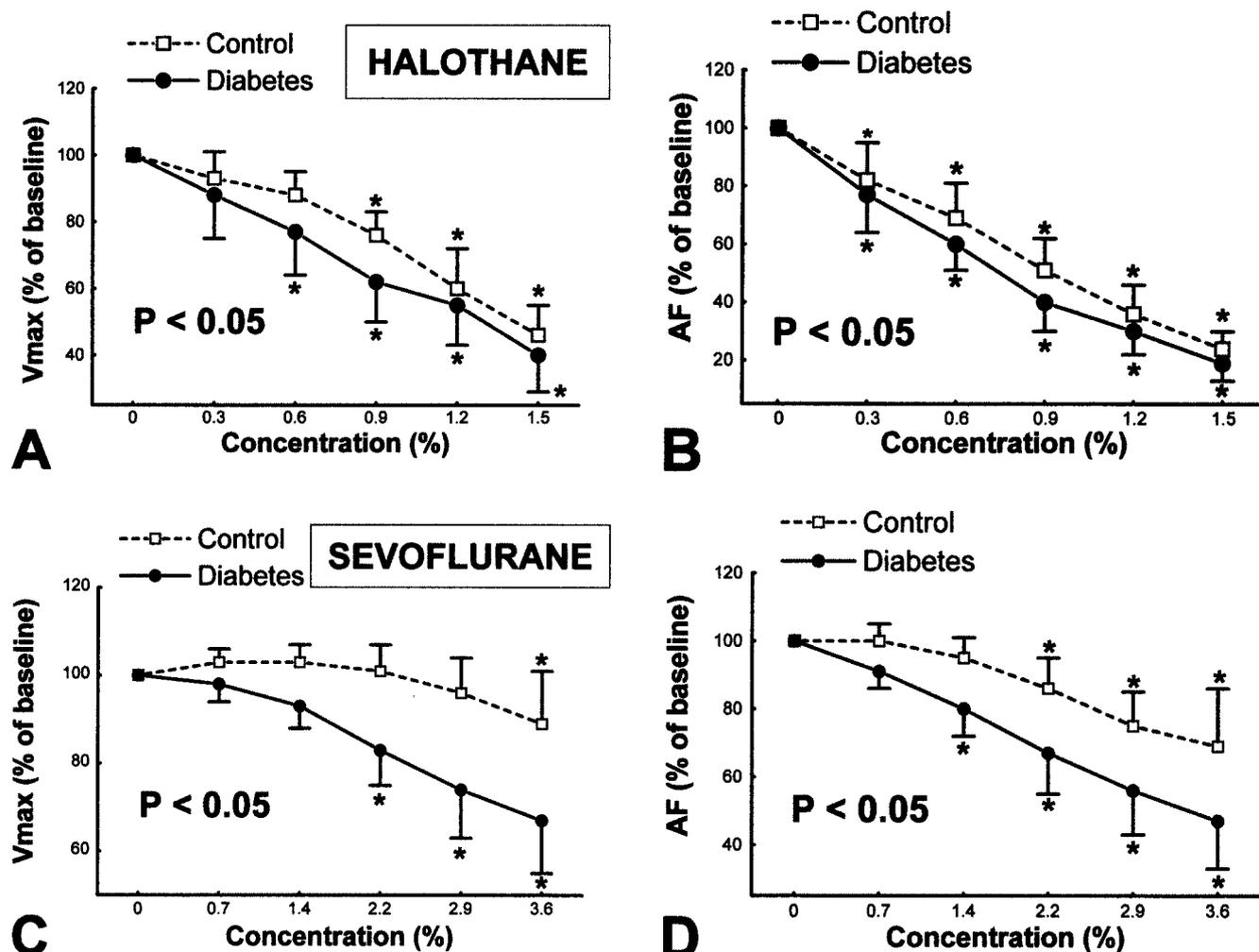


Fig. 1. Inotropic effects of halothane (A and B) and sevoflurane (C and D) under isotonic (maximum unloaded shortening velocity [V_{max}]) and isometric conditions (active isometric force normalized per cross-sectional area [AF]). Data are presented as mean percent of baseline \pm SD. P values refer to between-groups difference. * $P < 0.05$ versus baseline values.

were expressed as a percentage of these corresponding control values.

Statistical Analysis

Data are expressed as mean \pm SD. Comparison of two means was performed using the Student t test. Comparison of several means was performed using analysis of variance and the Newman-Keuls test. All P values were two-tailed, and a P value less than 0.05 was required to reject the null hypothesis. Statistical analysis was performed with NCSS 6.0 software (Statistical Solutions Ltd., Cork, Ireland).

Results

Papillary muscles and triton-skinned fibers were obtained from 17 control rats and 19 diabetic rats. Diabetic rats had significantly lower body and heart weight than control rats, but the ratio of the heart to body weight was not significantly different, indicating that no cardiac

hypertrophy occurred. Blood glucose concentrations were five times higher in diabetic rats than in control rats (table 1).

Papillary Muscles

The mean L_{max} and the mean ratio of resting force to total force were not significantly different between groups (table 2). We observed that shortening velocities in isotonic conditions were decreased in diabetic rats, whereas active force was not significantly modified compared with that of control rats (table 2). Prolongation of duration of contraction was observed in diabetic rats, as shown by the prolongation of time to peak force in isometric conditions and time to peak shortening in isotonic conditions. Time to half relaxation was also prolonged in diabetic rats (table 2). We observed significant differences in contraction-relaxation coupling in isometric (R2) but not in isotonic (R1) conditions.

A decrease in contractility was observed as $[Ca^{2+}]_o$ was decreased from 2.5 mM to 0.5 mM. The decrease in

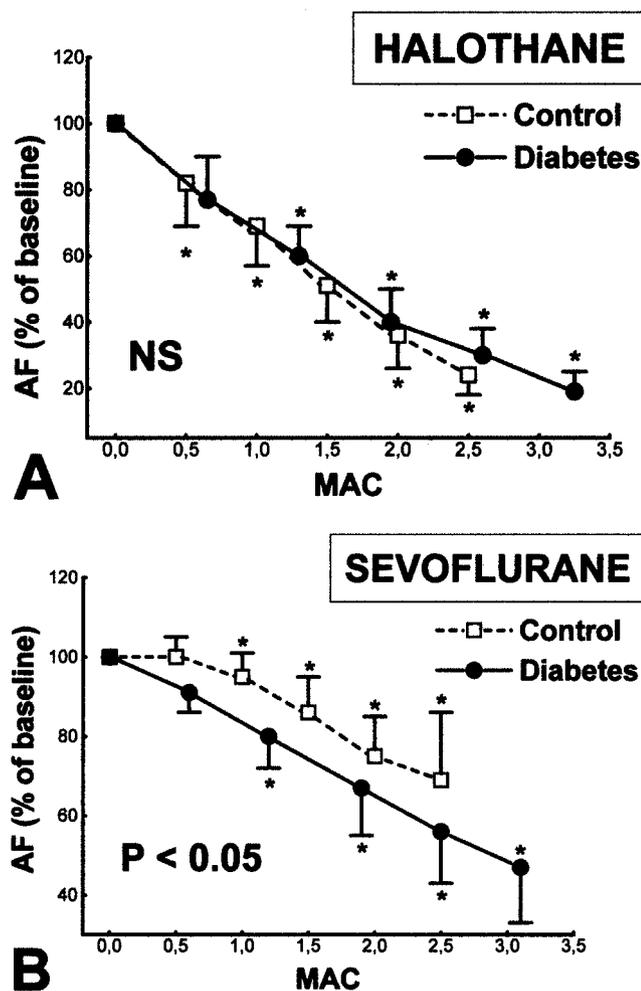


Fig. 2. Effects of halothane (A) and sevoflurane (B) on maximum isometric active force normalized per cross-sectional area (AF) according to the minimum alveolar concentration (MAC) in papillary muscle from control ($n = 8$) and diabetic ($n = 8$) hearts. Data are presented as mean percent of baseline \pm SD. P values refer to between-groups difference. * $P < 0.05$ versus baseline values. NS = not significant.

V_{\max} was not significantly different between control and diabetic rats (64 ± 8 vs. $69 \pm 5\%$ of the value at a $[\text{Ca}^{2+}]_o$ of 2.5 mM, not significant), but the decrease in AF was more pronounced in control rats (53 ± 10 vs. $65 \pm 10\%$ of the value at a $[\text{Ca}^{2+}]_o$ of 2.5 mM, $P < 0.05$). At a $[\text{Ca}^{2+}]_o$ of 0.5 mM, there was no significant difference between groups in baseline values of V_{\max} , AF, and R1, but R2 was significantly higher in diabetic rats in the halothane (2.56 ± 0.39 vs. 1.98 ± 0.36 , $P < 0.05$) and sevoflurane groups (2.66 ± 0.62 vs. 2.04 ± 0.35 , $P < 0.05$). At a $[\text{Ca}^{2+}]_o$ of 0.5 mM, the postrest potentiation was significantly impaired in diabetic rats (117 ± 9 vs. $163 \pm 25\%$ of AF, $P < 0.05$).

In control rats, halothane induced a marked negative inotropic effect that was significantly greater than that of sevoflurane, under both isotonic and isometric conditions (fig. 1). At the highest concentration, AF was $24 \pm 6\%$ of baseline values for halothane and $69 \pm 17\%$ of

baseline values for sevoflurane. In diabetic rats, the negative inotropic effects of halothane and sevoflurane were significantly more pronounced (fig. 1). At the highest concentration, AF was $19 \pm 6\%$ of baseline values for halothane and $47 \pm 14\%$ of baseline values for sevoflurane. Because the MAC of halogenated anesthetics is lower in diabetic rats, we also plotted the inotropic effect as a function of MAC values in each group, as previously reported.¹² At equipotent anesthetic concentrations, the negative inotropic effect of halothane was no longer significantly different between the two groups, in contrast with sevoflurane (fig. 2).

Under isotonic conditions and in control rats, halothane induced a significant negative lusitropic effect (increase in R1), whereas sevoflurane did not (fig. 3). There were no significant differences between control and diabetic rats (fig. 3).

Under isometric conditions and in control rats, halothane induced a significant positive lusitropic effect (decrease in R2), whereas sevoflurane did not (fig. 3). At the highest concentration, R2 was $78 \pm 11\%$ of baseline values ($P < 0.05$) for halothane and $90 \pm 12\%$ of baseline values (not significant) for sevoflurane. These effects were significantly more pronounced in diabetic rats (fig. 3). At the highest concentration, R2 was $64 \pm 11\%$ of baseline values ($P < 0.05$) for halothane and $79 \pm 13\%$ of baseline values for sevoflurane ($P < 0.05$).

In control rats, halothane did not significantly modify the postrest potentiation, whereas sevoflurane enhanced it (fig. 4). These effects were not significantly different between control and diabetic rats (fig. 4).

Myofilament Calcium Sensitivity

Experiments were performed in 17 skinned fibers (length, 1.72 ± 0.67 mm; diameter, 212 ± 62 μm) from 8 diabetic rats and 14 fibers (length, 1.84 ± 0.31 mm; diameter, 204 ± 63 μm) from 7 control rats. In baseline conditions (*i.e.*, in the absence of anesthetics), maximal Ca^{2+} -activated tension and pCa_{50} were not significantly different between diabetic and control rats (table 3). In both groups, halothane and sevoflurane produced a decrease in maximal developed tension and in pCa_{50} , with no significant difference between anesthetic agents (table 3). However, while their effects on maximal tension were moderate and identical in the two groups, myofilament Ca^{2+} sensitivity decreased to a greater extent in fibers from diabetic rats than in those from control rats (figs. 5A and B). This was attested by a larger decrease in pCa_{50} with each anesthetic in fibers from diabetic rats as compared with those from control rats, in the presence of halothane and sevoflurane (fig. 5C).

Discussion

In the current study, we showed that (1) halothane and sevoflurane induced a greater negative inotropic

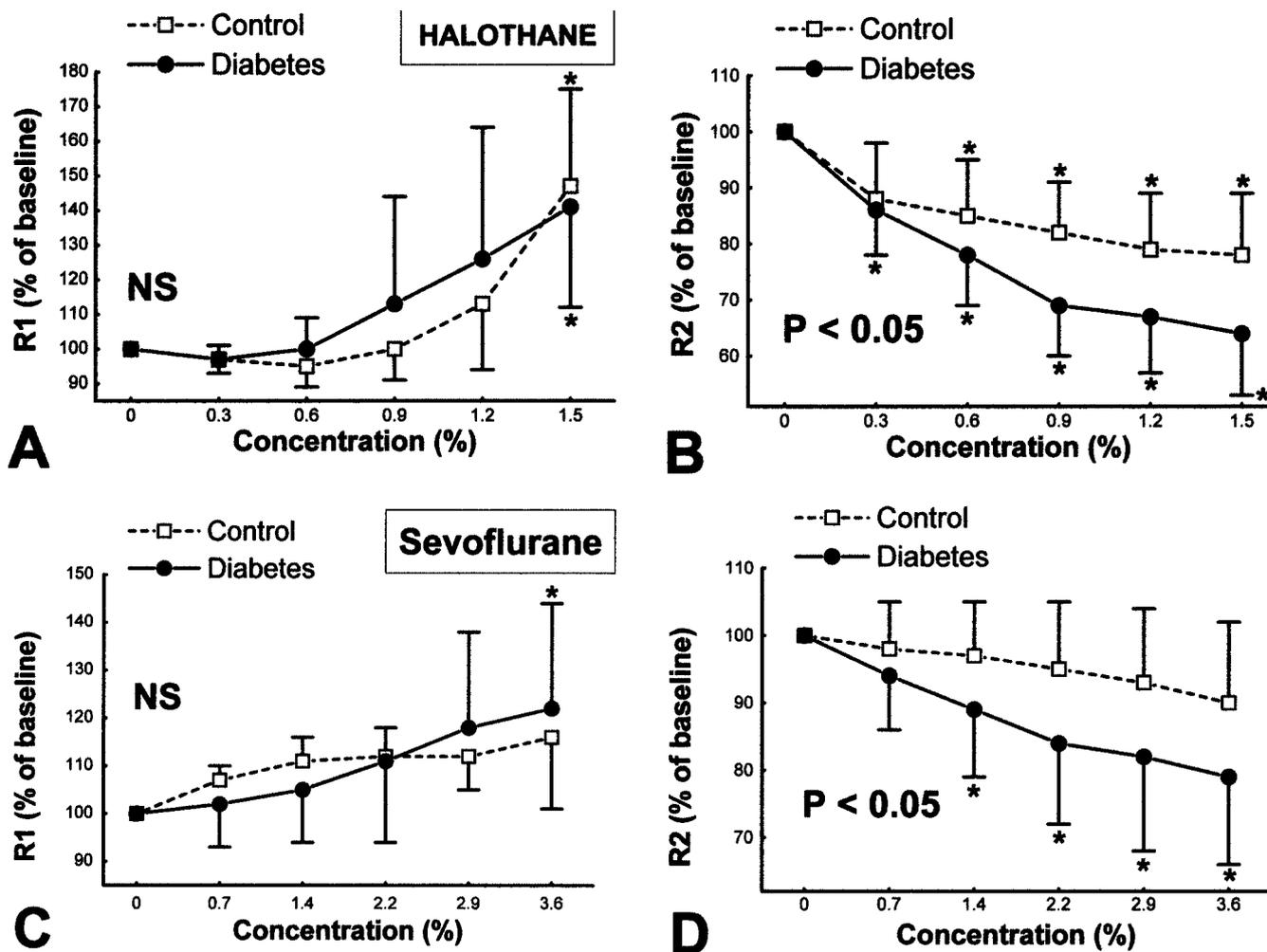


Fig. 3. Lusitropic effects of halothane (A and B) and sevoflurane (C and D) under low (R1) and high (R2) loads. R1 = ratio of maximum shortening velocity ($V_{\max}V_c$) to maximum lengthening velocity ($V_{\max}V_r$); R2 = ratio of the peak of the positive force derivative ($+dF \cdot dt^{-1}$) to the peak of the negative force derivative ($-dF \cdot dt^{-1}$). Data are presented as mean percent of baseline \pm SD. * $P < 0.05$ versus baseline values. P values refer to between-groups difference. NS = not significant.

effect in diabetic myocardium, but these differences were no longer significant for halothane when differences in MAC values were taken into account; (2) although the lusitropic effect under low load did not differ, the lusitropic effect under high load differed significantly between control and diabetic rats; (3) in both groups, halothane did not modify postrest potentiation, whereas sevoflurane enhanced it; and (4) halothane and sevoflurane decreased myofilament Ca^{2+} sensitivity to a greater extent in diabetic than in control rats.

We observed important alterations in the myocardium of diabetic rats, as previously reported.⁵ We observed a decrease in V_{\max} without change in AF, which was associated with a marked prolongation of the contraction phase (table 2). According to the Huxley theory, the absence of modification in AF suggests that there is no change in the total number of actomyosin cross-bridges, whereas the diminution in V_{\max} suggests a decrease in ATPase actomyosin activity.²³ An isomyosin shift from V1 (α dimer) to V3 (β dimer) has been consistently

reported in diabetic myocardium,²⁴ which results in a decreased ATPase activity and a decline in V_{\max} .²⁵ Prolongation of contraction has also been consistently reported in diabetic myocardium and could be related to a slower cross-bridge cycling rate and slower Ca^{2+} release from the SR. In our study, we also observed that postrest potentiation was markedly altered in diabetic myocardium (fig. 4), suggesting abnormalities of the cardiac SR calcium release channel (*i.e.*, ryanodine receptor), a decrease in the capacity of the SR to accumulate calcium during rest, or both. Abnormalities of SR, particularly those of the ryanodine receptor, have been already reported during diabetes.^{3,4}

We noted that relaxation was markedly prolonged only in isometry (increase in time to half relaxation), suggesting discrepancies between isotonic and isometric conditions (table 2). The results observed in isotonic conditions conflict with those seen in previous studies because they suggest that there was no modification or improvement in relaxation. It has been shown that ab-

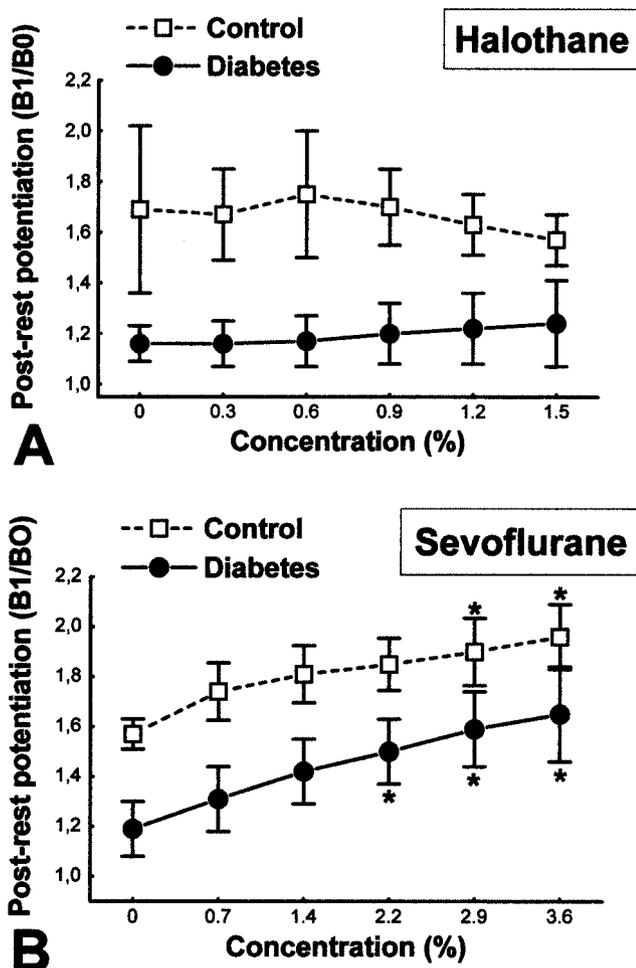


Fig. 4. Effects of halothane (A) and sevoflurane (B) on postrest potentiation in control (n = 6) and diabetic (n = 6) rats. AF = active isometric force normalized per cross-sectional area; B0 = last beat before rest; B1 = first beat after rest. Because baseline values were significantly different (P < 0.05), the between-group differences were significant (P < 0.05), but the interactions for halothane and sevoflurane were not significantly different, indicating that the response to halogenated anesthetic agents was not significantly modified between control and diabetic rats. Values are presented as mean ± SD. *P < 0.05 versus baseline values.

normalities of the SR in diabetic myocardium are gradual and sequential. Zhong *et al.*¹¹ showed that sarco(endo)plasmic reticulum ATPase protein concentration was unchanged in 4-week but decreased in 6-week diabetic rat hearts. Therefore, it is probable that in our 4-week streptozotocin-treated rats, modifications in SR were not sufficient to explain the discrepancies between isotony and isometry. A modification of Ca²⁺ sensitivity, as assessed by R2 and pCa₅₀, was probably not responsible for these discrepancies because we had shown that myofilaments calcium sensitivity was not different between diabetic and control rats (tables 2 and 3).

Halogenated anesthetic agents induce myocardial depression through different mechanisms, including Ca²⁺ homeostasis (inhibition of L-type Ca²⁺ channels and Na⁺-Ca²⁺ exchanger,^{26,27} sarcolemmal Ca²⁺-ATPase

Table 3. Effects of Halothane (0.65%) and Sevoflurane (1.80%) on Myofilament Calcium Sensitivity in Control and Diabetic Rats

	Control	Diabetes
AT _{max} , mN/mm ²		
Baseline	70 ± 18	69 ± 11
Halothane	62 ± 21*	65 ± 12*
Sevoflurane	65 ± 22*	64 ± 10*
pCa ₅₀ , log[Ca ²⁺]		
Baseline	5.72 ± 0.18	5.68 ± 0.11
Halothane	5.68 ± 0.23*	5.52 ± 0.11*
Sevoflurane	5.67 ± 0.23*	5.55 ± 0.12*
nH		
Baseline	1.50 ± 0.30	1.51 ± 0.30
Halothane	1.62 ± 0.28	1.97 ± 0.76*
Sevoflurane	1.51 ± 0.18	1.62 ± 0.44

Values are presented as mean ± SD, from 10 (maximal activated tension [AT_{max}]) and 8 (Ca²⁺ concentration for half-maximal tension [pCa₅₀]) and Hill coefficient [nH]) fibers from control rats, and 12 (AT_{max}) and 11 (pCa₅₀ and nH) fibers from diabetic rats.

* P < 0.05 vs. baseline.

and SR functions) and changes in Ca²⁺ sensitivity or cross-bridge cycling.^{16,28} We found that halothane was the more potent negative inotropic agent, as previously described,^{20,29} and that the effects of halogenated agents were more pronounced in diabetic rats (fig. 1). This suggests a greater sensitivity of diabetic myocardium for the same concentration of halogenated agent, although the mechanism for this difference remains unclear. It is interesting to note that diabetes mellitus and halogenated anesthetics share a common target (Ca²⁺ homeostasis, mitochondria, Ca²⁺ sensitivity and cross-bridge cycling) and that diabetes could have potentiated the action of halogenated anesthetics. For example, halothane might have accentuated depletion of SR calcium stores seen in diabetes by increasing the opening duration of the ryanodine receptor or may have depleted the amount of ATP available in the cell. In our study, we observed that, when [Ca²⁺]_o was decreased to 0.5 mM, AF decreased less in diabetic than in control rats, suggesting a calcium overload of the cytoplasm. It has been suggested that calcium overload occurs in diabetes.^{4,30} Because diabetes might alter SR uptake of Ca²⁺ in rat myocardium,³¹ it may have contributed to the negative inotropic effect of halogenated anesthetics in diabetes. However, it is difficult to define the contribution of each component implied in the different mechanisms responsible of the action of halogenated because our experimental model does not allow us to study directly L-type Ca²⁺ channels or the Na⁺-Ca²⁺ exchanger. Nevertheless, the study gave some insight into the SR and the sensitivity of myofilaments to calcium (table 3 and fig. 5). The fact that the responses of isotonic relaxation and postrest potentiation to halogenated anesthetic agents were not significantly modified in diabetic rats suggests that the SR is not the target and explains the observed

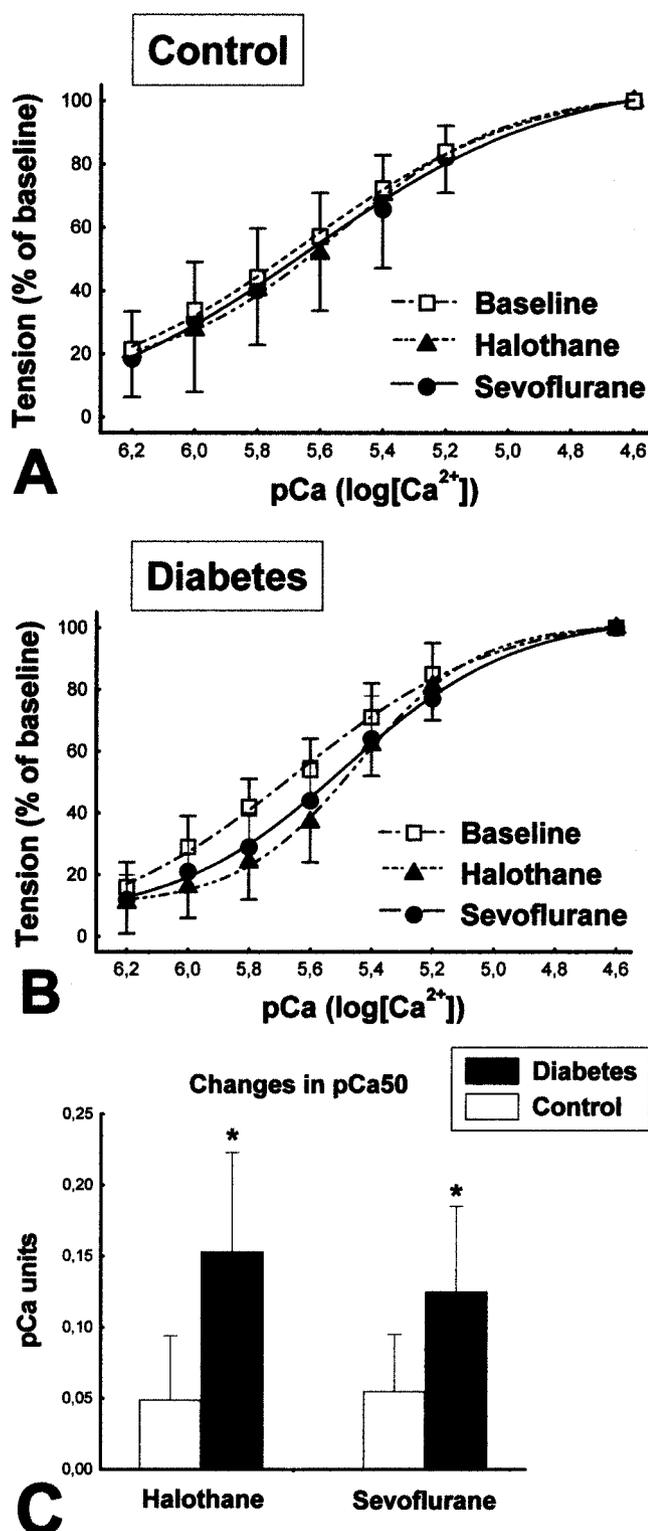


Fig. 5. Effects of halothane and sevoflurane on isometric tension-pCa relations in triton-skinned cardiac fibers from control (A; $n = 8$) and diabetic (B; $n = 11$) rats. A shift toward lower pCa values indicates a decrease in Ca^{2+} sensitivity of the contractile proteins. The decrease in pCa for half maximal tension (pCa_{50}) observed with each anesthetic was larger in fibers from diabetic rats (C). Values are presented as mean \pm SD. * $P < 0.05$ versus control group.

difference in the inotropic effect of halogenated anesthetics.

The absence of difference in Ca^{2+} sensitivity and maximal Ca^{2+} -activated tension between diabetic and control skinned fibers in basal conditions is in accord with the findings of recent studies.³⁰ Different results have also been reported,⁴ but the reason for these discrepancies is not clear. Nevertheless, the main finding of our experiments in skinned fibers was that the decrease in Ca^{2+} sensitivity produced by each anesthetic agent was more important in diabetic rats. This effect was also suggested by the more important decrease in R2 observed in diabetic rats (fig. 3),²¹ although changes in R2 are complex and influenced by the changes in force and not only by myofilament calcium sensitivity.¹⁸ This result may reflect a faster rate of release of Ca^{2+} by troponin on withdrawal of Ca^{2+} in diabetic myocardium or could be the result of a modification of the ratio of the two troponin T isoforms.¹⁵ Taken together, these results strongly suggest that volatile anesthetics alter the function of regulatory proteins (*i.e.*, the troponin-tropomyosin complex) to a greater extent in diabetic than in normal hearts. Murat *et al.*¹⁴ found no difference in the decrease in myocardial Ca^{2+} sensitivity produced by halothane in control and diabetic animals. The apparent discrepancy with our results may be accounted for by differences in halothane concentrations used. The effects of volatile anesthetics on Ca^{2+} sensitivity of cardiac skinned fibers have been shown to be dose dependent, with a major shift occurring at the lowest concentrations, suggesting some sort of saturating phenomenon.³² It is therefore conceivable that the difference between diabetic and control fibers observed using 0.65% halothane (in the current study) might have been missed with the higher concentrations (1 and 2%) used by Murat *et al.*¹⁴ Finally, a significant increase in the Hill coefficient was observed during halothane exposure in diabetic rats. This reflected the fact that halothane effects were more important at low Ca^{2+} concentrations, as found in other studies.³³ The reason for this observation remains unclear because Hill coefficients have no simple relation to the number of Ca^{2+} binding sites and cannot be attributed simply to the binding properties of the troponin complex.

The expression of AF as functions of MAC showed that the negative inotropic effects of halothane were not significantly different between control and diabetic rats, whereas the difference remained significant for sevoflurane. Because little information is available about anesthetic potency in diabetes, we believe that it is important to report our results both from a pharmacologic (fig. 1) and a clinical perspective (fig. 2).¹²

The following points must be considered when assessing the clinical relevance of our results. First, this *in vitro* study only dealt with intrinsic myocardial contractility. Observed changes in cardiac function also depend

on modifications in venous return, afterload, and compensatory mechanisms. Second, this study was conducted at low temperature and at a low-stimulation frequency. However, papillary muscles and skinned fibers must be studied at low temperature because stability of mechanical parameters is not sufficient at 37°C, and papillary muscles must be studied at a low frequency because high-stimulation frequency induces core hypoxia.³⁴ Third, this study was performed in rat myocardium, which differs from human myocardium, and the effects of halogenated anesthetics can differ between species.³⁵ Nevertheless, the myocardial effects of volatile anesthetics on the myocardium seem to be similar between rats and humans.^{20,29}

In conclusion, we found that the myocardial effects of halogenated anesthetics were more significant in diabetic rats, and these differences are probably related to a greater decrease in Ca²⁺ sensitivity produced by each anesthetic agent.

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