

# Interaction of Halogenated Anesthetics with $\alpha$ - and $\beta$ -Adrenoceptor Stimulations in Diabetic Rat Myocardium

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**Background:** Halogenated anesthetics potentiate the positive inotropic effects of  $\alpha$ - and  $\beta$ -adrenoceptor stimulations. Although diabetes mellitus induces significant myocardial abnormalities, the interaction of halogenated anesthetics and adrenoceptor stimulation in diabetic myocardium remains unknown.

**Methods:** Left ventricular papillary muscles were provided from healthy and streptozotocin-induced diabetic rats. Effects of 1 minimum alveolar concentration halothane, isoflurane, and sevoflurane on the inotropic and lusitropic responses of  $\alpha$  (phenylephrine)- and  $\beta$  (isoproterenol)-adrenoceptor stimulations were studied at 29°C with 12 pulses/min. Data shown are mean percentage of baseline active force  $\pm$  SD.

**Results:** Phenylephrine induced comparable positive inotropic effects in healthy and diabetic rats ( $143 \pm 8$  vs.  $136 \pm 18\%$ ; not significant), but the potentiation by halogenated anesthetics was abolished in the diabetic rats ( $121 \pm 20$ ,  $130 \pm 20$ , and  $123 \pm 20\%$  for halothane, isoflurane, and sevoflurane, respectively; not significant). In diabetic rats, the positive inotropic effect of isoproterenol was markedly diminished ( $109 \pm 9$  vs.  $190 \pm 18\%$ ;  $P < 0.05$ ), but its potentiation was preserved with isoflurane ( $148 \pm 21\%$ ;  $P < 0.05$ ) and sevoflurane ( $161 \pm 40\%$ ;  $P < 0.05$ ) but not with halothane ( $126 \pm 16\%$ ; not significant). Halothane induced a deleterious effect on the sarcoplasmic reticulum, as shown by its impairment in the lusitropic effect of isoproterenol, compared with isoflurane and sevoflurane.

**Conclusion:** Potentiation of the positive inotropic effect of  $\alpha$ -adrenoceptor stimulation by halogenated anesthetics is abolished in diabetic rats. In contrast, potentiation of  $\beta$ -adrenoceptor stimulation is preserved with isoflurane and sevoflurane but not with halothane, probably because of its deleterious effects on sarcoplasmic reticulum.

DIABETES mellitus is frequently associated with the development of cardiovascular diseases, and the development of a specific cardiomyopathy, which is independent of coronary artery disease, valve disease, and hypertension, is well established.<sup>1</sup> Diabetic cardiomyopathy results from a variety of alterations involving the

sarcoplasmic reticulum (SR),<sup>2</sup> calcium channels and intracellular calcium metabolism,<sup>3-5</sup> sodium-calcium exchange,<sup>6</sup> mitochondria,<sup>7</sup> and contractile proteins.<sup>8</sup> These anomalies lead to inotropic and lusitropic alterations, mainly slowing of contraction and relaxation velocities without significant change in developed tension.<sup>9</sup>

An increase in sympathetic drive represents an important mechanism for maintaining cardiac output in the compromised diabetic heart. Several studies have documented a depressed response of the myocardium to  $\beta$ -adrenoceptor stimulation,<sup>10</sup> but the literature is conflicting with reports of both depressed<sup>11</sup> and enhanced<sup>12</sup> responses to  $\alpha$ -adrenoceptor stimulation. These changes have been correlated with a decrease in the function of  $\alpha_1$  and  $\beta_1$  adrenoceptors<sup>10,11</sup> and an increase in the function of  $\beta_3$  adrenoceptors.<sup>13</sup>

During anesthesia, patients with diabetes have an increased incidence of intraoperative hypotension.<sup>14</sup> We have recently demonstrated that the negative inotropic effects of halogenated anesthetics are greater in diabetic rats, mainly because of a decrease in myofilament calcium sensitivity.<sup>15</sup> However, the myocardial effects of halogenated anesthetics also involve indirect effects, and it is known that halogenated anesthetics potentiate the positive inotropic effects of  $\alpha$ - and  $\beta$ -adrenoceptor stimulations.<sup>16,17</sup> This potentiation results from the Gi protein inhibition induced by halogenated anesthetics.<sup>18</sup> The interaction between halogenated anesthetics and  $\alpha$ - and  $\beta$ -adrenoceptor stimulation in diabetes remains unknown, whereas Gi proteins are markedly down-regulated in diabetic rat myocardium.<sup>10</sup> Therefore, this experimental study was designed to analyze the interaction between volatile anesthetics and  $\alpha$ - and  $\beta$ -adrenoceptor stimulation in myocardium from diabetic and control rats.

## Materials and Methods

The study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Helsinki Declaration. Therefore, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (B. R.).

### Animals

Six-week old male Wistar rats (Iffa Credo, L'arbresles, France) were each assigned to one of two groups, a healthy group and a diabetes mellitus group. In the

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Received from the Laboratory of Experimental Anesthesiology, Department of Anesthesiology and Critical Care, and Department of Emergency Medicine and Surgery, Centre Hospitalier Universitaire Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Université Pierre et Marie Curie, Paris, France; the Department of Anesthesiology and Critical Care, Centre Hospitalier Universitaire Edouard Herriot, Hospices Civils de Lyon, Lyon, France; and the Laboratory of Anesthesiology, Equipe d'Accueil 1896, Université Claude Bernard Lyon 1, Lyon, France. Submitted for publication February 13, 2004. Accepted for publication July 13, 2004. Supported by the Department of Anesthesiology, Centre Hospitalier Universitaire Pitié-Salpêtrière, Paris, France, and the Fondation pour la Recherche Médicale, Paris, France.

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diabetic group, streptozotocin (65 mg/kg; Sigma Chemical, L'Isle d'Abeau Chesnes, France) was injected intravenously, and rats were studied 4 weeks later, as previously reported.<sup>2,15</sup> 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 rats experienced diabetes (*i.e.*, blood glucose concentration > 25 mM). At the moment of killing, blood samples were withdrawn from diabetic and control rats and were centrifuged at 5,000g for 15 min, and then the plasma fraction was collected and stored at -20°C for further determination of glucose and bicarbonate concentration (Cobas Integra 400; Roche Diagnostic, Mannheim, Germany).

#### Experimental Protocol

After brief anesthesia with pentobarbital sodium, the hearts were quickly removed, and then 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 MgSO<sub>4</sub>, 1.1 mM KH<sub>2</sub>PO<sub>4</sub>, 25 mM NaHCO<sub>3</sub>, 2.5 mM CaCl<sub>2</sub>, and 4.5 mM glucose) maintained at 29°C with a thermostatic water circulator.<sup>19</sup> Preparations were field stimulated at 12 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 concentration of Ca<sup>2+</sup> was decreased from 2.5 mM to 0.5 mM because rat myocardial contractility is nearly maximal at 2.5 mM. Thereafter, the inotropic responses to either  $\alpha$ - or  $\beta$ -adrenoceptor stimulation were studied in separate groups in the absence or in the presence of halothane, isoflurane, or sevoflurane at 1 minimum alveolar concentration (MAC). Because baseline values differ from one muscle to another, mechanical responses were expressed as a percentage of baseline values, as previously reported.<sup>16,17</sup>

$\alpha$ -Adrenoceptor stimulation was induced with cumulative concentrations of phenylephrine (10<sup>-8</sup> to 10<sup>-4</sup> M) in the presence of propranolol (10<sup>-6</sup> M).  $\beta$ -Adrenoceptor stimulation was induced with cumulative concentrations of isoproterenol (10<sup>-8</sup> to 10<sup>-4</sup> M) in the presence of phentolamine (10<sup>-6</sup> M). The volume of drugs did not exceed 2% of the bath volume. All drugs were purchased from Sigma Chemical.

#### Administration of Halogenated Anesthetics

Halothane (Fluotec 3; Cyprane Ltd., Keighley, United Kingdom), isoflurane (Fortec 3; Cyprane Ltd.), and sevoflurane (Sevotec 3; Ohmeda, West Yorkshire, United Kingdom) were added to the carbon dioxide and oxygen

mixture using calibrated vaporizers, as previously described.<sup>16,17</sup> The gas mixture bubbled continuously in the bathing solution. To minimize evaporation of anesthetic vapors, the jacketed reservoir was covered with a thin paraffin sheet. Anesthetic concentrations in the gas phase were monitored continuously using an infrared calibrated analyzer (Artema MM206; Taema, Antony, France). Halothane, isoflurane, and sevoflurane concentrations used were 0.6, 0.8, and 1.4 vol%, respectively, corresponding to 1 MAC in the adult rat at 29°C.<sup>20</sup> A 30-min period of equilibration with halogenated anesthetics was allowed before  $\alpha$ - or  $\beta$ -adrenoceptor stimulation.

#### Mechanical Parameters

The electromagnetic lever system used in this study has been described previously.<sup>19</sup> All analyses were made from digital records of force and length obtained by computer. 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 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 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 negative ( $-dF \cdot dt^{-1}$ ) force derivative at  $L_{\max}$  normalized per cross-sectional area, the time to peak force, and the time to half relaxation were recorded from the isometric twitch. 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.<sup>19,21</sup> Thus, the R1 coefficient =  $_{\max}Vc / _{\max}Vr$  studies the coupling between contraction and relaxation under low load, and hence lusitropy, in a manner that is independent of inotropic changes.<sup>16</sup> R1 tests SR function.<sup>16,19</sup> The R2 coefficient =  $(+dF \cdot dt^{-1}) / (-dF \cdot dt^{-1})$  studies the coupling between contraction and relaxation under high load, and therefore lusitropy, in a manner that is less depends on inotropic changes.<sup>16</sup>

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

#### Statistical Analysis

Data are expressed as mean  $\pm$  SD. Concentration-response curves were determined by fitting the data to the Hill sigmoid pharmacologic model according to the following equation:

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

**Table 1. Characteristics of Healthy and Diabetic Rats**

	Healthy Rats (n = 38)	Diabetic Rats (n = 39)
Body weight, g	339 ± 18	222 ± 44*
Heart weight, mg	745 ± 74	495 ± 124*
Heart weight/body weight, mg/g	2.2 ± 0.2	2.2 ± 0.3
Serum glucose, mM	12.1 ± 1.8	48.9 ± 6.2*
Sodium bicarbonate, mM	29.0 ± 4.0	25.0 ± 5.0*

Values are presented as mean ± SD.

\*  $P < 0.05$  vs. healthy rats.

in which  $Eff_o$  is the observed effect,  $Eff_{max}$  is the maximum effect,  $C_{50}$  is the concentration that results in 50% of  $Eff_{max}$ , and  $n$  is the Hill coefficient.<sup>16,17</sup> Iterative non-linear regression curve fitting was used to obtain the best fit (Matlab 1.2c software; The MathWorks, South Natick, MA). As the baseline value of AF differs markedly from one papillary muscle to another, it is difficult to show a pharmacologic effect when using absolute values. Therefore, data were expressed as percentage of baseline, as previously reported.<sup>16,17</sup> Comparison of two means was performed using the Student  $t$  test. Comparison of several means was performed using one-way analysis of variance and the Dunnett test. All  $P$  values were two-tailed, and a  $P$  value of less than 0.05 was required to reject the null hypothesis. Statistical analysis was performed with NCSS 2001 software (Statistical Solutions Ltd., Cork, Ireland).

## Results

We studied 38 healthy and 39 diabetic rats. Diabetic rats had significantly lower body and heart weights than healthy rats, but the ratio of heart weight to body weight was not significantly different, indicating that no cardiac hypertrophy had occurred. Blood glucose concentrations were four times higher in diabetic rats than in healthy rats (table 1). We studied 131 left ventricular papillary muscles. The mean ratio of resting force to total force was not significantly different between groups (table 2). The mean  $L_{max}$  and the mean cross-sectional area were significantly lower in diabetic rats. We observed that shortening velocities in isotonic conditions were decreased in diabetic rats, whereas AF was not significantly modified compared with healthy rats (table 2). Prolongation of the duration of contraction was observed in diabetic rats as shown by the prolongation of time to peak shortening in isotonic conditions and time to peak force in isometric conditions. Time to half relaxation was also prolonged in diabetic rats (table 2). In contrast, we did not observe significant differences in contraction-relaxation coupling in isotonic (R1) or in isometric (R2) conditions (table 2). Baseline values (before inotropic stimulation) of  $V_{max}$ , AF, R1, and R2 are shown in table 3.

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

	Healthy (n = 64)	Diabetic (n = 67)
<b>Characteristics</b>		
$L_{max}$ , mm	5.8 ± 1.2	4.6 ± 1.3*
CSA, mm <sup>2</sup>	0.7 ± 0.3	0.6 ± 0.3*
RF/TF	0.14 ± 0.07	0.16 ± 0.05
<b>Contraction</b>		
$V_{max}$ , $L_{max}/s$	3.25 ± 0.28	3.05 ± 0.34*
$_{max}Vc$ , $L_{max}/s$	2.29 ± 0.23	1.83 ± 0.34*
TPS, ms	173 ± 12	207 ± 22*
AF, mN/mm <sup>2</sup>	69 ± 32	66 ± 32
+dF·dt <sup>-1</sup> , mN·s <sup>-1</sup> ·mm <sup>-2</sup>	1,028 ± 564	782 ± 420*
TPF, ms	149 ± 12	181 ± 22*
<b>Relaxation</b>		
$_{max}Vr$ , $L_{max}/s$	3.44 ± 0.66	2.88 ± 0.72*
-dF·dt <sup>-1</sup> , mN·s <sup>-1</sup> ·mm <sup>-2</sup>	356 ± 231	282 ± 129*
THR, ms	295 ± 38	360 ± 56*
<b>Relaxation-contraction coupling</b>		
R1 (low load)	0.68 ± 0.11	0.66 ± 0.15
R2 (high load)	3.00 ± 0.70	2.82 ± 0.83

Values are presented as mean ± SD. Baseline values were obtained at an extracellular calcium concentration of 2.5 mM.

\*  $P < 0.05$  vs. healthy.

AF = isometric active force normalized per CSA; CSA = cross-sectional area; +dF·dt<sup>-1</sup> = peak of the positive force derivative normalized per CSA; -dF·dt<sup>-1</sup> = peak of the negative force derivative normalized per CSA;  $L_{max}$  = initial length;  $_{max}Vc$  = maximum shortening velocity with preload only;  $_{max}Vr$  = maximum lengthening velocity of the twitch with preload only; R1 =  $_{max}Vc/_{max}Vr$ ; R2 = +dF·dt<sup>-1</sup>/-dF·dt<sup>-1</sup>; RF = resting force; RF/TF = ratio of resting force to total force; THR = time of half-relaxation; TPF = time to peak force; TPS = time to peak shortening;  $V_{max}$  = maximum unloaded shortening velocity.

### Effect of $\alpha$ - and $\beta$ -Adrenoceptor Stimulation

We observed a significant positive inotropic effect of  $\alpha$ -adrenoceptor stimulation in healthy rats as well as in diabetic rats, in isotonic and isometric conditions (table 4). There was no significant difference between these two groups.

We observed a significant positive inotropic effect of  $\beta$ -adrenoceptor stimulation in healthy rats. This inotropic effect was markedly diminished in diabetic rats (table 5).

$\beta$ -Adrenoceptor stimulation induced a positive lusitropic effect under low (decrease in R1) and high (decrease in R2) loads in healthy rats. These lusitropic effects were not significantly different in diabetic rats (table 6).

### Interaction of Halogenated Anesthetics

Halogenated anesthetics potentiated the positive inotropic effect of  $\alpha$ -adrenoceptor stimulation, and the magnitude of the potentiation was comparable for halothane, isoflurane, and sevoflurane in healthy rats (table 4 and fig. 1A). This potentiation was abolished in diabetic rats, both in isotonic and isometric conditions, regardless of the halogenated anesthetic used (table 4 and fig. 1B).

Halogenated anesthetics potentiated the positive inotropic effect of  $\beta$ -adrenoceptor stimulation, and the magnitude of the potentiation was comparable with halo-

**Table 3. Baseline Values\* of Mechanical Variables in the Different Groups of Papillary Muscles**

	Healthy Rats					Diabetic Rats				
	n	$V_{\max}$ , $L_{\max}/s$	AF, mN/mm <sup>2</sup>	R1	R2	n	$V_{\max}$ , $L_{\max}/s$	AF, mN/mm <sup>2</sup>	R1	R2
Phenylephrine										
Control	8	2.09 ± 0.24	43 ± 14	0.79 ± 0.07	2.19 ± 0.32	8	2.16 ± 0.33	49 ± 25	0.69 ± 0.09	2.19 ± 0.35
Halothane	8	1.10 ± 0.35	15 ± 7	0.83 ± 0.22	1.62 ± 0.14	10	1.73 ± 0.54	31 ± 9	0.60 ± 0.10	1.91 ± 0.46
Isoflurane	8	1.54 ± 0.36	26 ± 10	0.84 ± 0.15	1.72 ± 0.10	8	2.24 ± 0.33	37 ± 16	0.86 ± 0.24	1.72 ± 0.26
Sevoflurane	8	1.73 ± 0.32	26 ± 10	0.77 ± 0.13	1.66 ± 0.21	8	2.15 ± 0.36	39 ± 26	0.77 ± 0.10	2.04 ± 0.19
Isoproterenol										
Control	8	1.94 ± 0.22	32 ± 9	0.63 ± 0.10	1.87 ± 0.17	8	2.40 ± 0.42	47 ± 24	0.74 ± 0.12	2.39 ± 0.46
Halothane	8	1.10 ± 0.31	15 ± 3	0.86 ± 0.08	1.57 ± 0.12	10	1.53 ± 0.26	24 ± 9	0.51 ± 0.04	1.50 ± 0.13
Isoflurane	8	1.58 ± 0.32	35 ± 18	0.83 ± 0.14	1.59 ± 0.28	8	2.18 ± 0.42	47 ± 24	0.68 ± 0.11	1.93 ± 0.34
Sevoflurane	8	1.66 ± 0.18	23 ± 11	0.79 ± 0.06	1.62 ± 0.19	8	2.03 ± 0.39	52 ± 31	0.66 ± 0.07	1.78 ± 0.37

Values are presented as mean ± SD.

\* Baseline values correspond to those obtained at an extracellular calcium concentration of 0.5 mM and after halogenated anesthetics exposure (1 minimum alveolar concentration) in the halothane, isoflurane, or sevoflurane groups.

AF = isometric active force normalized per cross-sectional area; n = number of papillary muscles; R1 = ratio of maximum shortening velocity ( $v_{\max}$ ) to maximum lengthening velocity ( $v_{\max}$ ); R2 = ratio of peaks of the positive (+dF·dt<sup>-1</sup>) and negative (-dF·dt<sup>-1</sup>) force derivative at  $L_{\max}$ ;  $V_{\max}$  = maximum unloaded shortening velocity.

thane, isoflurane, or sevoflurane in healthy rats (table 5 and fig. 2A). We observed that this potentiation was preserved in diabetic rats with isoflurane and sevoflurane but not with halothane (table 5 and fig. 2B).

In the presence of 1 MAC halothane, isoflurane, or sevoflurane,  $\beta$ -adrenoceptor stimulation induced significant positive lusitropic effects under low and high loads in healthy rats. Under low load, the magnitude of these lusitropic effects was similar regardless of the halogenated anesthetic used in healthy rats (table 6 and fig. 3A). In diabetic rats, the lusitropic effects of  $\beta$ -adrenoceptor stimulation were not significantly different from control with isoflurane and sevoflurane (table 6 and fig. 3B). In contrast, halothane significantly impaired the lusitropic effects under low load of  $\beta$ -adrenoceptor stimulation (table 6 and fig. 3B). Under high load, halogenated anesthetics did not significantly potentiate the positive lusitropic effect of  $\beta$ -adrenoceptor stimulation in healthy or diabetic rats (table 6).

## Discussion

In the current study, we showed that (1) the inotropic response to  $\beta$ -adrenoceptor stimulation was markedly diminished in diabetic rats, whereas that of  $\alpha$ -adrenoceptor stimulation was preserved; (2) the potentiation of the positive inotropic effect of  $\alpha$ -adrenoceptor stimulation with halogenated anesthetics was abolished in diabetic rats; and (3) the potentiation of the positive inotropic effect of  $\beta$ -adrenoceptor stimulation was preserved in diabetic rats with isoflurane and sevoflurane but not with halothane.

Our study revealed important alterations in the myocardium of diabetic rats, as reported previously.<sup>2,15</sup> We observed a decrease in  $V_{\max}$  without change in AF, which was associated with a marked prolongation of the contraction phase. The decrease in  $V_{\max}$  has been associated with an isomyosin shift from V1 ( $\alpha\alpha$  dimer) to V3 ( $\beta\beta$  dimer),<sup>22</sup> whereas the prolongation of contraction

**Table 4. Effects of 1 MAC Halothane, Isoflurane, and Sevoflurane on the Inotropic Responses to  $\alpha$ -Adrenoceptor Stimulation in Healthy and Diabetic Rats**

	Healthy Rats		Diabetic Rats	
	$V_{\max}$	AF	$V_{\max}$	AF
Eff <sub>max</sub> , %				
Control (n = 8 vs. 8)	140 ± 6*	143 ± 8*	140 ± 11*	136 ± 18*
Halothane (n = 8 vs. 10)	166 ± 23*†‡	176 ± 33*†‡	123 ± 21*†	121 ± 20*†
Isoflurane (n = 8 vs. 8)	175 ± 10*†‡	173 ± 18*†‡	132 ± 20*†	130 ± 20*†
Sevoflurane (n = 8 vs. 8)	179 ± 30*†‡	176 ± 35*†‡	134 ± 16*†	123 ± 20*†
C <sub>50</sub> , $\mu$ M				
Control (n = 8 vs. 8)	3.1 ± 4.3	1.5 ± 1.6	7.7 ± 9.2	12.2 ± 26.6
Halothane (n = 8 vs. 10)	1.7 ± 1.9	7.1 ± 14.3	17.7 ± 32.3	4.0 ± 2.5
Isoflurane (n = 8 vs. 8)	1.2 ± 1.4	1.0 ± 0.8	8.5 ± 16.4	4.2 ± 8.5
Sevoflurane (n = 8 vs. 8)	0.9 ± 0.7	1.1 ± 1.7	8.1 ± 17.6	1.7 ± 1.8

Values are presented as mean ± SD.

\*  $P < 0.05$  vs. baseline value. †  $P < 0.05$  vs. healthy rats. ‡  $P < 0.05$  vs. control group.

AF = active force per cross-sectional area; C<sub>50</sub> = concentration of phenylephrine that results in 50% of Eff<sub>max</sub>; Eff<sub>max</sub> = maximum effect in percentage of baseline value; MAC = minimum alveolar concentration;  $V_{\max}$  = maximum unloaded shortening velocity.

**Table 5. Effects of 1 MAC Halothane, Isoflurane, and Sevoflurane on the Inotropic Response to  $\beta$ -Adrenoceptor Stimulation in Healthy and Diabetic Rats**

	Healthy Rats		Diabetic Rats	
	$V_{max}$	AF	$V_{max}$	AF
$Eff_{max}$ , %				
Control (n = 8 vs. 8)	188 ± 13*	190 ± 18*	127 ± 10*†	109 ± 9*†
Halothane (n = 8 vs. 8)	222 ± 34*‡	239 ± 50*‡	139 ± 16*†	126 ± 16*†
Isoflurane (n = 8 vs. 9)	231 ± 27*‡	238 ± 34*‡	159 ± 12*†‡	148 ± 21*†‡
Sevoflurane (n = 8 vs. 8)	230 ± 15*‡	234 ± 25*‡	162 ± 13*†‡	161 ± 40*†‡
$C_{50}$ , $\mu M$				
Control (n = 8 vs. 8)	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.3	0.2 ± 0.5
Halothane (n = 8 vs. 8)	0.3 ± 0.2	0.4 ± 0.3	0.1 ± 0.1†	0.1 ± 0.1†
Isoflurane (n = 8 vs. 9)	0.5 ± 0.7	0.6 ± 0.6	0.3 ± 0.3	0.4 ± 0.5
Sevoflurane (n = 8 vs. 8)	0.3 ± 0.7	0.3 ± 0.4	0.2 ± 0.1	0.5 ± 0.6

Values are presented as mean ± SD.

\*  $P < 0.05$  vs. baseline value. †  $P < 0.05$  vs. healthy rats. ‡  $P < 0.05$  vs. control group.

AF = active force per cross-sectional area;  $C_{50}$  = concentration of isoproterenol that results in 50% of  $Eff_{max}$ ;  $Eff_{max}$  = maximum effect in percentage of baseline value; MAC = minimum alveolar concentration;  $V_{max}$  = maximum unloaded shortening velocity.

has been related to a slower cross-bridge cycling rate, slower  $Ca^{2+}$  release from the SR,<sup>2</sup> and to an alteration of Ito potassium current.<sup>23</sup> We observed that the ratio R1, which reflects the  $Ca^{2+}$  uptake by the SR, was not significantly modified in diabetic rats. Abnormalities of the SR in diabetic myocardium are gradual and sequential, and Zhong *et al.*<sup>24</sup> showed that the SR calcium adenosine triphosphatase protein concentration was unchanged in 4-week but decreased in 6-week diabetic rat hearts. Therefore, it is possible that, in our 4-week streptozotocin-treated rats, modifications of the SR could have been not sufficient to induce a significant alteration in R1. Nevertheless, it should be pointed out that the low stimulation rate, which is lower than that in physiologic conditions, could have also minimized the SR dysfunction. The lack of significant difference in R2 suggests that myofilament calcium sensitivity was not modified in diabetic rats, as previously demonstrated.<sup>15</sup>

In previous studies, the positive inotropic effect of

$\alpha$ -adrenoceptor stimulation has been shown to be either enhanced<sup>25</sup> or decreased<sup>11</sup> in diabetes. Brown *et al.*<sup>26</sup> suggested that these discrepancies may be explained by differences in experimental conditions (such as the use of auricular *vs.* ventricular myocardium, and different calcium concentrations). In our study, we observed that the positive inotropic effect of  $\alpha$ -adrenoceptor stimulation was not significantly modified in diabetic rats, although a decrease in the density of  $\alpha_1$  adrenoceptors has previously been reported.<sup>11</sup> The positive inotropic effect of  $\beta$ -adrenoceptor stimulation was nearly abolished in diabetic rats, as previously reported.<sup>10,27</sup> Gando *et al.*<sup>10</sup> have suggested that this abolition does not result from a decrease in the number of  $\beta_1$  adrenoceptors but from perturbations of  $\beta$ -adrenoceptor transduction pathway beyond cyclic adenosine monophosphate (cAMP) production. In the absence of halogenated anesthetics exposure, we also observed a positive lusitropic effect of  $\beta$ -adrenoceptor stimulation both in healthy and diabetic

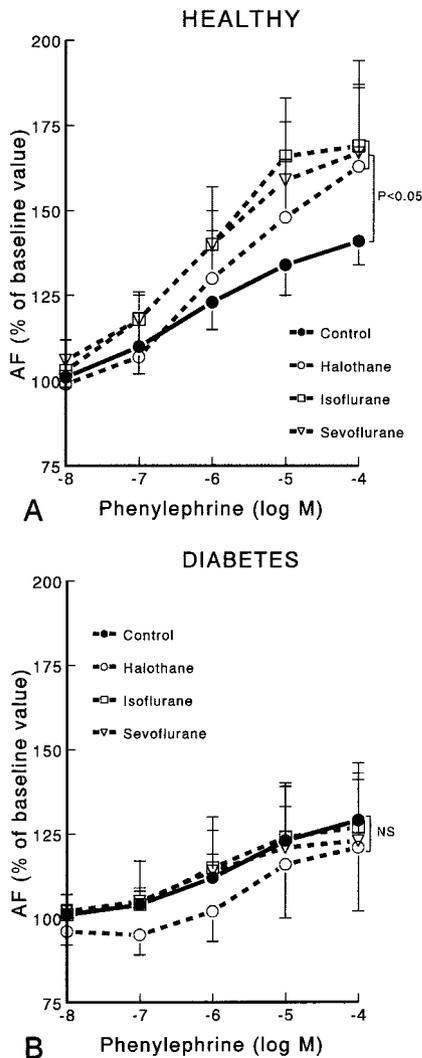
**Table 6. Effects of 1 MAC Halothane, Isoflurane, and Sevoflurane on the Lusitropic Response to  $\beta$ -Adrenoceptor Stimulation in Healthy and Diabetic Rats**

	Healthy Rats		Diabetic Rats	
	R1	R2	R1	R2
$Eff_{max}$ , %				
Control (n = 8 vs. 8)	57 ± 8*	91 ± 8*	61 ± 8*	81 ± 15*
Halothane (n = 8 vs. 8)	41 ± 6*‡	86 ± 11*	71 ± 6*†‡	82 ± 4*
Isoflurane (n = 8 vs. 9)	43 ± 5*‡	89 ± 17	53 ± 10*†	84 ± 15*
Sevoflurane (n = 8 vs. 8)	47 ± 5*‡	87 ± 16	54 ± 8*	83 ± 11*
$C_{50}$ , $\mu M$				
Control (n = 8 vs. 8)	0.09 ± 0.09	1.90 ± 2.22	0.63 ± 0.72	0.63 ± 0.88
Halothane (n = 8 vs. 8)	0.07 ± 0.03	1.88 ± 3.24	0.16 ± 0.11†	0.50 ± 0.40
Isoflurane (n = 8 vs. 9)	0.25 ± 0.34	1.35 ± 1.95	0.35 ± 0.28	1.96 ± 2.85
Sevoflurane (n = 8 vs. 8)	0.25 ± 0.24	0.92 ± 0.64	0.30 ± 0.20	1.74 ± 2.40

Data are presented as mean ± SD.

\*  $P < 0.05$  vs. baseline value. †  $P < 0.05$  vs. healthy rats. ‡  $P < 0.05$  vs. control group.

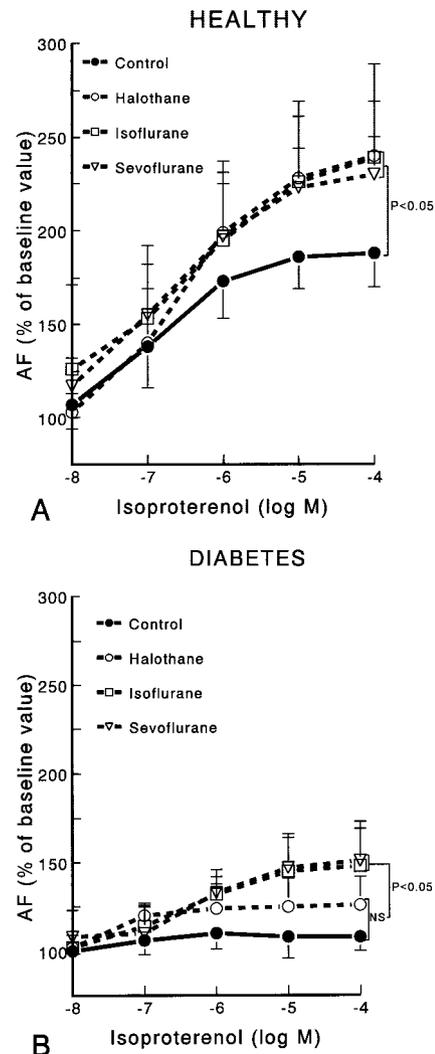
$C_{50}$  = concentration that results in 50% of  $Eff_{max}$ ;  $Eff_{max}$  = maximum effect in percentage of baseline value; MAC = minimum alveolar concentration; R1 = ratio of maximum shortening velocity ( $_{max}Vc$ ) to maximum lengthening velocity ( $_{max}Vr$ ); R2 = ratio of peaks of the positive (+dF·dt<sup>-1</sup>) and negative (-dF·dt<sup>-1</sup>) force derivative at  $L_{max}$ .



**Fig. 1.** Effects of 1 minimum alveolar concentration halothane, isoflurane, and sevoflurane on the inotropic response to  $\alpha$ -adrenoceptor stimulation in healthy (A) and diabetic (B) rats. AF = isometric active force normalized per cross-sectional area. The control group was not exposed to halogenated anesthetics. Data are presented as mean percentage of baseline value  $\pm$  SD.

rats, without significant difference between these two groups (table 5). This result markedly contrasts with the abolition of the inotropic effect of  $\beta$ -adrenoceptor stimulation (fig. 2 and table 4). Several arguments may explain these results. First, in a 4-week diabetic rat model, the SR calcium adenosine triphosphatase concentration is not markedly decreased.<sup>24</sup> Second, positive lusitropic effects are maximal with smaller cAMP production than that required for inotropic effects.<sup>28</sup> Finally, the perturbations of  $\beta$ -adrenoceptor transduction pathway are thought to occur mainly beyond cAMP production.<sup>10</sup> However, the preservation of the lusitropic effect of  $\beta$ -adrenoceptor stimulation is important because diabetic cardiomyopathy is associated with diastolic dysfunction.<sup>1</sup>

Halogenated anesthetics potentiated the positive ino-



**Fig. 2.** Effects of 1 minimum alveolar concentration halothane, isoflurane, or sevoflurane on the inotropic response to  $\beta$ -adrenoceptor stimulation in healthy (A) and diabetic (B) rats. AF = isometric active force normalized per cross-sectional area. The control group was not exposed to halogenated anesthetics. Data are presented as mean percentage of baseline value  $\pm$  SD.

tropic effect of  $\alpha$ -adrenoceptor stimulation, and the magnitude of these potentiations were similar with halothane, isoflurane, or sevoflurane in healthy rats, as previously reported.<sup>16,17</sup> We observed that this potentiation disappears in diabetic rats (fig. 1). A decrease in the number of  $\alpha_1$  adrenoceptors is not likely because we did not observe significant difference in the inotropic response of  $\alpha$ -adrenoceptor stimulation between healthy and diabetic rats (fig. 1). Moreover, we did not observe any significant shift in the concentration-response curves (table 3), suggesting no modification in the affinity or number of receptors.<sup>16,17</sup> Because halogenated anesthetics are known to target G protein and because G proteins are known to be altered in diabetes, we suggest that the abolition of the potentiation of  $\alpha$ -adrenoceptor stimulation by halogenated anesthetic may be related to

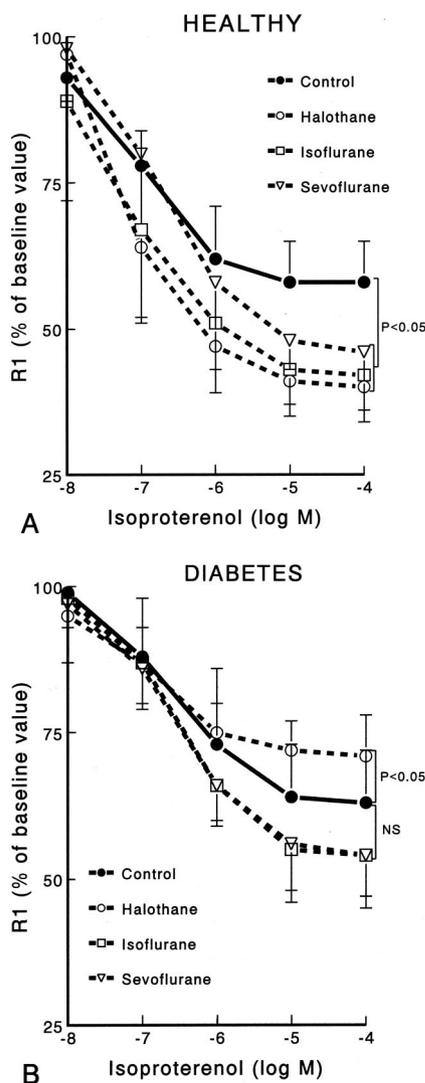


Fig. 3. Effects of 1 minimum alveolar concentration halothane, isoflurane, or sevoflurane on the lusitropic response under low load to  $\beta$ -adrenoceptor stimulation in healthy (A) and diabetic (B) rats. R1 = ratio of maximum shortening velocity ( $v_{\max}$ ) to maximum lengthening velocity ( $v_{\max}$ ), tested the lusitropic effect under low load. The control group was not exposed to halogenated anesthetics. Data are presented as mean percentage of baseline value  $\pm$  SD.

their effects on this specific entity. Further studies are needed to confirm this hypothesis.

As previously reported,<sup>16,17</sup> halogenated anesthetics potentiated the positive inotropic effect of  $\beta$ -adrenoceptor stimulation, and the magnitude of these potentiations were similar with halothane, isoflurane, and sevoflurane in healthy rats.  $\beta$  Adrenoceptors are coupled with Gs and Gi proteins, which modulate adenylyl cyclase and thus cAMP production, which in turn activates cAMP-dependent protein kinase A. Schmidt *et al.*<sup>18</sup> have reported that volatile anesthetics decrease the inhibitory effect of Gi protein on adenylyl cyclase activity. Although the positive inotropic effect of  $\beta$ -adrenoceptor stimulation was abolished and Gi proteins are quantitatively decreased in diabetes,<sup>10</sup> we observed that the potentiation by haloge-

nated anesthetics was preserved, at least with isoflurane and sevoflurane. Halogenated anesthetics induce myocardial depression through different mechanisms, including  $\text{Ca}^{2+}$  homeostasis (inhibition of L-type  $\text{Ca}^{2+}$  channels and  $\text{Na}^{+}$ - $\text{Ca}^{2+}$  exchanger,<sup>29,30</sup> sarcolemmal calcium adenosine triphosphatase and SR functions) and changes in  $\text{Ca}^{2+}$  sensitivity or cross-bridge cycling.<sup>31,32</sup> The potentiation induced by halothane was significantly lower than that induced by isoflurane and sevoflurane. However, this difference is probably explained by the specific action of halothane on the myocardium because this agent is known to induce dysfunction of ryanodine receptors and SR calcium adenosine triphosphatase and to induce a decrease in SR calcium stores.<sup>33,34</sup> This hypothesis is supported by the alteration in R1 observed with halothane, but not with isoflurane or sevoflurane, during  $\beta$ -adrenoceptor stimulation (fig. 3) and is in accord with our previous observation.<sup>16</sup>

The following points should 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 with  $\alpha$ - and  $\beta$ -adrenoceptor stimulations and their interaction with halogenated anesthetics *in vivo* depend also on modifications in venous return, afterload, and compensatory mechanisms. This point is important because diabetes also leads to vascular and nervous system dysfunctions. Second, this study was conducted at 29°C and at a low-stimulation frequency because high-stimulation frequency induces core hypoxia.<sup>35</sup> Third, it was performed in rat myocardium, which differs from human myocardium, but the effects of volatiles anesthetics on the myocardium seem to be very similar among species, at least for halothane and isoflurane.<sup>36-38</sup> Fourth, streptozotocin induces a diabetes of type I, which is not the most frequently encountered clinical form. Nevertheless, the streptozotocin-induced diabetic rat is widely used and recognized as an appropriate animal model of diabetes, most of myocardial dysfunctions being present after 4 weeks without any streptozotocin toxicity.

In conclusion, this study has shown that the positive inotropic effect of  $\beta$ -adrenoceptor stimulation is markedly diminished in diabetic rats, whereas that of  $\alpha$ -adrenoceptor stimulation is preserved. In addition, the potentiation of the positive inotropic effect of  $\alpha$ -adrenoceptor stimulation by halogenated anesthetics is abolished in diabetic rats. In contrast, the potentiation of the positive inotropic effect of  $\beta$ -adrenoceptor stimulation is preserved in diabetes rats with isoflurane and sevoflurane but not with halothane, probably because of its deleterious effect on SR.

The authors thank David Baker, D.M., F.R.C.A. (Staff Anesthesiologist, Department of Anesthesiology and Critical Care, Centre Hospitalier Universitaire Necker-Enfants Malades, Paris, France), for reviewing the manuscript.

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