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Myocardial Effects of Halothane and Isoflurane in Hamsters with Hypertrophic Cardiomyopathy

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Background: **The effects of halothane and isoflurane on myocardial contraction and relaxation in diseased myocardium are not completely understood.**

Methods: **The effects of equianesthetic concentrations of halothane and isoflurane on inotropy and lusitropy in left ventricular papillary muscles of healthy hamsters and those with genetically induced cardiomyopathy (strain BIO 14.6) were investigated *in vitro* (29°C; pH 7.40; Ca^{2+} 2.5 mM; stimulation frequency, 3/min) in isotonic and isometric conditions.**

Results: **Halothane induced a negative inotropic effect that was greater in cardiomyopathic than in healthy hamsters (1.5 vol%, active isometric force (AF): 19 ± 8% *vs.* 28 ± 11% of control values; $P < 0.05$). Isoflurane induced a negative inotropic effect that was greater in cardiomyopathic than in healthy hamsters (2.0 vol%, AF: 64 ± 13% *vs.* 75 ± 11% of control values; $P < 0.01$). However, the negative inotropic effects of halothane and isoflurane were not different for cardiomyopathic or healthy hamsters when their concentrations were corrected for minimum alveolar concentration (MAC) values in each strain. Halothane induced a negative lusitropic effect under low load, which was more important in cardiomyopathic hamsters, suggesting a greater impairment in calcium uptake by the sarcoplasmic reticulum. In contrast, isoflurane induced a moderate positive lusitropic effect under low load in healthy but not in cardiomyopathic hamsters. Halothane and isoflurane induced no significant lusitropic effect under high load.**

Conclusions: **Halothane and isoflurane had greater negative inotropic effects in cardiomyopathic than in healthy hamsters. Nevertheless, no significant differences in their inotropic effects were noted when concentrations were correlated as a multiple of MAC in each strain. (Key words: Anesthetics, volatile: halothane; isoflurane; Heart: cardiomyopathy; papillary muscle; contractility; relaxation.)**

HALOGENATED anesthetic agents have myocardial depressant effects that are observed *in vivo* and *in vitro*.[1-4] Halothane is recognized as a more potent negative inotropic agent than other halogenated anesthetic agents including isoflurane.[1-4] Nevertheless, most previous studies were conducted in normal myocardium. We recently showed that the effects of intravenous anesthetic agents can be different in diseased myocardium.[5,6] Previous *in vivo* studies have reported that the negative inotropic effects of halogenated anesthetics are more pronounced in ischemic myocardium[7,8] and during pacing-induced cardiomyopathy.[9] The negative inotropic effect of isoflurane *in vitro* is enhanced during congestive heart failure,[10] but this is not true for halothane.[1] In contrast, Hattori *et al.*[12] showed that the effects of halothane and isoflurane were less pronounced on cardiac muscles from diabetic rats than those observed in healthy rats. Murat *et al.*[13] reported that halothane produced a similar dose-dependent decrease in maximal calcium-activated tension in healthy and cardiomyopathic hamsters. Nevertheless, they studied skinned myocardial fibers in which sarcoplasmic reticulum (SR) and sarcolemma were removed, and the effects of halogenated anesthetic agents on myofilament are believed to occur only at high supratherapeutic concentrations.[14,15]

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The various strains of Syrian hamsters with hereditary cardiomyopathy offer an opportunity to investigate the effects of anesthetic agents on intrinsic myocardial contractility.[5,6,13] Contractility, cellular biochemistry, molecular biology, and pathophysiology have been studied extensively in this model, the time course of heart failure is well known, and impairment in contractility is due primarily to cardiac muscle cell disease, and thus may be more relevant to clinical cardiomyopathies.[16] Therefore, we conducted an *in vitro* study of the effects of halothane and isoflurane on the contractility of left ventricular papillary muscles from healthy hamsters and those with hypertrophic cardiomyopathy. The experimental model that we used allowed us to assess the effects of halothane and isoflurane on both contraction (inotropy) and relaxation (lusitropy).

Materials and Methods

Thirteen healthy Syrian hamsters (strain FIB) and 12 cardiomyopathic Syrian hamsters (strain BIO 14.6) were used (Bio Breeders, Fitchburg, MA). In the cardiomyopathic strain, all animals of both sexes develop hypertrophic cardiomyopathy from the age of 6 weeks. 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. All healthy and cardiomyopathic hamsters were 6 months old. Body weight, heart weight, and left ventricular weight were determined at the moment of killing. Heart weight-to-body weight and left ventricular weight-to-body weight ratios were calculated. The degree of cardiac hypertrophy was determined by dividing the heart weight-to-body weight value of each cardiomyopathic hamster by the mean heart weight-to-body weight value in healthy hamsters, as previously reported.[5,6]

Experimental Protocol

Forty left ventricular papillary muscles (one or two from each hamster) were studied. 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 containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.1 mM KH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and 4.5 mM glucose. The Krebs-Henseleit solution was prepared daily with highly purified water (Ecopure, Barnstead/Thermolyne Corp., Dubuque, IA). The jacketed reservoir was maintained at 29°C with a thermostatic water circulator (Polystat 5HP; Bioblock, Illkirch, France) with continuous monitoring of the solution temperature. Muscles were field stimulated at 3 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 7.40. After a 90-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.

The control values of each mechanical parameter were recorded at an extracellular calcium concentration ($[Ca^{++}]_o$) of 2.5 mM. A preliminary study (data not shown) showed that hamster myocardial contractility is nearly maximum at a $[Ca^{++}]_o$ of 3.5 mM and that a $[Ca^{++}]_o$ of 2.5 mM corresponds to the upper point of the linear part of the $[Ca^{++}]_o$-active isometric force relation, where active isometric force is 75% of its maximal value. This $[Ca^{++}]_o$ enabled us to study the negative inotropic effects of volatile anesthetics over a range of concentrations. Equianesthetic concentrations were studied in a cumulative manner in separate groups of papillary muscles for halothane (n = 20) and isoflurane (n = 20).

Administration of Halogenated Anesthetics

Halothane (Fluotec 3; Cyprane Ltd, Keighley, UK) and isoflurane (Fortec 3, Cyprane Ltd) were added to the carbon dioxide-oxygen mixture with calibrated vaporizers. The gas mixture bubbled continuously in the bathing solution. To minimize evaporation of anesthetic vapors, the jacketed reservoir was covered with a paraffin sheet. Anesthetic concentrations in the gas phase were monitored continuously using an infrared analyzer (Artema MM 206SD; Taema, Antony, France). Minimum alveolar concentration (MAC) values of halothane and isoflurane were determined previously for rodents at 37°C, 1.1% and 1.4%, respectively.[17] In the present study, halothane concentrations used were 0.3, 0.6, 0.9, 1.2, and 1.5 vol%, and those of isoflurane were 0.4, 0.8, 1.2, 1.6, and 2 vol%. These concentrations are equivalent to 0.5, 1, 1.5, 2, and 2.5 MAC, respectively, in healthy adult rodents at 29°C.[18] However, we have shown that MAC values of halothane and isoflurane are lower in cardiomyopathic than in healthy hamsters.[19] Therefore two points of view were chosen in the present study: (1) a pharmacologic perspective in which comparison of halogenated anesthetics was performed

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Table 1. Correspondence between Halothane and Isoflurane Concentrations in the Gas Phase (in vol %) and in the Krebs-Henseleit Solution (in mM)

Halothane (vol %)	0.6	1.2
Halothane (mM)	0.35 ± 0.04	0.71 ± 0.06
Isoflurane (vol %)	0.8	1.6
Isoflurane (mM)	0.36 ± 0.17	0.72 ± 0.21

Values are mean ± SD.

at the same concentrations (expressed as vol%) between normal and cardiomyopathic hamsters; (2) a clinical perspective in which comparison of halogenated anesthetics was performed at equipotent concentrations (expressed as MAC) between normal and cardiomyopathic hamsters. Because MAC values have been determined by measuring expired concentrations,[19] corrections of 11% for halothane and 4% for isoflurane were applied to estimate real MAC values, as previously reported.[17] Thus we used the following values for MAC at 29°C: halothane 0.54% in healthy and 0.42% in cardiomyopathic hamsters; isoflurane 0.90% in healthy and 0.77% in cardiomyopathic hamsters. A 20-min period of equilibration was allowed between each anesthetic concentration and mechanical parameter recordings.

Anesthetic Concentrations in the Krebs-Henseleit Solution

Halothane and isoflurane concentrations in the Krebs-Henseleit bicarbonate buffer solution were measured by gas-liquid chromatography. The coefficient of variation in the measurement was 5.5% and the limit of quantification was 0.02 mM. Table 1 reports anesthetic concentrations measured in the Krebs-Henseleit solution after 20 min of continuous bubbling.

Electromagnetic Lever System and Recording

The electromagnetic lever system has been described previously.[20] Briefly, the load applied to the muscle was determined using a servomechanism-controlled current through the coil of an electromagnet. Muscular shortening displaced 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.[21]

Mechanical Parameters

Conventional mechanical parameters at L_{max} were calculated from three twitches. The first twitch was isotonic and 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 critical damping to slow the first and rapid shortening overshoot resulting from the recoil of series passive elastic components, as previously reported[22]; the maximum unloaded shortening velocity (V_{max}) was determined from this twitch. The third twitch was fully isometric at L_{max}.

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

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

Relaxation Phase. We determined maximum lengthening velocity of the twitch with preload only ($_{max}Vr$), and peak of the negative force derivative at L_{max} normalized per cross-sectional area (−dF/dt). These two par-

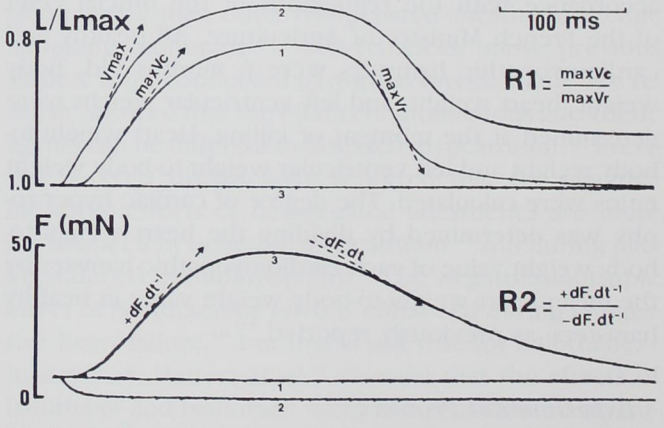

Fig. 1. Mechanical parameters of contraction and relaxation. (*Upper*) Muscle shortening length (L/L_{max}) plotted *versus* time. (*Lower*) Force (F) plotted *versus* time. Twitch 1 was loaded with preload only at L_{max}. Twitch 2 was loaded with the same preload as twitch 1 but was abruptly clamped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

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ameters studied relaxation under low- and high-loading conditions, respectively. Nevertheless, because changes in the contraction phase induce coordinated changes in the relaxation phase, $_{max}Vr$ and $-dF/dt$ cannot assess lusitropy, and thus variations in contraction and relaxation must be considered simultaneously to quantify drug-induced changes in myocardial lusitropy. Indexes of contraction-relaxation coupling thus have been developed to study lusitropy.[23]

Contraction–Relaxation Coupling. Coefficient R1 = $_{max}Vc/_{max}Vr$ studied the coupling between contraction and relaxation under low load, and thus lusitropy, in a manner that is independent of inotropic changes. Under isotonic conditions, the amplitude of sarcomere shortening is markedly greater than that observed under isometric conditions.[24] 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 because of the rapid uptake of calcium by the SR. Thus, in hamster myocardium, R1 tests SR calcium uptake function. Coefficient R2 = $(+dF/dt)/(-dF/dt)$ studied the coupling between contraction and relaxation under high load, and thus lusitropy, in a manner that is less dependent on inotropic changes. When the muscle contracts isometrically, sarcomeres shorten very little.[24] Because of a higher sensitivity of myofilament for calcium,[25] the relaxation time course is determined by calcium unbinding from troponin C rather than by calcium sequestration by the SR. Thus R2 reflects myofilament calcium sensitivity.[22,26] The contraction-relaxation coupling parameters R1 and R2 have been validated as indexes of myocardial lusitropy in a mathematical model.[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, force in mN/mm^2, and force derivative in $mN \cdot mm^{-2} \cdot s^{-1}$.

Statistical Analysis

Data are expressed as means ± SD. Comparison of two means was performed using the Student's *t* test. Comparison of several means was performed using repeated-measures analysis of variance and the Newman-Keuls test (concentrations of anesthetic agents expressed as vol%) or multivariate analysis of variance (concentrations of anesthetic agents expressed as multiples of MAC). All probability values were two tailed, and a value less than 0.05 was required to reject the null hypothesis. Statistical analysis was performed on a computer using NCSS 6.0 software (Statistical Solutions, Ltd, Cork, Ireland).

Table 2. Characteristics of Normal Hamsters and Those with Cardiomyopathy

Parameter	Normal (n = 13)	Cardiomyopathy (n = 12)	*P* Value
BW (g)	117 ± 12	101 ± 10	0.001
HW (mg)	382 ± 74	419 ± 82	NS
LVW (mg)	265 ± 46	286 ± 48	NS
HW/BW (10^{-3})	3.24 ± 0.41	4.13 ± 0.50	0.001
LVW/BW (10^{-3})	2.26 ± 0.31	2.82 ± 0.30	0.001
Cardiac hypertrophy (%)	100	127 ± 15	0.001

Values are means ± SD.

BW = body weight; HW = heart weight; LVW = left ventricular weight; NS = not significant.

Results

Body weight was significantly lower in cardiomyopathic hamsters than in controls (table 2). Heart weight and left ventricular weight were not significantly different between healthy hamsters and those with cardiomyopathy. Consequently, the heart weight-to-body weight ratio was significantly greater in cardiomyopathic hamsters, indicating cardiac hypertrophy (table 2).[5,6]

The intrinsic mechanical performance of papillary muscles from hamsters with cardiomyopathy was significantly lower in isometric (AF, $+dF/dt$) and isotonic (V_{max}, $_{max}Vc$) conditions. R1, which tests the contraction-relaxation coupling under low load and thus SR function, was slightly but significantly greater in cardiomyopathic hamsters than in controls. In contrast, R2, which tests the contraction-relaxation coupling under high load, and thus the myofilament calcium sensitivity, was not significantly different between normal hamsters and those with cardiomyopathy (table 3).

Effects of Halothane

In healthy hamsters, halothane induced a marked dose-dependent negative inotropic effect, as shown by the decrease in V_{max} (fig. 2) and AF (fig. 3). V_{max} and AF observed at 1.5 vol% in healthy hamsters were 51 ± 12% and 28 ± 11% of control values, respectively. The negative inotropic effect was significantly greater in cardiomyopathic hamsters in isotonic (fig. 2) and isometric (fig. 3) conditions. Because contractile properties vary from one papillary muscle to another in cardi-

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Table 3. Mechanical Parameters of Papillary Muscles in Normal Hamsters and Those with Cardiomyopathy

Parameter	Normal (n = 20)	Cardiomyopathy (n = 20)	*P* Value
Characteristic			
L_{max} (mm)	3.6 ± 1.3	2.3 ± 0.8	0.003
CSA (mm^2)	0.67 ± 0.12	0.78 ± 0.20	0.04
RF/TF	0.18 ± 0.06	0.28 ± 0.11	0.001
Contraction			
V_{max} ($L_{max} \cdot s^{-1}$)	3.68 ± 0.41	2.68 ± 0.85	0.001
$_{max}Vc$ ($L_{max} \cdot s^{-1}$)	2.46 ± 0.27	1.66 ± 0.58	0.001
AF ($mN \cdot mm^{-2}$)	43 ± 22	21 ± 13	0.002
+dF/dt ($mN \cdot s^{-1} \cdot mm^{-2}$)	575 ± 269	289 ± 155	0.001
Relaxation			
$_{max}Vr$ ($L_{max} \cdot s^{-1}$)	3.32 ± 0.60	1.96 ± 0.96	0.001
−dF/dt ($mN \cdot s^{-1} \cdot mm^{-2}$)	397 ± 155	196 ± 122	0.001
Contraction–relaxation coupling			
R1 (low load)	0.75 ± 0.09	0.86 ± 0.09	0.001
R2 (high load)	1.44 ± 0.21	1.53 ± 0.21	NS

Values are mean ± SD.

NS = not significant; L_{max} = initial length; CSA = cross-sectional area; RF/TF = ratio of resting force to total force; V_{max} = maximum unloaded shortening velocity; $_{max}Vc$ = maximum shortening velocity; AF = isometric active force normalized per cross-sectional area (CSA); +dF/dt = peak of the positive force derivative normalized per CSA; $_{max}Vr$ = maximum lengthening velocity; −dF/dt = peak of the negative force derivative normalized per CSA; R1 = $_{max}Vc/_{max}Vr$; R2 = (+dF/dt)/(−dF/dt).

omyopathic hamsters,[6] papillary muscles were divided into two groups: those with severe myocardial failure (*i.e.*, baseline AF ≤ 15 mN/mm^2; n = 4, AF = 12 ± 2 mN/mm^2) and those with moderate myocardial failure (*i.e.*, baseline AF > 15 mN/mm^2; n = 6; AF = 31 ± 13 mN/mm^2). At the highest concentration of halothane (1.5 vol%), there was no significant difference in the negative inotropic effect of halothane between papillary muscles with severe myocardial failure and those with moderate myocardial failure (fig. 4).

Under isotonic conditions, halothane induced a significant negative lusitropic effect (increase in R1) in healthy and cardiomyopathic hamsters (fig. 5). This impairment became significant at 1.5 vol% in healthy hamsters and at 1.2 vol% in cardiomyopathic hamsters and was significantly more important in cardiomyopathic hamsters than in healthy hamsters (fig. 5). Under isometric conditions, halothane induced no significant lusitropic effect, either in healthy or in cardiomyopathic hamsters (fig. 6).

Effects of Isoflurane

In healthy hamsters, isoflurane induced a moderate significant negative inotropic effect, as shown by the decreases in V_{max} (fig. 2) and AF (fig. 3). V_{max} and AF observed at 2 vol% in healthy hamsters were 87 ± 9%, and 75 ± 9% of control values, respectively. The decrease in AF was significantly greater in cardiomyopathic hamsters (fig. 3), but not that of V_{max} (fig. 2). As stated previously, papillary muscles were divided into two groups: those with a severe myocardial failure (n = 5; AF = 12 ± 3 mN/mm^2) and those with moderate myocardial failure (n = 5; AF = 26 ± 17 mN/mm^2). At the highest concentration of isoflurane (2 vol%), there was no significant difference in the negative inotropic effect of isoflurane between papillary muscles with severe myocardial failure and those with moderate myocardial failure (fig. 4).

Under isotonic conditions, isoflurane induced no significant lusitropic effect, except at the highest concentration (2 vol%) in healthy hamsters (fig. 5). Nevertheless, a global and significant difference in R1 was noted between normal and cardiomyopathic hamsters (fig. 5). Under isometric conditions, isoflurane induced no significant lusitropic effect, either in healthy or in cardiomyopathic hamsters (fig. 6).

Expression of Anesthetic Concentration as a Multiple of Minimum Alveolar Concentration in Each Strain

Because we showed that MAC of halothane and isoflurane were 23% and 14% lower, respectively, in cardiomyopathic hamsters,[19] we also plotted V_{max} and AF as functions of MAC values determined in each strain (fig. 7). Accordingly, the negative inotropic effects of halothane and isoflurane were not significantly different between healthy hamsters and those with cardiomyopathy.

Discussion

Our main finding was that halothane and isoflurane had greater negative inotropic effects in cardiomyopathic hamsters than in healthy hamsters. Nevertheless, no significant differences in the inotropic effects of halothane and isoflurane were noted when concentrations were corrected as MAC multiples for each strain. Furthermore, halothane induced a significant negative lusitropic effect under low load, which was greater in cardiomyopathic hamsters than in healthy ones.

The cardiovascular effects of halothane and isoflurane have been studied extensively in animals and humans *in vivo* and *in vitro*.[1–4,28] Because of concomitant

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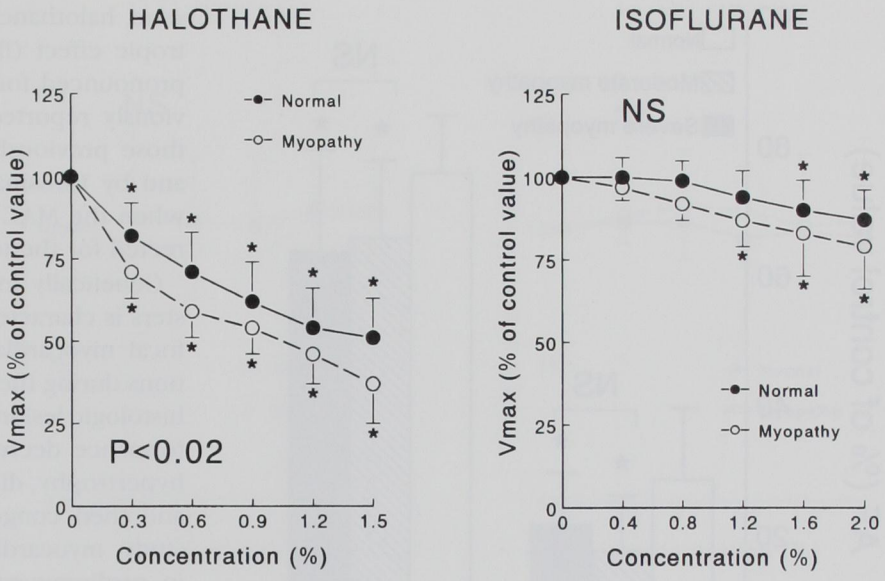

Fig. 2. Comparison of the effects of halothane and isoflurane on maximum unloaded shortening velocity (V_{max}) in papillary muscles from healthy hamsters and those with cardiomyopathy. Halothane and isoflurane concentrations are reported in vol%. Data are mean ± SD. Probability values refer to between-group differences. NS = not significant. *$P < 0.05$ *versus* control values.

changes in preload, systemic resistance, heart rate, sympathetic activity, and central nervous system activity, the precise effects of halogenated anesthetics on intrinsic myocardial contractility are difficult to assess *in vivo*. Volatile anesthetics induce myocardial depression *via* profound alterations in the main cellular components involved in intracellular calcium homeostasis: (1) a depression of the inward Ca^{++} current (I_{Ca}) through the sarcolemma, *via* inhibition of both L-type Ca^{++} channels and Na^{+}/Ca^{++} exchanger[29,30]; (2) a decrease in SR function,[31] which has been shown to occur to a lesser extent with isoflurane than with halothane; furthermore, halothane, but not isoflurane, increases the Ca^{++} permeability of SR membrane, which contributes to depleting the SR of Ca^{++} stores[32]; this effect may be related to the fact that halothane, but not isoflurane, gates the cardiac SR Ca^{++} release channel into the open state[33]; and (3) a decrease in the calcium myofilament sensitivity,[34] which is thought to occur only at high supratherapeutic concentrations.[14,15] In healthy ham-

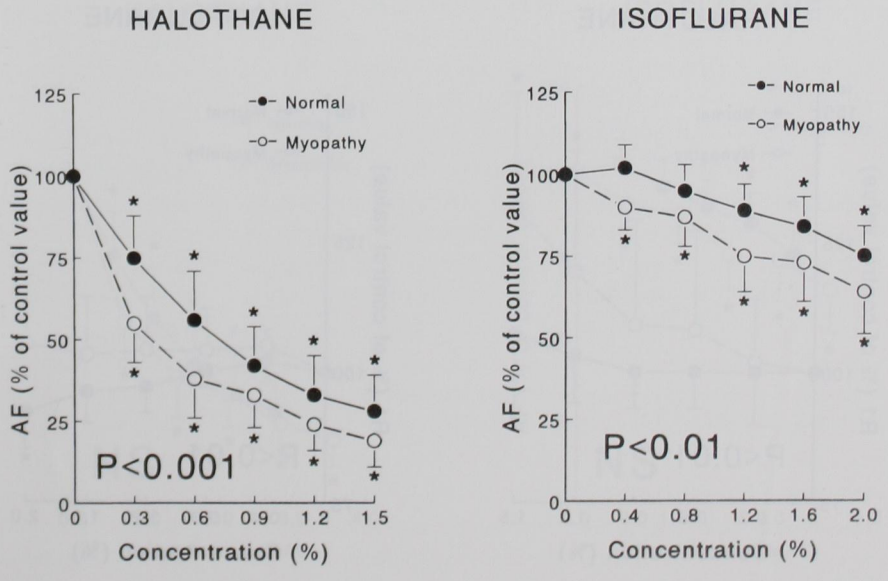

Fig. 3. Comparison of the effects of halothane and isoflurane on isometric active force (AF) normalized per cross-sectional area of papillary muscles from healthy hamsters and those with cardiomyopathy. Halothane and isoflurane concentrations are reported in vol%. Data are mean ± SD. Probability values refer to between-group differences. *$P < 0.05$ *versus* control values.

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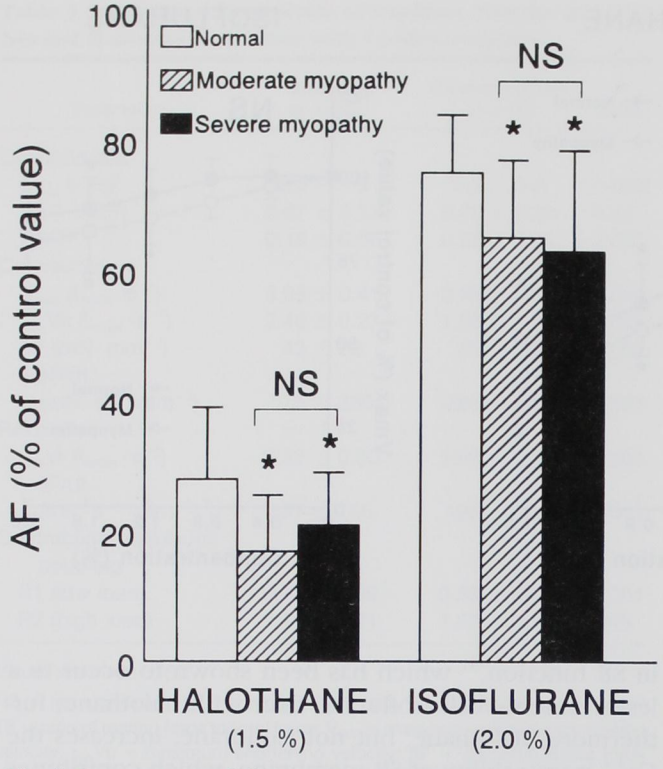

Fig. 4. Comparison of the negative inotropic effects (assessed by percentage change in isometric active force [AF]) of halothane (1.5 vol%) and isoflurane (2 vol%), corresponding to 2.5 MAC in healthy rodents, in healthy papillary muscles and cardiomyopathic papillary muscles with a moderate (AF > 15 mN/mm^2) or severe (AF ≤ 15 mN/mm^2) decrease in contractility. Data are mean ± SD. NS = not significant. *$P < 0.05$.

sters, halothane and isoflurane induced a negative inotropic effect (figs. 2 and 3), which was much more pronounced for halothane than for isoflurane, as previously reported.[1-3,14,28] Our results correspond with those previously noted by Lynch[2] in rat myocardium and by Housmans *et al.*[3,35] in the ferret myocardium when the MAC of halothane and isoflurane were corrected for the temperature of the experiment.

Genetically induced cardiomyopathy in Syrian hamsters is characterized by the progressive occurrence of focal myocardial degeneration, fibrosis, and calcifications during the life of the animal.[16] At age 30-40 days, histologic lesions become apparent and myocardial performance decreases. Further cardiac changes include hypertrophy, dilation, or both, depending on the strain, and then congestive heart failure and death. In our study, myocardial contractility was markedly impaired in cardiomyopathic left ventricular papillary muscles (table 2). The decreased myocardial contractility in cardiomyopathic hamsters may be explained by the decreased activities of G proteins,[36] sarcolemmal Ca^{++}, and Na^+-K^+ ATPase,[37] alterations in Na^+/Ca^{++} exchange,[38] decreased conductance and density of voltage-sensitive calcium channels,[38] and alteration in myofilament regulatory proteins.[39] Nevertheless, a decrease in SR function is not observed in all hamster strains, and the strain used in the present study (BIO 14.6), which develops hypertrophic cardiomyopathy, has been shown to exhibit normal SR function.[40] In the present study, we observed a moderate but significant

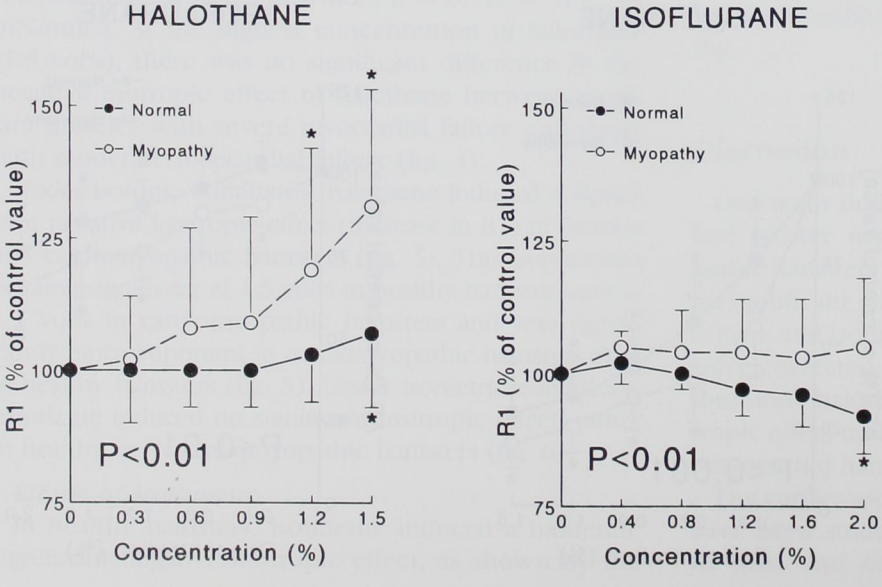

Fig. 5. Comparison of the lusitropic effects under low load (R1 = $_{max}Vc/_{max}Vr$) of halothane and isoflurane in papillary muscles from healthy hamsters and those with cardiomyopathy. Halothane and isoflurane concentrations are reported in vol%. Data are mean ± SD. Probability values refer to between-groups difference. $P < 0.05$ *versus* control values.

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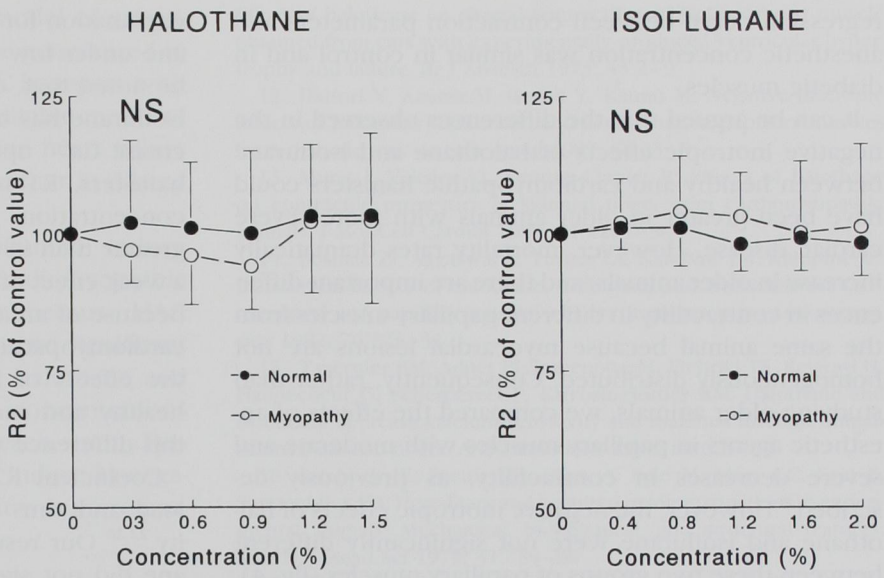

Fig. 6. Comparison of the lusitropic effects under high load (R2 = [+dF/dt]/[−dF/dt]) of halothane and isoflurane in papillary muscles from healthy hamsters and those with cardiomyopathy. Halothane and isoflurane concentrations are reported in vol%. Data are mean ± SD. Probability values refer to between-groups difference. NS = not significant. No significant differences *versus* control values.

increase in R1 in cardiomyopathic hamsters. This result suggests that SR function is moderately impaired in hamsters with hypertrophic cardiomyopathy, whereas it is severely impaired in hamsters with dilated cardiomyopathy.[40]

In cardiomyopathic hamsters, we observed that the negative inotropic effect of halothane was more pronounced than in healthy hamsters (figs. 2 and 3). This result is contrary to that reported *in vitro* by Kemmotsu *et al.*[10] on isolated cat papillary muscle in an experimental model of hypertrophic heart failure. We also noted that the negative inotropic effect of isoflurane was greater in cardiomyopathic hamsters (fig. 3). These results concord with those reported by Shimosato *et al.*[11] on isolated cat papillary muscle in an experimental model of congestive heart failure. On the other hand, Hattori *et al.*[12] reported that myocardium from diabetic rats is less sensitive to the negative inotropic effects of halothane and isoflurane than the myocardium of healthy rats; however, in that study, the slope of the

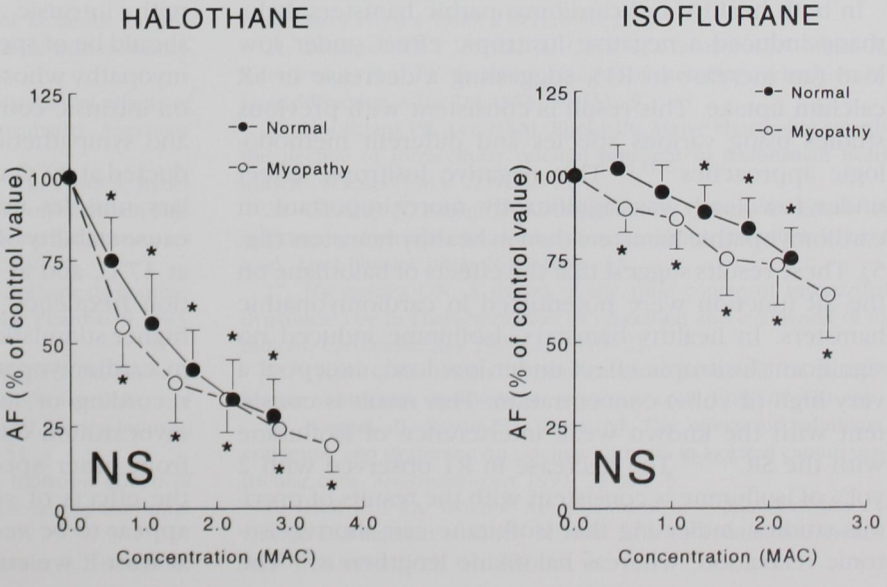

Fig. 7. Comparison of the effects of halothane and isoflurane on isometric active force (AF) normalized per cross-sectional area of papillary muscles from healthy hamsters and those with cardiomyopathy. Halothane and isoflurane concentrations are reported as minimum alveolar concentration multiples in each strain. Data are mean ± SD. Probability values refer to between-group difference (multivariate analysis of variance). NS = not significant. *$P < 0.05$ *versus* control values.

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regression curve between contraction parameters and anesthetic concentration was similar in control and in diabetic muscles.

It can be argued that the differences observed in the negative inotropic effects of halothane and isoflurane between healthy and cardiomyopathic hamsters could have been greater in older animals with more severe cardiac disease. However, mortality rates dramatically increase in older animals, and there are important differences in contractility in different papillary muscles from the same animal because myocardial lesions are not homogeneously distributed. Consequently, rather than studying older animals, we compared the effects of anesthetic agents in papillary muscles with moderate and severe decreases in contractility, as previously described.[6] However, the negative inotropic effects of halothane and isoflurane were not significantly different between these two groups of papillary muscles (fig. 4).

We have shown that the MAC of halothane and isoflurane are decreased in cardiomyopathic hamsters.[19] The expression of V_{max} and AF as functions of MAC in each strain showed that the negative inotropic effects of halothane and isoflurane were not significantly different between healthy and cardiomyopathic hamsters (fig. 7). This important point (*i.e.*, the increase in anesthetic potency in cardiomyopathy) has been overlooked in previous experimental and clinical studies. However, because little information is available about anesthetic potency in cardiovascular diseases, we believe it was important to report our results both from a pharmacologic (fig. 3) and a clinically relevant perspective (fig. 7).

In both healthy and cardiomyopathic hamsters, halothane induced a negative lusitropic effect under low load (an increase in R1), suggesting a decrease in SR calcium uptake. This result is consistent with previous studies using various species and different methodologic approaches.[3,31,35] This negative lusitropic effect under low load was significantly more important in cardiomyopathic hamsters than in healthy hamsters (fig. 5). These results suggest that the effects of halothane on the SR function were potentiated in cardiomyopathic hamsters. In healthy hamsters, isoflurane induced no significant lusitropic effect under low load, except at a very high (2 vol%) concentration. This result is consistent with the known weak interference of isoflurane with the SR.[3,31,33] The decrease in R1 observed with 2 vol% of isoflurane is consistent with the results of previous studies, indicating that isoflurane can shorten isotonic relaxation, whereas halothane lengthen it.[35] The explanation for this positive lusitropic effect of isoflurane under low load is not fully understood. It should be noted that, depending on experimental conditions, isoflurane has been reported to either increase or decrease Ca^{++} uptake by the SR.[41,42] In cardiomyopathic hamsters, R1 remained unchanged regardless of the concentration of isoflurane, and was significantly greater than in healthy hamsters (fig. 5). Thus despite a weak effect of isoflurane on SR function, and probably because of an already slightly impaired SR function in cardiomyopathic hamsters, a significant difference in the effects of isoflurane on R1 was noted between healthy and cardiomyopathic hamsters. Nevertheless, this difference was very moderate (fig. 5).

Coefficient R2 tests the lusitropic state under higher load and thus reflects myofilament calcium sensitivity.[22,26] Our results showed that halothane and isoflurane did not significantly modify R2 (fig. 6), either in healthy or in cardiomyopathic hamsters, suggesting that they did not interfere with myofilament calcium sensitivity. Using skinned rat ventricular trabeculae, Murata *et al.*[34] reported that halothane and isoflurane decreased calcium myofilament sensitivity, but the importance of this phenomenon in intact muscular preparation is debated.[14,15] Furthermore, species differences may also explain observed discrepancies because halothane and isoflurane induced an abbreviation of isometric relaxation in the ferret myocardium[3,35] but not in the hamster and rat myocardium.[43]

The following points must be considered in the assessment of the clinical relevance of our results. First, because this study was conducted *in vitro*, it dealt only with intrinsic myocardial contractility. This point should be of special importance in patients with cardiomyopathy whose cardiac function does not depend only on intrinsic contractility but also on preload, afterload, and sympathetic activity. Second, this study was conducted at 29°C at a low-stimulation frequency; yet papillary muscles must be studied at this temperature because stability of mechanical parameters is not sufficient at 37°C, and at a low frequency because high-stimulation frequency induces core hypoxia.[44] Furthermore, higher stimulation rates frequently induce extrasystoles in cardiomyopathic muscles, resulting in an unreliable recording of mechanical parameters. Third, hamster myocardium differs somewhat in its cardiac behavior from other species, including humans. Nevertheless, the effects of volatile anesthetics on the myocardium appear to be very similar from one species to another, at least if we consider mammalian species. Fourth, the

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results obtained in this experimental model of genetically induced cardiomyopathy cannot be generalized to all types of cardiac failure. Nevertheless, hamsters with cardiomyopathy may be considered a suitable model of human cardiomyopathy with progressive heart failure for a prolonged period, as is observed either in dilated or hypertrophic cardiomyopathies.

In conclusion, halothane and isoflurane induce greater negative inotropic effects in cardiomyopathic than in healthy hamsters. Nevertheless, because MAC values are decreased in cardiomyopathy, no significant differences in the inotropic effects of halothane and isoflurane were noted, providing that concentrations are corrected as a multiple of MAC in each strain. Furthermore, halothane, but not isoflurane, induced a negative lusitropic effect under low load that was more pronounced in cardiomyopathic hamsters.

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