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Myocardial Effects of Halothane and Isoflurane in Senescent Rats

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Background: **Aging is associated with marked alterations in myocardial contraction and relaxation, whereas halogenated anesthetics depress myocardial contractility. However, their effects on aging myocardium are unknown.**

Methods: **Mechanical variables of left ventricular papillary muscles from adult and senescent rats (29°C; pH 7.40; Ca^{2+} 1.0 or 0.5 mM; stimulation frequency, 12 pulses/min) were studied. The expression of genes coding for the α- and β-myosin heavy chain (MHC) and Ca^{2+}–ATPase of the sarcoplasmic reticulum (SR) were studied. The effects of halothane and isoflurane were studied. The inotropic effects were compared under low and high loads, using the maximum unloaded shortening velocity (V_{max}) and maximum isometric active force (AF). The lusitropic effects were compared in isotonic and isometric conditions.**

Results: **Senescent rats had a decrease in contraction and relaxation velocities, associated with a reexpression of β-MHC mRNAs and a decrease in SR Ca^{2+}–ATPase mRNAs. Halothane induced a lower negative inotropic effect in senescent rats (1.5 vol%, AF: 53 ± 14% *vs.* 39 ± 12% of baseline values; $P < 0.01$) whereas isoflurane induced a similar negative inotropic effect (1.5 vol%, AF: 81 ± 7% *vs.* 87 ± 7% of baseline values; NS). Halothane induced a negative lusitropic effect in isotonic conditions in adult, but not in senescent, rats.**

Conclusions: **The inotropic and lusitropic effects of halothane were less important in senescent than in adult rats, whereas the effects of isoflurane were similar. These differences are probably related to differences in SR function and in the effects of halogenated anesthetics on the SR.**

HALOGENATED anesthetics induce negative inotropic effects on the myocardium that have been reported *in vivo* and *in vitro* in various animal species and humans.[1–3] The negative inotropic effects of halogenated anesthetics are more pronounced in several myocardial diseases such as ischemia,[4] and pacing-induced[5] and genetically-induced cardiomyopathies.[6] The effects of halogenated anesthetics on senescent myocardium remain unknown, whereas senescence *per se* can induce important myocardial changes, including: (1) a loss of myocytes and fibrosis associated with myocyte hypertrophy, which is more significant in the left ventricle than in the right ventricle[7]; (2) abnormal accumulation of fibrillar collagen, both around the vessels and within the interstitium,[7] which is the major determinant of both the alteration in diastolic compliance and occurrence of arrhythmias; (3) prolongation of the myoplasmic calcium transient, without any modification of its amplitude[8]; (4) slowing of the contraction,[8] enabling the senescent muscle to maintain a normal active tension to the detriment of the velocity at which this tension is developed and related to reexpression of β-myosin heavy chain[9]; (5) impairment in relaxation,[9] related to a decreased activity of Na^+–Ca^{2+} exchanger and sarcoplasmic reticulum (SR) Ca^{2+}–ATPase.[10]

The myocardial consequences of senescence during aging contrast with our poor understanding of the effects of halogenated anesthetics. Epidemiologic studies have recently emphasized the public health problems of the aged population, suggesting a growing use of anesthesia in elderly patients.[11] The need to develop our knowledge in geriatric anesthesia has also been recently pointed out.[12] Therefore, we conducted an *in vitro* study of the myocardial effects of halothane and isoflurane in adult and senescent rats. We hypothesized that the effects of halogenated anesthetics differ in senescent myocardium.

Materials and Methods

Adult (3-month-old) and senescent (24-month-old) male Wistar rats from the same origin (Iffa Credo, Lyon, France) were studied. Care of the animals conformed to the recommendations of the Helsinski Declaration, and the study was performed in accordance with the regulations of the official edict of the French Ministry of Agriculture. Body weight was determined at the moment of killing; heart weight, and left ventricular weight were determined after. Heart weight to body weight and left ventricular weight to body weight ratios were calculated.

Experimental Protocol

Left ventricular papillary muscles were studied in a Krebs-Henseleit bicarbonate buffer solution (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) maintained at 29°C with a thermostatic water circulator. Muscles 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

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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.[2,6] In a preliminary study, we verified that the stability of the preparation was obtained over 120 min in papillary muscles from adult (n = 7) and senescent (n = 6) rats (data not shown). The extracellular calcium concentration ($[Ca^{2+}]_0$) was decreased from 2.5 to 1 mM because rat myocardial contractility is nearly maximum at 2.5 mM, and hence it is difficult to quantify inotropic changes without previously decreasing $[Ca^{2+}]_0$. We performed additional experiments at a $[Ca^{2+}]_0$ of 0.5 mM, because the lusitropic effects of halothane are modulated by $[Ca^{2+}]_0$, and postrest potentiation is more sensitive at low $[Ca^{2+}]_0$.[13]

Volatile Anesthetic Agent Administration

Halothane (Fluotec 3; Cyprane Ltd, Keighley, UK) and isoflurane (Fortec 3, Cyprane Ltd) were added to the carbon dioxide–oxygen mixture with calibrated vaporizers.[2,6] Anesthetic concentrations in the gas phase were monitored continuously using a infrared analyzer (Artema MM 206SD; Taema, Antony, France). Halothane concentrations ranged from 0.3 to 1.5 vol%, and those of isoflurane from 0.4 to 2 vol%, equivalent to 0.5 to 2.5 minimum alveolar concentration (MAC), respectively.[14] Since MAC values of halogenated anesthetics are lower in senescent rats,[15] we also compared halogenated anesthetics at equipotent concentrations (expressed as MAC). A correction factor of −17% was applied to estimate real MAC values in senescent rats.[15] A 20-min equilibration period was allowed between each anesthetic concentration.

Mechanical Parameters

The electromagnetic lever system has been described previously.[13] All analyses were made from digital records of force and length obtained with a computer.[13] Conventional 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 fully isometric at L_{max}. The third twitch was abruptly clamped to zero-load just after the electrical stimulus; the muscle was released from preload to zero-load with critical damping.[13] 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 per cross-sectional area (+dF/dt). 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). Because changes in the contraction phase induce coordinated changes in the relaxation phase, indexes of contraction–relaxation coupling thus have been developed to study lusitropy.[16] R1 coefficient = $_{max}Vc/_{max}$ Vr studied the coupling between contraction and relaxation under low load, and thus lusitropy, in a manner that is independent of inotropic changes. R1 tests SR uptake function.[2,13,16] R2 coefficient = (+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. R2 indirectly reflects myofilament calcium sensitivity.[2,13,16]

During rest in the rat myocardium, SR accumulates calcium and the first beat after the rest interval (B1) is more forceful than the last beat before the rest interval (B0).[13] During postrest recovery, the SR-dependent part of activator calcium decreases somewhat toward a steady state, which is achieved in a few beats. AF during postrest recovery was studied at a $[Ca^{2+}]_0$ of 0.5 mM, after a 1-min rest duration, and the rate constant of the exponential decay of AF (τ) was determined.[13] τ is assumed to represent the time required for the SR to reset Ca^{2+} homeostasis and thus was used to test SR function.[13] At the end of the study, the muscle cross-sectional area was calculated from its length and weight, assuming a density of 1.

Quantification of α- and β-Myosin Heavy Chain and SR Ca^{2+}-ATPase mRNAs

Total RNA was isolated from the individual left ventricle.[17] cDNA probes were labeled by random priming or nick translation with an α [^{32}P] dCTP and the synthetic oligonucleotides with T4 polynucleotide kinase and γ [^{32}P] ATP. For Northern Blot analysis, 10 μg of total RNA were submitted to electrophoresis and then transfer was performed on Nylon Hybond-N membrane (Amersham; Les Ulis, France). For Slot Blot analysis, 1, 2, 4, and 8 μg of total RNA were directly applied to the membrane (Nylon Hybond-N membrane; Amersham). After ultraviolet irradiations to covalently link the RNA samples, the RNA blots were hybridized sequentially with an α-myosin heavy chain (MHC) oligonucleotide probe,[9] a β-MHC cDNA probe,[9] a SR Ca^{2+}–ATPase cDNA probe,[18] a 18S rRNA oligonucleotide probe,[9,18] and a 25- to 30-mer oligo(dT) probe.[9,18] The last two probes were used to quantitate 18S rRNA and total poly(A)$^+$ mRNA, respectively, in a given left ventricle and to normalize the measurements.[9,18] Prehybridization, hybridization, and washing conditions were performed, as previously reported.[9,18] The washed blots were exposed to x-ray films (Hyperfilm; Amersham) with Quanta III intensifying screens for 16–48 h at −70°C. The relative level of each mRNA species was determined in slot blots by scanning densitometry within the linear response range of the x-ray films. The densitometric scores of each specific mRNA were normalized to that of 18S rRNA or poly(A)$^+$ mRNA measured by oligo(dT).

Table 1. Baseline Mechanical Variables of Papillary Muscles in Adult and Senescent Rats

Variables	Adult (n = 43)	Senescent (n = 42)
Characteristics		
L_{max} (mm)	6.3 ± 1.8	6.1 ± 1.8
CSA (mm^2)	0.60 ± 0.20	0.73 ± 0.21*
RF/TF	0.15 ± 0.09	0.33 ± 0.18*
Contraction		
V_{max} (L_{max}/s)	3.46 ± 0.62	2.53 ± 0.70*
$_{max}Vc$ (L_{max}/s)	2.23 ± 0.52	1.21 ± 0.37*
Δ L (% L_{max})	19 ± 3	13 ± 2*
AF ($mN \cdot mm^{-2}$)	76 ± 45	49 ± 29*
+dF/dt ($mN \cdot s^{-1} \cdot mm^{-2}$)	1003 ± 63	549 ± 342*
Relaxation		
$_{max}Vr$ (L_{max}/s)	2.86 ± 0.84	1.67 ± 0.68*
−dF/dt ($mN \cdot s^{-1} \cdot mm^{-2}$)	439 ± 256	267 ± 119*
Contraction–relaxation coupling		
R1 (low load)	0.76 ± 0.08	0.74 ± 0.10
R2 (high load)	2.27 ± 0.58	1.96 ± 0.55*

Values are mean ± SD.

* $P < 0.05$ *vs.* adult rats.

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 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 = $(+dF/dt_{max})/(-dF/dt_{max})$.

Statistical Analysis

Data are expressed as mean ± SD. Baseline values between groups were compared using the Student *t* test. Comparison of several means was performed using a 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 *P* values less than 0.05 were required to reject the null hypothesis.

Results

Comparison between Adult and Senescent Rats

Body weight (490 ± 118 *vs.* 328 ± 38 g, $P < 0.05$), heart weight (1060 ± 180 *vs.* 710 ± 110 mg, $P < 0.05$), and left ventricular weight (760 ± 150 *vs.* 520 ± 90 mg, $P < 0.05$) were significantly higher in senescent than in adult rats. The heart weight to body weight ratio (2.23 ± 0.55 *vs.* 2.10 ± 0.38 mg/g, NS) and the left ventricular weight to body weight ratio (1.58 ± 0.33 *vs.* 1.58 ± 0.20 mg/g, NS) were not significantly different between senescent and adult rats.

The L_{max} was not significantly different between papillary muscles from senescent and adult rats. On the other hand, CSA and RF/TF were higher in the senescent group (table 1). The intrinsic mechanical inotropic performance of papillary muscles from senescent rats was significantly lower in isometric (AF, +dF/dt) and isotonic (V_{max}, $_{max}$Vc, ΔL) conditions (table 1). Relaxation velocities were lower in senescent rats in isometric (-dF/dt) and isotonic ($_{max}Vr$) conditions (table 1). R1 was not significantly different between senescent and adult groups. In contrast, R2 was lower in senescent group (table 1).

Compared with the adult group, α-MHC mRNA concentration was lower in the senescent group whereas β-MHC mRNA concentration was higher (fig. 1). In addition, SR Ca^{2+}–ATPase mRNA relative concentration was lower in the senescent group (fig. 1), even when these mRNA species were normalized to rRNAs (data not shown).

Inotropic Effects of Halogenated Anesthetics

At $[Ca^{2+}]_0$ of 0.5 or 1.0 mM, halothane induced a marked concentration-dependent negative inotropic effect in adult and senescent rats, as shown by the decreases in AF (fig. 2). The negative inotropic effect was less pronounced in senescent rats only in isotonic conditions at $[Ca^{2+}]_0$ of 0.5 mM (V_{max} at 1.5%: 63 ± 22% of baseline in senescent rats *vs.* 48 ± 15% of baseline in adult rats, $P < 0.01$) and in both isotonic and isometric conditions at $[Ca^{2+}]_0$ of 1 mM (fig. 2). At a $[Ca^{2+}]_0$ of 0.5 mM, isoflurane induced a moderate but significant negative inotropic effect, as shown by the decrease in AF (fig. 3) without significant differences between adult and senescent groups. At a $[Ca^{2+}]_0$ of 1 mM, isoflurane induced a moderate negative inotropic effect only in isometric conditions (AF), without significant difference between adult and senescent rats (fig. 3).

Since MAC of halogenated anesthetic agents is 17% lower in senescent rats, we also plotted the inotropic effect as a function of MAC values in each group. At equipotent anesthetic concentrations, the negative inotropic effects of halothane remained significantly less pronounced in senescent rats compared with adult rats (fig. 4). In contrast, there was no significant difference between adult and senescent rats with isoflurane (fig. 4).

Lusitropic Effects of Halogenated Anesthetics

At a $[Ca^{2+}]_0$ of 1.0 mM and under isotonic conditions, halothane induced a complex effect in adult rats: a positive lusitropic effect at low concentrations and a negative lusitropic effect at high concentrations (fig. 5). These effects significantly differed in senescent rats since halothane induced only a moderate negative lusitropic effect at high concentrations (fig. 5). At a $[Ca^{2+}]_0$ of 0.5 mM and in isotonic conditions, halothane induced a marked negative lusitropic effect (increase in R1) in adult rats, which was significant at 2.0 vol% (fig. 5). On the other hand, in isotonic conditions, halothane induced no significant lusitropic effect in senescent rats (fig. 5).

Under isometric conditions, whatever was the $[Ca^{2+}]_0$ concentration, halothane induced a moderate but significant negative lusitropic effect (decrease in R2) similar in adult and senescent rats (data not shown). For example,

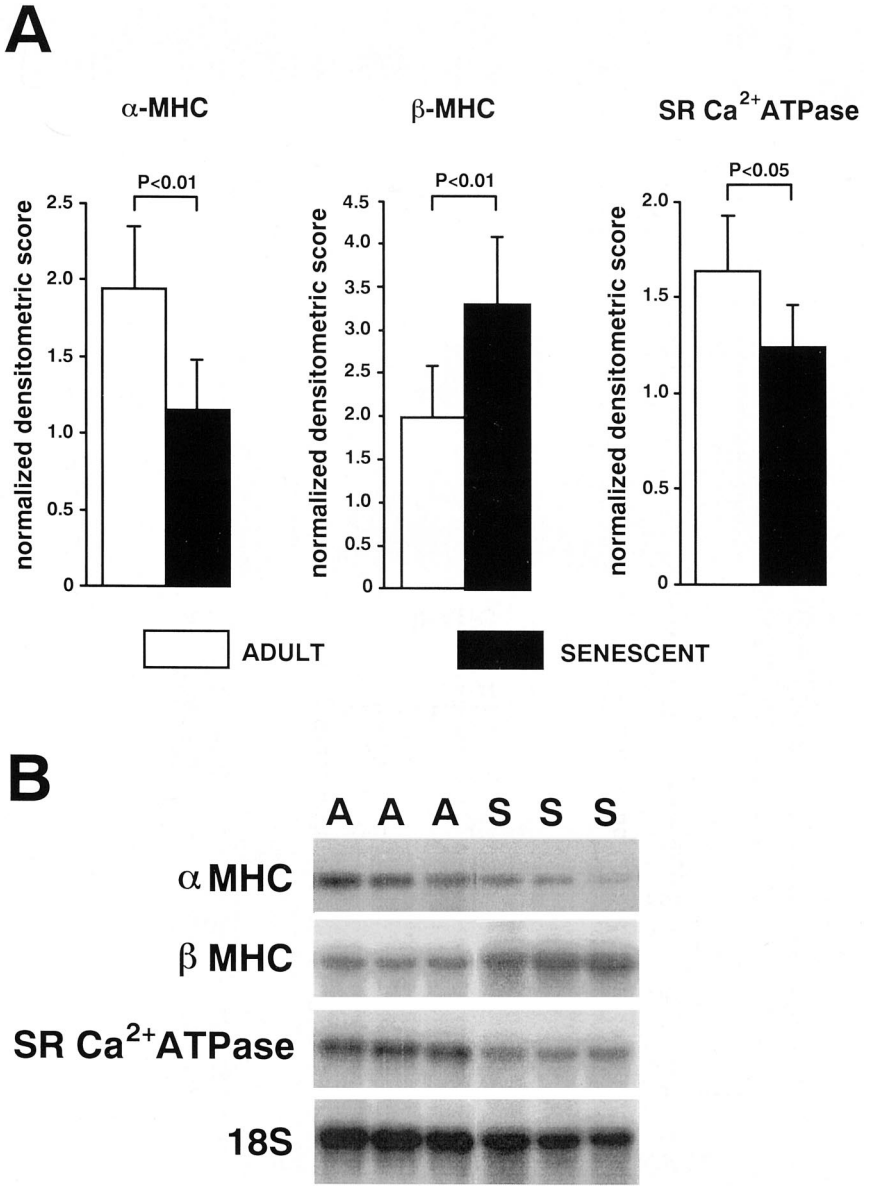

Fig. 1. Comparison of α- and β-myosin heavy chain (MHC) and sarcoplasmic reticulum (SR) Ca^{2+}–ATPase mRNA concentrations in left ventricle from adult and senescent rats. (*A*) αMHC, βMHC, and SR Ca^{2+}–ATPase mRNA concentrations are expressed in densitometric scores normalized to poly (A+) mRNA densitometric score, obtained by slot blot hybridization with the oligo d(T) probe. (*B*) Northern blot analysis of 20 μg of total RNA, isolated from left ventricles of adult (A) and senescent (S) rats. Blots were sequentially hybridized with αMHC, βMHC, SR Ca^{2+}–ATPase, and 18S probes. Data are mean ± SD. *P* values = between-group differences.

at a $[Ca^{2+}]_0$ of 1 mM, the decrease in R2 induced by halothane (1.5%) was 79 ± 8% of baseline in the senescent group and 84 ± 9% of baseline in the adult group.

At a $[Ca^{2+}]_0$ of 0.5 mM under low load, isoflurane induced a negative lusitropic effect at high concentrations similar in each group of rats (R1 at 2.0%: 112 ± 2% of baseline in the senescent group *vs.* 108 ± 22% in the adult group), whereas it induced no significant effect at a $[Ca^{2+}]_0$ of 1 mM (data not shown). Under isometric conditions and whatever the $[Ca^{2+}]_0$, isoflurane induced no significant lusitropic effect in both groups (data not shown).

Effects of Halogenated Anesthetics on Postrest Potentiation

Isoflurane, but not halothane, slightly improved the postrest potentiation in both adult and senescent rats (table 2). There was no significant difference between the two groups of rats. Whatever the group studied, halothane and isoflurane did not significantly modify τ (table 2).

Discussion

In the present study, we showed that despite marked alterations in myocardial contraction and relaxation in senescent myocardium, associated with reexpression of β-MHC mRNAs and a decrease in SR Ca^{2+}–ATPase mRNA concentrations, halothane induced less pronounced negative inotropic and lusitropic effects in senescent than in adult rats, while isoflurane induced similar effects in both groups.

The mortality rate is 50% in 24-month old rats, approximately corresponding to humans aged 75 yr, both in terms of mortality and molecular and cellular alterations.[19] We observed important alterations in the myo-

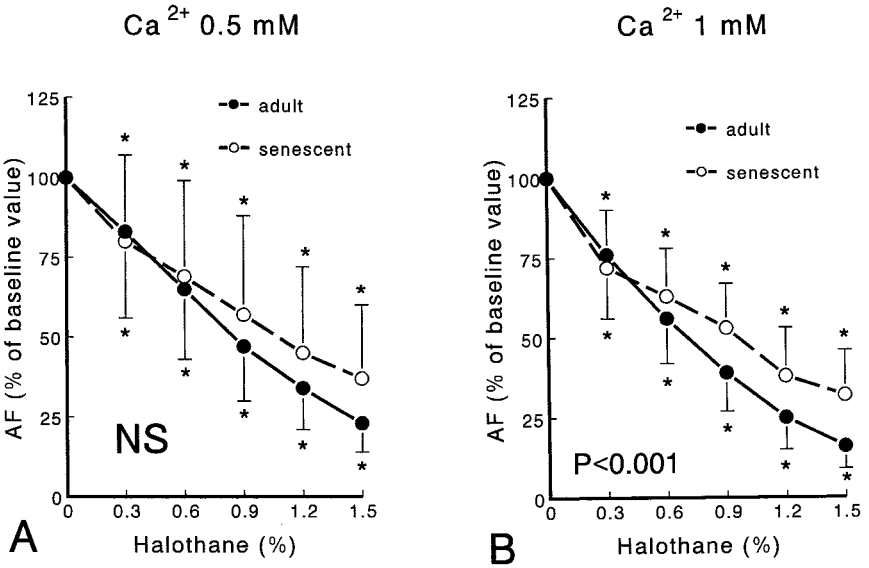

Fig. 2. Comparison of the effects of halothane in adult and senescent rats on isometric active force normalized per cross-sectional area (AF), at a calcium concentration of 0.5 (*A*; adult n = 12, senescent n = 12) or 1 mM (*B*; adult n = 8, senescent n = 6). Concentrations are reported in vol%. Data are mean ± SD. *P* values refer to between-group differences. NS = not significant. * = $P < 0.05$ *versus* baseline values.

cardium of these senescent rats. The rat ventricle contains two isoforms of myosin heavy chains, α and β isoforms within a high and slow ATPase activity, respectively.[20] During aging, our results showed an inactivation of the gene coding for αMHC associated with a reexpression of the βMHC gene. This isomyosin shift results in a decreased ATPase activity which correlates with the decline in V_{max}, as previously reported.[9] Several hypothesis can explain the decrease in force in senescent rats: (1) an important fibrosis, responsible for a decrease in the myofibrillar content[9]; (2) actomyosin cross-bridge kinetic alterations, but this hypothesis remains to be tested. In contrast, several studies found no significant change in myofilament calcium sensitivity in the senescent rat.[21,22] As shown by the increase in R1, the relaxation velocity is decreased in senescent papillary muscles.[9] Calcium extrusion by Na^+-Ca^{2+} exchanger and uptake by the SR Ca^{2+}-ATPase are the two major determinants of relaxation. However, in the rat, the SR plays a more important role in the relaxation process than in other mammalian species. We and others[9,23] have observed a significant decrease in SR Ca^{2+}-ATPase mRNA concentrations and Heyliger *et al.*[24] have reported a reduction in the activity of the Na^+-Ca^{2+} exchanger during aging.

The cardiac effects of halothane and isoflurane have been widely studied *in vitro*.[1–6] Vivien *et al.*[6] showed that their negative inotropic effects were more pronounced in cardiomyopathic hamsters. Our study is the first to assess the effects of volatile anesthetics in senescent myocardium. Volatile anesthetics induce myocardial depression *via* marked alterations in calcium homeostasis: (1) a decrease in the inward calcium current (I_{Ca}), related to an inhibition of both L-type calcium channels and Na^+-Ca^{2+} exchanger[25]; (2) a decrease in SR function, which occurs to a lesser extent with isoflu-

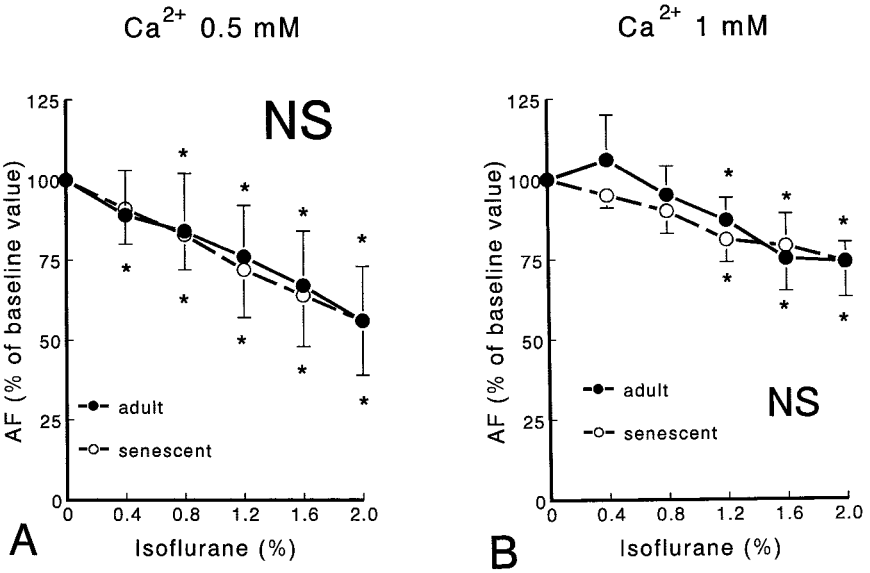

Fig. 3. Comparison of the effects of isoflurane in adult and senescent rats on isometric active force normalized per cross-sectional area (AF), at a calcium concentration of 0.5 (*A*; adult n = 10, senescent n = 12) or 1 mM (*B*; adult n = 6, senescent n = 6). Concentrations are reported in vol%. Data are mean ± SD. *P* values refer to between-group differences. NS = not significant. * = $P < 0.05$ *versus* baseline values.

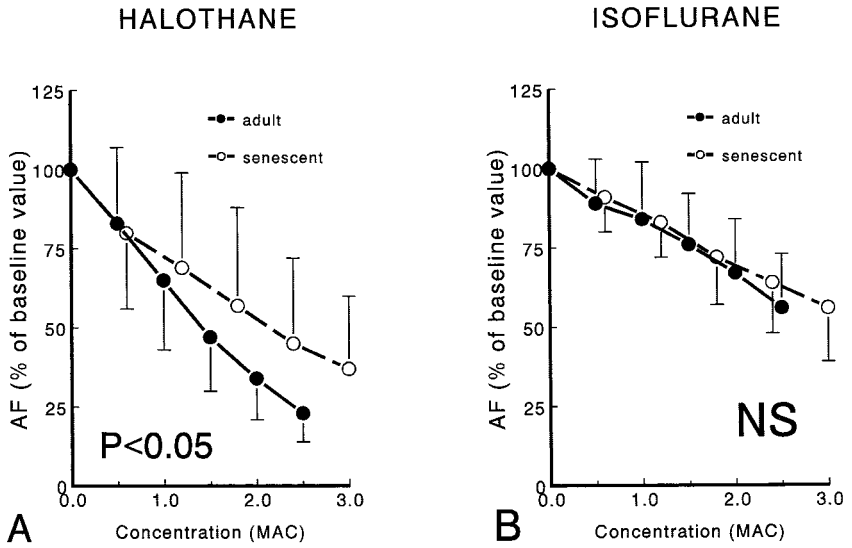

Fig. 4. Comparison of the effects of halothane (*A*) and isoflurane (*B*) on isometric active force (AF) normalized per cross-sectional area of papillary muscles from adult (halothane n = 12; isoflurane n = 10) and senescent (halothane n = 12; isoflurane n = 12) rats at a calcium concentration of 1 mM. Halothane and isoflurane concentrations are reported as minimum alveolar concentration (MAC) multiples in each group. Data are mean ± SD. *P* values refer to between-group differences. NS = not significant. * = $P < 0.05$ *versus* baseline values.

rane than with halothane[26]; furthermore, halothane, but not isoflurane, increases calcium permeability of SR membrane, which contributes to depleting the SR calcium stores[26]; this effect may be related to the fact that halothane, but not isoflurane, induces an increase in the opening duration of the cardiac SR calcium release channel (*i.e.*, ryanodine receptor) without altering the conductance or the opening likelihood[27]; and (3) a decrease in Ca^{2+} myofilament sensitivity, although the magnitude of this effect is still debated.[3] In our study, halothane induced a lower negative inotropic effect in senescent than in adult rats, in spite of the marked contractile proteins alterations observed during senescence. During aging, ryanodine receptor density and activity are decreased[28,29] without changes in mRNA concentrations (posttranscriptional regulation).[18] On the other hand, in hypertrophied senescent myocytes, Walker *et al.*[30] observed a proportional increase in the number of Ca^{2+} channels while the action potential duration is prolonged in relation with a slowed inactivation of L-type Ca^{2+} channel current, and consequently, the Ca^{2+} current density is maintained. These differences may, at least partly, explain the lower negative inotropic effect of halothane in senescent rat.

In our study, isoflurane induced lower negative inotropic effects than halothane, in both groups, as previously reported.[2] However, the inotropic effects of isoflurane were not significantly different between adult and senescent rats. The inotropic effects of isoflurane are mainly related to its effects on Ca^{2+} channel and myofilament Ca^{2+} sensitivity, without any significant effect on SR. The difference observed between halothane and isoflurane may be related to the absence of effect of isoflurane on the SR, or to the weak negative inotropic effect of isoflurane, which might have precluded us to show a difference during aging.

The MAC of halothane is decreased in senescent rats.[15] The expression of AF as a function of MAC in each group

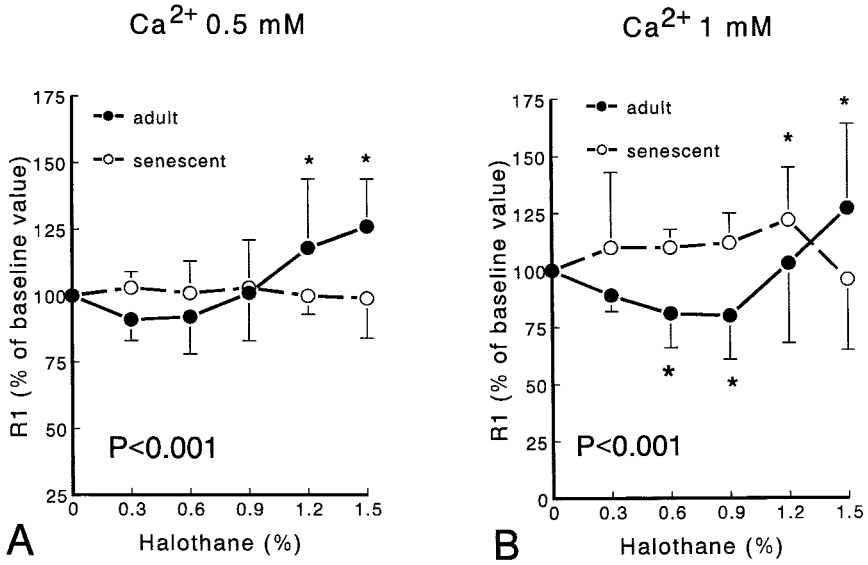

Fig. 5. Comparison of the lusitropic effects of halothane under low load under isotonic conditions (R1= $_{max}Vc/_{max}Vr$) in adult and senescent at a calcium concentration of 0.5 mM (*A*; adult n = 12, senescent n = 12) or 1.0 mM (*B*; adult n = 8, senescent n = 6). Halothane concentrations are reported in vol%. Data are mean ± SD. *P* values refer to between-group differences. NS = not significant. * = $P < 0.05$ *versus* baseline values.

Table 2. Effects of Halothane (0.6%) and Isoflurane (0.8%) on Postrest Potentiation

	B1/B0 (%)		τ (Beats)	
	Adult	Senescent	Adult	Senescent
	(n = 10)	(n = 12)	(n = 10)	(n = 12)
Baseline	173 ± 38	151 ± 38	4.5 ± 0.8	4.3 ± 0.8
Halothane	162 ± 26	161 ± 33	3.7 ± 0.9	3.5 ± 0.6
	(n = 12)	(n = 10)	(n = 10)	(n = 10)
Baseline	159 ± 16	145 ± 34	3.9 ± 1.1	4.4 ± 1.0
Isoflurane	176 ± 16*	177 ± 46*	4.1 ± 1.2	4.2 ± 0.6

Values are mean ± SD.

* = $P < 0.05$ *vs.* baseline values. No significant differences between groups.

B1 = first isometric contraction after rest; B0 = last isometric contraction before rest: τ = rate constant of the exponential decay in active isometric force (AF) after postrest potentiation: τ = number of beats required for postrest contraction to decay to one-tenth of its maximum (B1).

confirmed that the negative inotropic effect of halothane is lower in senescent rats and also confirmed the lack of significant difference observed with isoflurane. As previously discussed in diseased myocardium,[6] because little information is available about anesthetic potency during senescence in humans, we believe that it was important to report our results from a pharmacological (fig. 2) and a clinically relevant (fig. 4) point of view.

We observed that the lusitropic effect of halothane on R1 differs between adult and senescent papillary muscles. At a $[Ca^{2+}]_0$ of 0.5 mM, halothane induced a negative lusitropic effect observed only in adult rats. This result is consistent with previous studies in adult rats,[2] suggesting a decrease in SR calcium uptake. Halothane induces an alteration of cytosolic calcium concentration return during the relaxation phase,[31] resulting from an inhibition of the SR Ca^{2+}–ATPase.[32] During senescence the activity of Ca^{2+}–ATPase is reduced.[10] Nevertheless, even if capture and extrusion lastings are increased,[10] the amount of calcium pumped by the SR is unchanged in senescent rats. Moreover, the halothane-induced inhibition of SR Ca^{2+}–ATPase is enhanced when phospholamban is phosphorylated,[32] and Xu *et al.*[33] have reported a significant age-related decrease in the phosphorylation of phospholamban by the endogenous SR-associated calmoduline kinase, which may be attributed to the decrease in the amount of δ-calmoduline kinase II, the predominant isoform present in cardiac cytosol and SR. These important senescence-induced differences may explain the lower negative lusitropic effects of halothane observed in senescent rats. In addition, we also demonstrated that the lusitropic effect of halothane on R1 depends on the Ca^{2+} concentration. Using cardiac SR vesicles, Karon *et al.*[32] have also reported that the halothane-induced inhibition of Ca^{2+} uptake was more pronounced at low than at high Ca^{2+} concentrations. These results can explain, at least partly, the different effects of halothane on R1 at 0.5 and 1.0 mM of calcium. However, at a high Ca^{2+} concentration, no significant difference in the lusitropic effects of halothane was noted between adult and senescent rat. Isoflurane did not induce any significant lusitropic effect on R1 in senescent and adult rats, as previously reported.[2] These results concord with the absence of reported effect of isoflurane on SR.

We showed that isoflurane, but not halothane, slightly improved postrest potentiation in both adult and senescent rats. Postrest potentiation provides a useful tool for examining complex underlying cellular processes, such as SR calcium release in cardiac muscle.[2,13] In both groups, halothane and isoflurane did not affect postrest recovery, assessed by the rate constant, τ, of the exponential decay of force. As previously reported, in the adult rat,[2] these results suggest that halogenated anesthetics do not significantly alter the recirculation fraction of calcium within the SR whatever the age of the animals.

Coefficient R2 tests the lusitropic state under isometric conditions and thus reflects myofilament Ca^{2+} sensitivity.[2,16] Several studies have reported that myofilament calcium sensitivity is not significantly modified during senescence.[21,22] This is in accordance with data from our laboratory, showing that the active force-Ca^{2+} relationship is similar between adult and senescent rats (S. Rozenberg, personal communication, 2002). Our results showed that halothane, but not isoflurane, induced a small decrease in R2 whatever the Ca^{2+} concentration in both adult and senescent rats, but this effect has been previously related to the decrease in force and not to a decrease in myofilament Ca^{2+} sensitivity.[6,34] These results suggest that the difference in the myocardial effects of halogenated anesthetics do not rely on different action on myofilament Ca^{2+} sensitivity.

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 addressed only intrinsic myocardial contractility. Observed changes in cardiac function after anesthetic administration also depend on modifications in heart rate, venous return, afterload, sympathetic nervous system activity, and compensatory mechanisms. Second, this study was carried out at 29°C and at low-stimulation frequency; however, papillary muscles must be studied at this temperature because the stability of mechanical parameters is not sufficient at 37°C and at low frequency because high-stimulation frequency may induce

core hypoxia.[2,13] Third, we studied 24-month-old Wistar rats whose ventricular function is not altered,[18] and this is not the case in very old rats (>28 month old).[35] Further studies may be useful in older animals. Fourth, it was performed on rat myocardium, which differs from human myocardium, but the effects of volatile anesthetics on the myocardium appear to be very similar among species, at least for halothane and isoflurane.[36]

In conclusion, in spite of marked alterations in the senescent myocardium, the inotropic and lusitropic effects of halothane were less important in senescent than in adult rats, whereas the effects of isoflurane were similar. These differences (adult *vs.* senescent rats and halothane *vs.* isoflurane) are probably related to differences in SR function and in the halogenated anesthetics-induced effects on the SR.

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