Effects of Desflurane in Senescent Rat Myocardium

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Background: The myocardial negative inotropic effects of desflurane are less pronounced than those of other halogenated anesthetics, partly because of intramyocardial catecholamine store release. However, the effects of desflurane on aging myocardium are unknown, whereas aging is known to be associated with an attenuation of catecholamine responsiveness.

Methods: The effects of desflurane (1.9–9.3 vol%) were studied in left ventricular papillary muscle of adult and senescent rats (29°C; 0.5 mm Ca2+; stimulation frequency 12 pulses/min). The inotropic effects were compared under low and high loads, using the maximum unloaded shortening velocity and maximum isometric active force, and without or with α- and β-adrenoceptor blockade.

Results: Desflurane induced a moderate positive inotropic effect in adult rats but a negative inotropic effect in senescent rats. After α- and β-adrenoceptor blockade, desflurane induced a comparable negative inotropic effect in adult and senescent rats. No lusitropic effect under low load was observed, whereas desflurane induced a slight but significant positive lusitropic effect under high load similar between the two groups of rats. This positive effect was abolished by adrenoceptor blockade.

Conclusion: The authors' study suggests that desflurane does not induce significant intramyocardial catecholamine release in senescent myocardium, a result that should be integrated in the well-known alteration in the catecholamine response during aging.

A LOW blood gas solubility allowing rapid adjustments in the depth of anesthesia and quicker time to awakening, associated with remarkable metabolic stability, make desflurane attractive for clinical use.1 The cardiovascular effects of desflurane have been widely studied in vitro and in vivo.2–7 Desflurane decreases systemic vascular resistance, an effect that is less pronounced than that of isoflurane and may contribute to its maintenance of higher arterial blood pressure.2–4 A rapid increase in desflurane concentrations can induce increases in heart rate and arterial blood pressure due to sympathetically activation.9 The mechanisms of this sympathetic activation may involve airway irritation by this pungent agent, transient inhibition of centers modulating sympathetic efferent outflow, and peripheral actions on sympathetic nerve endings.9,10

Because of concomitant changes in preload, systemic resistance, and central nervous system activity, the precise effects of anesthetic agents on intrinsic myocardial contractility are difficult to assess in vivo. Moreover, the myocardial effect of desflurane seems to be unique because it is able to induce intramyocardial catecholamine release, a phenomenon that occurs at low concentrations and not during a rapid increase in desflurane concentration. This may participate to a less pronounced negative inotropic effect than that of isoflurane.5 However, the inotropic effects of halogenated anesthetics may be markedly different in healthy and diseased myocardium, including the aging myocardium.11 Among different alterations related to aging such contraction and relaxation dysfunctions,13 the cardiovascular effects of adrenergic stimulation are attenuated, even though plasma catecholamine concentration increases with age.14

Therefore, we conducted an in vitro study of the myocardial effects of desflurane in adult and senescent rats. We hypothesized that the effects of desflurane differ in senescent rats in relation with an attenuation of adrenergic response.

Materials and Methods

Twenty-nine male Wistar rats were studied: 13 adult (3-month-old) rats and 16 senescent (24-month-old) rats from the same origin (Laboratoires Charles River, Saint Germain sur l’Arbresle, France). Care of the animals conformed to the recommendations of the Declaration of Helsinki, and the study was performed in accordance with the regulations of the official edict of the French Ministry of Agriculture (Paris, France). Body weight was determined at the time of death. Heart weight and left ventricular weight were determined later. Heart weight-to-body weight and left ventricular weight-to-body weight ratios were calculated, as previously described.12

Experimental Protocol

Thirty-four left ventricular papillary muscles (17 adult and 17 senescent) were studied in a Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.1

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mm KH₂PO₄, 25 mm NaHCO₃, 2.5 mm CaCl₂, and 4.5 mm glucose) maintained at 29°C with a thermostatic water circulator (Polystat SHP; Bioblock, Illkirch, France) with continuous monitoring of the solution temperature. Muscles were field stimulated at 12 pulses/min by two platinum electrodes with 5-ms rectangular wave pulses 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 (Lmax), papillary muscles recovered their optimal mechanical performance, which are stable for several hours in both young and elderly rats.12,13,15 The extracellular calcium concentration was decreased from 2.5 to 0.5 mm because rat myocardial contractility is nearly maximum at 2.5 mm, and hence it is difficult to quantify inotropic changes without previously decreasing extracellular calcium concentration. In addition, in rat myocardium, a postrest potentiation study is more sensitive at low extracellular calcium concentration.15 Thereafter, we studied the effects of equianesthetic concentrations (0.5, 1.0, 1.5, 2.0, 2.5 minimum alveolar concentration [MAC]) of desflurane (n = 8 from adult and n = 10 from senescent rats). Because we showed previously that desflurane induces intramyocardial catecholamine release in adult rat myocardium,5 we looked for a similar effect in senescent rat by additional experiments. Alpha and β adrenoceptors were blocked with phentolamine (5 μM) and propranolol (5 μM), which were added to the bathing solution at the end of the stabilization period (n = 9 from adult and n = 7 from senescent rats).

Volatile Anesthetic Agent Administration
Desflurane (TEC 6; Ohmeda, Steeton, United Kingdom) was added to the carbon dioxide–oxygen mixture with a calibrated vaporizer. 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). In the current study, desflurane concentrations used were 1.9, 3.7, 5.6, 7.5, and 9.3 vol%. These concentrations are equivalent to 0.5, 1.0, 1.5, 2.5, and 3.0 MAC, respectively, in adult rodents at 29°C.16 A 20-min equilibration period was allowed between each anesthetic concentration and mechanical parameter recordings.

Mechanical Parameters
The electromagnetic lever system has been described previously.15 Briefly, the load applied to the muscle was determined using a servomechanism-controlled current through the coil of an electromagnet. Muscular shortening induced a displacement of the lever, which modulated the light intensity of a photoelectric transducer. All analyses were made from digital records of force and length obtained with a computer.15

Conventional mechanical variables at Lmax were calculated from three twitches. The first twitch was isometric and was loaded with the preload corresponding to Lmax. The second twitch was fully isometric at Lmax. 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 to slow the first and rapid shortening overshoot resulting from the recoil of series passive elastic components.15 The mechanical parameters characterizing the contraction and relaxation phases and the coupling between contraction and relaxation are defined as follows.

Contraction Phase. We determined Vmax using the zero-load clamp technique and maximum shortening velocity (Vmax/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). Vmax and AF tested the isotropic state under low (isotony) and high (isometry) loads, respectively. We also calculated the ratio of resting force (RF) to total force (TF = RF + AF).

Relaxation Phase. We determined maximum lengthening velocity of the twitch with preload only (Vmax/Vr) and peak of the negative force derivative at Lmax normalized per cross-sectional area (−dF/dt). These two parameters studied relaxation under low- and high-loading conditions, respectively. Nevertheless, 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.17

Contraction–Relaxation Coupling. R1 coefficient = maxVc/maxVr studied the coupling between contraction and relaxation under low load, and thus lusitropy, in a manner that is independent of inotropic changes. As previously reported, R1 tests sarcoplasmic reticulum (SR) uptake function.5–7,11,12,15,17,18 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.5–7,12,15,17,18 Moreover, a decrease in R2 is constantly observed with the positive inotropic effect of β-adrenoceptor stimulation.19

Postrest Potentiation. Recovery of stable, reproducible isometric contraction after a rest interval (1 min) was studied to identify the effects of volatile anesthetics on SR functions. During rest in the rat myocardium, SR accumulates calcium above and beyond that accumulated with regular stimulation, and the first beat after the rest interval (B1) is more forceful than the last beat before the rest interval (B0).6–15 During postrest recovery (B1, B2, B3, . . .), the SR-dependent part of activator
calcium decreases somewhat toward a steady state, which is achieved in a few beats. The maximal isometric AF during postrest recovery was studied at an extracellular calcium concentration of 0.5 mM, after a 1-min rest duration, and at a stimulation frequency of 12 pulses/min, and the rate constant of the exponential decay of AF was determined. The number of beats required for the postrest potentiation to decay to one tenth of its maximum (τ) is assumed to represent the time required for the SR to reset itself and thus was used to test SR function.

At the end of the study, the muscle cross-sectional area was calculated from length and weight of papillary muscle, assuming a density of 1. Shortening and lengthening velocities were expressed in Lmax/s, and force derivative was expressed in mN · s⁻¹ · mm⁻².

**Statistical Analysis**

Data are expressed as mean ± SD. The Student t test was used to compare two means. Comparison of several means was performed using a repeated-measures analysis of variance and the Newman-Keuls test. The beat-to-beat decay of isometric force during postrest recovery was plotted against the number of beats and fitted to an exponential curve, and regression was performed using the least-squares method. All probability values were two-tailed, and P values less than 0.05 were required to reject the null hypothesis. Statistical analysis was performed on a computer using NCSS 6.0 software (Statistical Solutions, Ltd., Cork, Ireland).

**Results**

**Comparison between Adult and Senescent Rats**

Body weight (466 ± 120 vs. 345 ± 33 g; P < 0.05), heart weight (1010 ± 190 vs. 740 ± 140 mg; P < 0.05), and left ventricular weight (730 ± 140 vs. 540 ± 110 mg; P < 0.05) were significantly higher in senescent than in adult rats. The heart weight-to-body weight ratio (2.24 ± 0.45 vs. 2.15 ± 0.34) and the left ventricular weight-to-body weight ratio (1.60 ± 0.24 vs. 1.57 ± 0.25) were not significantly different between senescent and adult rats.

The Lmax was not significantly different between papillary muscles from senescent and adult rats. On the other hand, cross-sectional area and the ratio of resting force to total force 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 (Vmax, maxVc) conditions (table 1). Relaxation parameters were lower in senescent rats in isometric (−dF/dt) and isotonic (maxVr) conditions (table 1). R1 was not significantly different between senescent and adult groups. In contrast, R2 was lower in the senescent group (table 1).

**Inotropic Effects of Desflurane**

In adult rats, desflurane induced a moderate positive inotropic effect only at low concentrations in isotonic conditions (fig. 1). Only under isometric conditions and at high concentrations, desflurane induced a moderate negative inotropic effect. In contrast, desflurane induced a concentration-dependent negative inotropic effect in senescent rats, as shown by the decrease in Vmax and AF (fig. 1).

We studied the effect of desflurane after α- and β-adrenoceptor blockade. In adult rats, the positive inotropic effect of desflurane was abolished by adrenoceptor blockade. Under adrenoceptor blockade, the inotropic effect of desflurane was comparable in adult and senescent rats (fig. 1). Moreover, the negative inotropic effect of desflurane in senescent rats with adrenoceptor blockade was comparable with that previously observed with isoflurane (2.5 MAC, AF: 68 ± 12% vs. 56 ± 17% of baseline).12

**Lusitropic Effects of Desflurane**

Desflurane induced slight significant negative lusitropic effects under isometric conditions only at higher concentrations in senescent rats, but no significant difference were observed between adult and senescent rats (fig. 2). Under isometric conditions, desflurane induced a moderate but significant positive lusitropic effect (de-
crease in R2) in adults and senescent rats. These effects were nearly completely abolished by adrenoceptor blockade (fig. 2).

Effects of Desflurane on Postrest Potentiation
Desflurane 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, desflurane did not significantly modify \( V_{\text{max}} \) (table 2).

Discussion
In the current study, we showed that desflurane induced a moderate positive inotropic effect in adult rats but a negative inotropic effect in senescent rats. After adrenoceptor blockade, desflurane induced a comparable negative inotropic effect in both adults and senescent rats, suggesting that intramyocardial catecholamine release was not observed in the senescent rat myocardium.

The mortality rate is 50% in 24-month old rats, approximately corresponding to humans aged 75 yr in terms of both mortality and molecular and cellular alterations. Myocardial senescence is associated with important alterations of myocardial contraction and relaxation. As previously reported, we observed a decrease in \( V_{\text{max}} \) which is related to a reexpression of the \( \beta \)MHC gene. The decrease in active force is mainly caused by the decrease in myofibrillar content associated with fibrosis and resulting from myocyte loss. The relaxation velocity is decreased in senescent papillary muscles. In previous studies, we and others have observed a significant decrease in SR Ca\(^{2+}\)-adenosine triphosphatase messenger RNA and protein levels in senescent myocardium, leading to a reduced calcium uptake of SR. In addition, the activity as well as the protein content of the Na\(^+\)-Ca\(^{2+}\) exchanger is reduced during aging. These alterations in the cellular determinants of the relaxation are associated with an increased twitch duration.

The cardiovascular effects of an adrenergic stimulation, whether induced by exercise or directly by isoproterenol infusion, are also attenuated during senescence.
though plasma catecholamine concentration increases with age.\textsuperscript{21} Indeed, aging is associated with exaggerated plasma levels of norepinephrine and epinephrine due to an increased spillover into the circulation and to a reduced plasma clearance during senescence.\textsuperscript{21} A deficient norepinephrine reuptake at nerve endings is the primary mechanism for its increased spillover.\textsuperscript{21} In the senescent Wistar rat heart, the density of the total $\beta$ adrenoceptors are diminished.\textsuperscript{26,29} Nevertheless, the major limiting modifications of this signaling pathway occurring with senescence seem to be the coupling of $\beta$ adrenoceptor to adenyl cyclase \textit{via} the Gs protein and a decrease in activity of the catalytic unit of adenyl cyclase protein. This leads to a reduction in the ability to sufficiently augment cell adenosine 3',5'-cyclic monophosphate formation and to activate protein kinase A to drive the phosphorylation of key proteins that are required to augment cardiac contractility.\textsuperscript{21,28,29} $\alpha_1$-adrenoceptor density is also decreased in senescent myocardium, leading to a decline in $\alpha_1$-adrenergic responsiveness.\textsuperscript{30} A selective translocation of protein kinase C and protein kinase C in response to $\alpha_1$-adrenergic stimulation is disrupted in the senescent myocardium.\textsuperscript{31} Thus, both $\alpha$- and $\beta$-adrenergic responsiveness are attenuated during senescence.

It has been previously reported that desflurane may directly stimulate release of intramyocardial catecholamine stores, which could partially attenuate its direct negative inotropic effect.\textsuperscript{5} Indeed, after adrenoceptor blockade, desflurane induced a negative inotropic effect comparable to that of isoflurane in left ventricular rat heart.
papillary muscles. This phenomenon is distinct from the increase in sympathetic activity observed in vivo, because it occurred at low concentrations and was not observed during a rapid increase in desflurane concentration. Our results suggest that such intramyocardial catecholamine release did not occur in the senescent rat and/or that this release was not sufficient to induce any significant inotropic effect, perhaps because of a decrease in receptor density or responsiveness. These results are in accord with our current knowledge that the response to α1 and β adrenoceptors is attenuated in the senescent myocardium.

Halogenated anesthetics induce myocardial depression via marked alterations in the main cellular components involved in calcium homeostasis: (1) a decrease in the inward calcium current (I_{calc}) through the sarcolemma, related to an inhibition of both L-type calcium channels and Na⁺-Ca²⁺ exchanger; (2) a decrease in SR function, which has been demonstrated to occur to a greater extent with halothane; furthermore, only halothane markedly enhances the open time of the cardiac SR calcium release channel (i.e., ryanodine receptor) without altering the conductance or the opening likelihood, and (3) a decrease in calcium myofilament sensitivity, although the magnitude of this effect at therapeutic concentrations is still debated. After adrenoceptor blockade, the inotropic effect of desflurane was comparable between adult and senescent rats, as previously observed with isoflurane. Moreover, the negative inotropic effect of desflurane (with adrenoceptor blockade) was comparable with that previously observed with isoflurane.

During senescence, the activity of Ca²⁺-adenosine triphosphatase, which correlates to the maximum relaxation velocity, is reduced. Capture and extrusion durations are increased, but the amount of calcium pumped by the SR is unchanged in senescent rat myocardium. We have shown that isoflurane had no significant effect on R1 in senescent rats, whereas halothane altered it. Desflurane induces a moderate significant negative lusitropic effect on R1 and only at high concentrations in senescent rats, as previously reported. These results are in accord with the absence of reported effect of desflurane on SR.

Desflurane 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. In both groups, desflurane did not affect postrest recovery, assessed by the rate constant τ of the exponential decay of force. As previously reported in adult rats, these results suggest that halogenated anesthetics do not significantly alter the recirculation fraction of calcium within the SR whatever the age of the animals. These results are also consistent with the absence of effect of desflurane on R1 coefficient in both adult and senescent rats.

Desflurane induced a slight but significant decrease in R2 in both adult and senescent rats. The R2 coefficient tests the lusitropic state under isometric conditions and thus reflects myofilament calcium sensitivity. We and others have reported that myofilament calcium sensitivity is not significantly modified during senescence. The decrease in R2 observed in conjunction of a decrease in AF is not surprising, because these two variables are linked. The decrease in R2 that occurred at low concentrations of desflurane without significant changes in force should be interpreted differently. This decrease was no longer observed after β-adrenoceptor blockade, suggesting a possible role of intramyocardial catecholamine release. Indeed, β-adrenoceptor stimulation increases adenosine cyclic monophosphate level, allowing the phosphorylation of the regulatory protein phospholamban and leading to an increased rate of calcium uptake by the SR, and the phosphorylation of troponin I, resulting in a decrease in calcium sensitivity of contractile proteins. However, the concentration of isoproterenol required to increase myocardial relaxation rate is 100-fold lower in the isometric twitch than in the isotonic twitch. Therefore, our results suggest that desflurane may induce a moderate intramyocardial catecholamine release that was sufficient to induce a small decrease in R2 coefficient but not sufficient to induce any significant decrease in R1 or even any significant inotropic effect. Such dissociation between inotropic and lusitropic effects of β-adrenoceptor stimulation have been previously noted.

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 performed at 29°C and at low-stimulation frequency; however, papillary muscles must be studied at this temperature because the stability of mechanical variables is not sufficient at 37°C and at low frequency because high-stimulation frequency may induce core hypoxia. Third, we studied 24-month-old Wistar rats whose ventricular function is not altered, and this is not the case in very old rats (≥28 months old). Further studies may be useful in older animals. Fourth, it was performed in rat myocardium, which differs from human myocardium, and some species differences have been noted even for the effects of halogenated anesthetics on the myocardium.

In conclusion, desflurane induced a moderate positive inotropic effect in adult rats but a negative inotropic effect in senescent rats. Our study suggests that desflu-
rane did not induce significant intramyocardial catecholamine release in senescent myocardium, a result that should be integrated in the well-known alteration in the catecholamine response during aging.

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