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Effect of Volatile Anesthetics on the Force–Frequency Relation in Human Ventricular Myocardium

The Role of the Sarcoplasmic Reticulum Calcium-release Channel

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Background: **In human ventricular myocardium, contractile force increases at higher stimulation frequencies (positive force–frequency relation). In failing hearts, the force–frequency relation (FFR) is negative. Data on the effect of volatile anesthetics on FFR are very limited.**

Methods: **The authors obtained left ventricular tissue from 18 explanted hearts from patients undergoing cardiac transplantation and tissue of 8 organ donors. The negative inotropic effect of halothane, isoflurane, and sevoflurane on isometric force of contraction of isolated muscle preparations at a stimulation frequency of 1 and 3 Hz and the effect of each anesthetic on the FFR were studied. Ryanodine and verapamil were studied for comparison. In addition, the effect of the anesthetics on Ca^{2+}-dependent ^{3}H-ryanodine binding was investigated.**

Results: **In nonfailing myocardium, halothane was the strongest negative inotropic compound, and the positive FFR was not affected by either drug. In failing myocardium, halothane also showed the strongest negative inotropic effect, but the positive shape of FFR was restored by halothane and ryanodine. In contrast, isoflurane, sevoflurane, and verapamil did not change FFR. Only halothane shifted the Ca^{2+}-dependent ^{3}H-ryanodine binding curve toward lower Ca^{2+} concentrations.**

Conclusion: **In nonfailing human myocardium, none of the anesthetics affect FFR, but halothane is the strongest negative inotropic compound. In failing myocardium, halothane, but not isoflurane or sevoflurane, restores the positive shape of FFR. Both the more pronounced negative inotropic effect of halothane and the restoration of the positive shape of FFR in failing myocardium in the presence of halothane can be explained by its interaction with the myocardial sarcoplasmic reticulum calcium-release channel.**

THE cardiodepressant effect of volatile anesthetics can be explained by an inhibition of the Ca^{2+}-inward current,[1] Ca^{2+} depletion of the sarcoplasmic reticulum (SR) by an interaction with the Ca^{2+}-release channel of the SR,[2,3] and by a reduction of the Ca^{2+} sensitivity of the myofibrils.[4] Beyond these changes of the excitation–contraction coupling, volatile anesthetics interact with other regulatory mechanisms controlling contractile force. For example, the anesthetics modulate the β-adrenergic signal transduction pathway.[5,6]

A further important mechanism that regulates myocardial contractility in the heart is the force–frequency relation (FFR). In healthy human isolated myocardium, an increase in stimulation frequency results in an increase in developed contractile force. The positive FFR could also be demonstrated *in vivo*[7] and is crucial for the adaptation of the heart during exercise. Several investigators have demonstrated a flat or even negative FFR in human myocardium of patients suffering from heart failure[8] or cardiac hypertrophy.[9] The negative FFR has been suggested to contribute to the maladaptation of the heart at high rates. Accordingly, a reduction of heart rate may improve cardiac performance in patients with depressed cardiac function.[10] Whether this improvement of myocardial contractility at low frequencies also occurs during anesthesia is currently not known.

The effect of halogenated anesthetics on mechanical restitution has been addressed by a number of studies showing that halothane inhibits the postrest potentiation of contractile force by impairing the function of the SR.[11] However, data on the interaction of volatile anesthetics with the FFR in healthy or diseased human myocardium are very limited. In one study, halothane restored the positive shape of the FFR in failing human myocardium.[12] However, it is not known whether the halogenated anesthetics differentially alter the FFR in human myocardium or whether this depends on changes present in failing myocardium. Moreover, the negative inotropic effect of sevoflurane has only been evaluated in human atrial myocardium.[13]

In the light of the considerable differences in cardiac physiology in different species, it appears necessary to evaluate the mechanisms of cardiac depression by volatile anesthetics and their interaction with the FFR in human cardiac tissue. Accordingly, the importance of studies on human tissue samples has recently been stressed by several investigators.[13,14]

In the current study, we compared the negative inotropic effects of halothane, isoflurane, and sevoflurane on isolated ventricular human myocardium from healthy organ donors and from patients with end-stage heart failure at different stimulation frequencies. In search for an explanation for the different extent of the negative inotropic effects, we studied the influence of the three

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anesthetics on the Ca^{2+} sensitivity of the human Ca^{2+}-release channel of the SR.

Methods

Patients

Left ventricular myocardium was obtained from 18 patients suffering from end-stage heart failure. The patients underwent cardiac transplantation as a result of dilated (n = 10) or ischemic (n = 8) cardiomyopathy. Nonfailing left ventricular myocardium was obtained from eight multiorgan donors whose hearts could not be transplanted for technical reasons. All organ donors showed a normal left ventricular pump function as shown by echocardiography (ejection fraction > 55%), and none of the donors had any pathologic cardiovascular history. Because brain death may lead to a pronounced depression of cardiac performance, all patients with brain death–induced cardiac dysfunction were excluded.[15] The study was approved by the institutional review board on human research of the University Hospital, Aachen. All patients gave written informed consent. In case of the organ donors, consent was obtained from the relatives.

Contraction Experiments

Immediately after surgical resection, the tissue was placed into bathing solution (2.5 mM $CaCl_2$, 119.8 mM NaCl, 1.04 mM $MgCl_2$, 5.36 mM KCl, 22.6 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, 5.05 mM glucose, 0.28 mM ascorbic acid, 0.05 mM Na_2EDTA, pH 7.4, gassed with 5% CO_2–95% O_2, room temperature) and transferred to the laboratory. In parallel to muscle fiber direction, thin myocardial strips were prepared under stereomicroscopic control. The diameters of the muscle preparations were less than 0.6 mm to avoid core hypoxia and were comparable to those used by other investigators in similar experiments.[16] The muscle preparations were transferred into an organ bath filled with prewarmed (37°C) bathing solution and electrically stimulated at 1 Hz with rectangular impulses (5-ms duration, 5–10% above threshold voltage). After an equilibration period of 30 min, the muscles were stretched by increasing the resting tension from 1 mN stepwise by 0.5 mN until the muscle length providing maximal active force generation was reached. Table 1 shows the main mechanical characteristics of the muscle preparations. In a first group of muscle preparations, the concentration-dependent negative inotropic effect of the volatile anesthetics was studied (10–12 muscle preparations from 5 donor hearts and 22–23 muscle preparations from 13 failing hearts). The anesthetics were added to the carbogen using vaporizers (Dräger [Lübeck, Germany] for halothane and sevoflurane or Ohmeda [Herts, United Kingdom] for isoflurane) and bubbled through the bathing solution. Gas chromatography measurements revealed that equilibration of the anesthetics with the bathing solution was complete within 5 min. The concentration of the anesthetics was increased in steps of 0.5 minimum alveolar concentration (MAC) up to 3 MAC for isoflurane and halothane. In the case of sevoflurane, 2.5 MAC was the maximal concentration that could be delivered by the vaporizer. If force of contraction after wash-out of the anesthetic was less than 5% lower than the predrug value, the next anesthetic was studied. Halothane, isoflurane, and sevoflurane were studied in a random order. In a second group of muscle preparations, the effect of the anesthetics on the FFR was studied (10–12 muscle preparations from 5 donor hearts and 19–24 muscle preparations from 13 failing hearts). The stimulation frequency was increased from 0.5 to 3 Hz at control conditions, at 1.5 MAC, and at 2.5 MAC of each anesthetic. In 12 muscle preparations from 5 donor hearts and 20 muscle preparations from 13 failing hearts, the effect of ryanodine (10 nM) and verapamil (100 nM) on the FFR was studied for comparison. The effect of low Ca^{2+} concentrations in the bathing solution (1.5 and 0.5 mM) on the FFR was studied in 10 muscle preparations from 3 failing hearts.

Table 1. Dimensions and Baseline Forces (37°C, 1 Hz) of All Muscle Preparations

	Nonfailing Myocardium	Failing Myocardium
Patients (n)	5	16
Muscle preparations (n)	34	68
Length (mm)	5.3 ± 0.4	5.2 ± 0.4
Diameter (mm)	0.43 ± 0.04	0.44 ± 0.05
Resting force (mN/mm^2)	6.7 ± 2.1	7.3 ± 1.9
Active force (mN/mm^2)	14.3 ± 3.8	11.8 ± 3.3*
Ratio (active/resting force)	2.2 ± 0.2	1.6 ± 0.1*

* $P < 0.05$ *versus* nonfailing myocardium.

To exclude that hypoxia occurs in the preparations, at the end of the experiment the muscle strips were kept in a condition with a high oxygen demand (*i.e.*, a high stimulation frequency). After force of contraction had reached steady state, the gas bubbling (95% O_2, 5% CO_2) was changed to 80% O_2 plus 15% N_2 and 5% CO_2 for a period of 30 min. All muscle preparations showing a decline in force of contraction of more than 5% during this period were excluded from the study (< 5% of all muscle preparations).[17]

^{3}H-ryanodine Binding

Immediately after explantation, the myocardium was cut into small pieces, immersed in liquid nitrogen, and stored at −80°C until use. ^{3}H-ryanodine binding experiments were performed as previously described.[18] Briefly, 70 mg myocardium of each heart were homogenized three times for 15 s with an Ultraturrax (IKA, Staufen, Germany) and afterward with 25 bursts with a

glas potter at 1,100 rpm in 3 ml ice-cold buffer A: 1 M KCl, 20 mM HEPES, and 1 mM EGTA at pH 7.4. For saturation binding experiments, the free Ca^{2+} concentration was adjusted to 10^{-5} M according to the calculations of Fabiato.[19] Radioligand binding was started by addition of 150 μl of the heart homogenates (100 μg protein) to 100 μl buffer A containing 0.3–40 nM ^{3}H-ryanodine. The reaction was stopped by the addition of 5 ml ice-cold buffer A, immediately followed by rapid filtration through Whatman GF/C filters. The filters were washed twice with 5 ml buffer A and counted after the addition of 5 ml Hionic Fluor (Canberra-Packard, Dreieich, Germany). Nonspecific radioactivity was determined by the addition of 10 μM unlabeled ryanodine into the binding assays and was approximately 15% at 1 nM ^{3}H-ryanodine. To investigate Ca^{2+}-dependent ^{3}H-ryanodine binding, 100 μg protein was incubated with 12 nM ^{3}H-ryanodine in the presence of 15 different Ca^{2+} concentrations ranging from 10^{-9} to 10^{-1} M. Ca^{2+} ions were buffered with 1 mM EGTA and adjusted to the respective final free concentrations according to Fabiato.[19] All other steps were performed as described previously. To study the effect of volatile anesthetics on Ca^{2+}-dependent binding, the membrane preparation was gassed for 15 min with anesthetic in nitrogen or with pure nitrogen for control to avoid oxidative alterations of membrane lipids. The radioligand binding experiments were performed in anesthetic–nitrogen atmosphere or pure nitrogen, respectively. As determined by gas chromatography, equilibration of anesthetic concentration in the medium was achieved within 15 min. In these experiments, the assays were performed in the presence of 10 different Ca^{2+} concentrations ranging from 10^{-8} to 10^{-5} M.

Protein content of the homogenates was measured by the method of Bradford[20] using γ-globulin as standard.

Materials

^{3}H-ryanodine (68.4–74.8 Ci/mmol) was obtained from NEN DuPont de Nemours (Bad Homburg, Germany), and ryanodine was obtained from Calbiochem-Novabiochem (Bad Soden, Germany). All chemicals were of analytical or best commercial grade available. Deionized water was used throughout.

Statistical Analysis

Saturation curves, Ca^{2+}-dependent ^{3}H-ryanodine binding curves, and Scatchard plots[21] were fitted with computer-assisted least square regression analysis using software designed by Graphpad (San Diego, CA). Data are expressed as mean ± SD. For EC_{50} and EC_{40} values, 95% confidence intervals are given. Statistical significance was determined with the unpaired Student *t* test (for comparison of two groups) or by one-way analysis of variance with Newman-Keuls test for comparison of multiple groups. A P value < 0.05 was considered statistically significant.

Results

Negative Inotropic Effect of Halogenated Anesthetics

A total of 34 muscle preparations from 5 donor hearts (in 3 donors only frozen tissue was obtained) and 68 preparations from 16 failing hearts were studied. Length, diameter, and resting force at the maximal active force generation of the preparations did not differ between nonfailing and failing myocardium, whereas the active force at a stimulation frequency of 1 Hz and the ratio between active and resting force were significantly higher in nonfailing myocardium (table 1).

Figure 1 (top) shows the concentration-dependent negative inotropic effect of all three anesthetics in left ventricular myocardium of five organ donors. The negative inotropic effect of halothane was more pronounced than that of isoflurane or sevoflurane. At a physiologic rate (1 Hz), 0.72 MAC halothane reduced contractile force by 40% (EC_{40}), whereas approximately 1.4 MAC isoflurane or sevoflurane was needed to elicit the same effect ($P < 0.05$; table 2). This difference was even more pronounced at a stimulation frequency of 3 Hz. Interestingly, only the first part of the concentration–response curve of halothane, at concentrations up to 1.5 MAC, was steeper compared with the other compounds. At greater than 1.5 MAC, the contractile force decreased

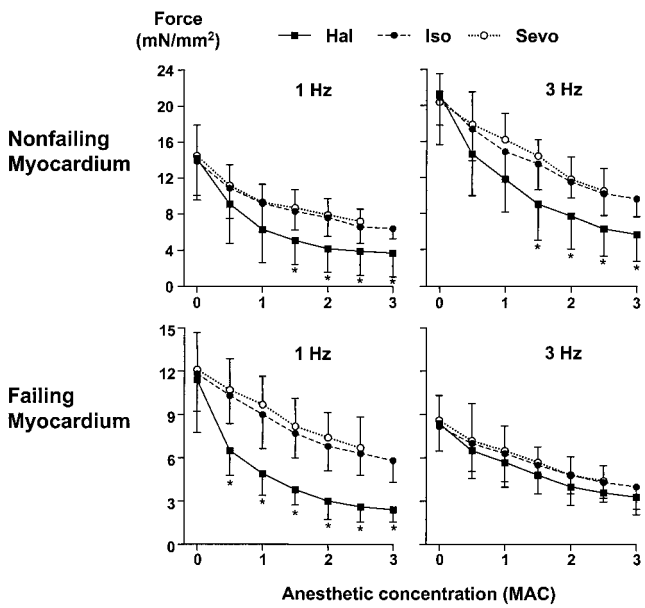

Fig. 1. Negative inotropic effect of anesthetics on force of contraction of nonfailing (10–12 preparations, 5 organ donors, *top*) and failing myocardium (22–23 preparations from 13 patients, *bottom*) at 1 or 3 Hz stimulation frequency. *$P < 0.05$. Hal = halothane; Iso = isoflurane; Sevo = sevoflurane; MAC = minimum alveolar concentration.

Table 2. Negative Inotropic Potency of Volatile Anesthetics

	Nonfailing Myocardium (5 Patients, 10–12 Preparations)		Failing Myocardium (13 Patients, 22–23 Preparations)	
	1 Hz	3 Hz	1 Hz	3 Hz
Halothane	0.72 (0.68–0.77)	0.90 (0.82–1.06)	0.48 (0.42–0.58)	1.50 (1.40–1.65)
Isoflurane	1.37 (1.30–1.49)*	1.81 (1.65–2.12)*	1.72 (1.45–1.89)*	1.71 (1.56–1.90)
Sevoflurane	1.41 (1.35–1.53)*	1.90 (1.70–2.27)*	2.05 (1.88–2.34)*	1.79 (1.71–1.90)

Concentration of each anesthetic (times minimum alveolar concentration) eliciting a 40% reduction in contractile force (EC_{40} value).

* $P < 0.05$ *versus* halothane.

parallel to the decrease with isoflurane and sevoflurane. In failing human myocardium, at a stimulation frequency of 1 Hz, the negative inotropic effect of halothane was also most pronounced (fig 1, bottom). Approximately 0.5 MAC halothane was sufficient to reduce contractile force by 40% (table 2). Similar as in nonfailing myocardium, the negative inotropic potency of isoflurane and sevoflurane was lower ($P < 0.05$; table 1). Again, only the initial part of the concentration–response curve of halothane was steeper. At concentrations greater than 1.5 MAC, force of contraction decreased parallel in all three anesthetics. At 3 Hz, the negative inotropic effect of all three anesthetics was similar. The concentration–response curves were nearly superimposable. Forty percent reduction of contractile force was reached at equianesthetic concentrations of halothane, isoflurane, and sevoflurane (nonsignificant; table 2). Interestingly, the negative inotropic effect of halothane was much less pronounced at 3 Hz compared with 1 Hz.

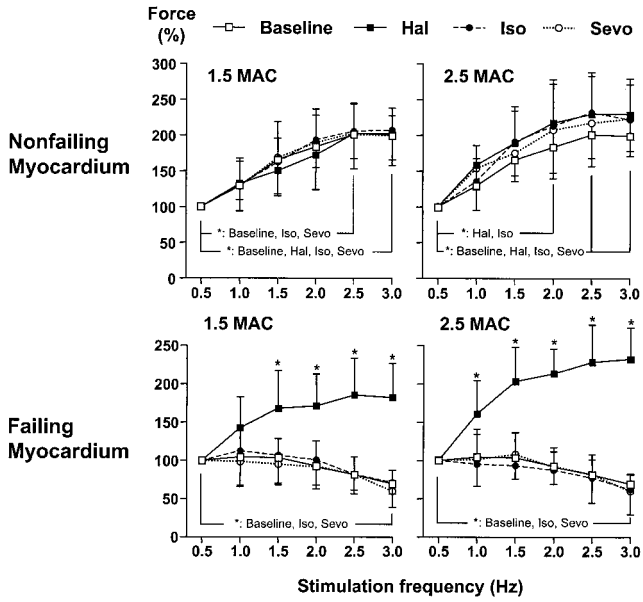

Fig. 2. Effect of 1.5 and 2.5 minimum alveolar concentration (MAC) of each anesthetic on the force–frequency relation in nonfailing (10–12 preparations from 5 donor hearts) and failing myocardium (19–24 muscle preparations, 13 patients). *$P < 0.05$. Hal = halothane; Iso = isoflurane; Sevo = sevoflurane.

Effect of Halogenated Anesthetics on Force–Frequency Relation

Figure 2 shows the influence of the anesthetics on the FFR. In nonfailing myocardium and in the absence of anesthetics (baseline), the FFR was positive. All three anesthetics reduced contractile force equally at all stimulation frequencies, with halothane being the strongest compound, but none of them changed the positive shape of the FFR. In contrast, in failing myocardium, the FFR was differentially affected by the anesthetics. In the absence of anesthetics, the FFR was negative in failing myocardium. As previously mentioned, the negative inotropic effect of halothane was relatively strong at 1 Hz, whereas it was much less pronounced at 3 Hz. As a result, the shape of the FFR became positive in the presence of halothane, although the baseline FFR was negative. The restoration of the positive shape of the FFR could be demonstrated at 1.5 and 2.5 MAC halothane. In contrast, isoflurane and sevoflurane did not alter the negative shape of the FFR.

Effects of Verapamil, Ryanodine, and Low Ca^{2+} Concentrations on Force–Frequency Relation

In nonfailing myocardium, neither verapamil nor ryanodine changed the positive shape of the FFR, although both drugs showed a strong negative inotropic effect (fig. 3). In failing myocardium, verapamil also did not alter the (negative) shape of the FFR, but blockade of the SR by ryanodine reduced contractile force preferentially at low stimulation frequencies. Similar to halothane, ryanodine restored the positive shape of the FFR. In failing myocardium, lowering the availability of Ca^{2+} for the contraction by low Ca^{2+} concentrations in the bathing solutions (1.5 and 0.5 mM) did not change the shape of the FFR despite the pronounced negative inotropic effect (table 3).

^{3}H-ryanodine Binding Studies

Binding of the radioligand ^{3}H-ryanodine to left ventricular homogenates showed saturation characteristics (fig. 4A), and the Scatchard plot (fig. 4B) was linear, indicating that ^{3}H-ryanodine bound to one distinct receptor binding site. Similar to our previous observation,[18] the ^{3}H-ryanodine binding site density was the same in nonfailing (86 ± 11 fmol/mg protein; n = 8) and failing

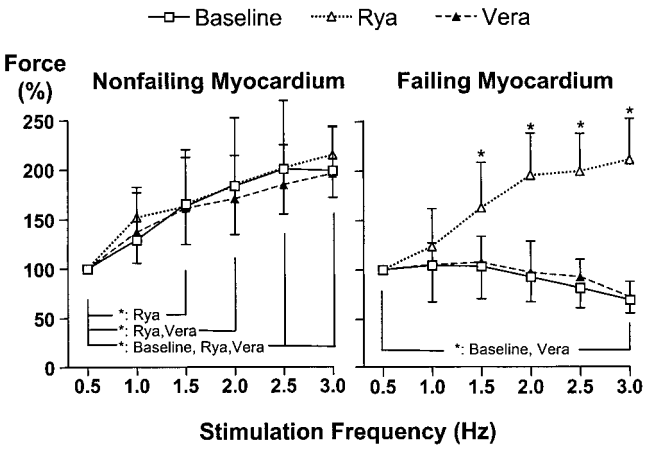

Fig. 3. Effect of verapamil (Vera; 100 nM) and ryanodine (Rya; 10 nM) on the force–frequency relation in nonfailing (12 muscle preparations, 5 organ donors) and failing (20 muscle preparations, 13 patients) myocardium. Forces in nonfailing myocardium at 0.5 Hz were 12.4 ± 4.9 mN/mm^2 (baseline), 3.0 ± 1.8 mN/mm^2 in the presence of ryanodine ($P < 0.05$ *vs.* baseline), and 5.8 ± 2.9 mN/mm^2 in the verapamil group ($P < 0.05$ *vs.* baseline). Forces in failing myocardium at 0.5 Hz were 11.3 ± 4.3 mN/mm^2 (baseline), 1.8 ± 1.0 mN/mm^2 in the presence of ryanodine ($P < 0.05$ *vs.* baseline), and 7.0 ± 2.7 mN/mm^2 in the verapamil group ($P < 0.05$ *vs.* baseline and ryanodine). *$P < 0.05$.

myocardium (91 ± 30 fmol/mg protein; n = 11; nonsignificant).

A representative example of Ca^{2+}-dependent ^{3}H-ryanodine binding to the human Ca^{2+}-release channel is shown in figure 4C. ^{3}H-ryanodine binding showed a bell-shaped pattern with an increase in specific binding at submicromolar Ca^{2+} concentrations and a decrease at Ca^{2+} concentrations higher than 0.5 mM.

Because it has been shown that increasing specific ^{3}H-ryanodine binding at submicromolar Ca^{2+} concentrations reflects increasing open probability of the channel and the role of decreasing ^{3}H-ryanodine binding at Ca^{2+} concentrations in the millimolar range is still unknown, in the following experiments we concentrated on Ca^{2+} concentrations ranging from 10^{-8} to 10^{-5} M. Ca^{2+}-dependent ^{3}H-ryanodine binding to the SR Ca^{2+}-release channel from nonfailing and failing human myocardium

Table 3. Effect of Low Ca^{2+} Concentrations on the FFR in Failing Human Myocardium

	Force of Contraction (mN/mm^2 or % of 0.5 Hz)		
	2.5 mM Ca^{2+}	1.5 mM Ca^{2+}	0.5 mM Ca^{2+}
0.5 Hz (mN/mm^2)	11.7 ± 2.4	6.3 ± 1.5*	2.2 ± 0.5*
0.5 Hz (%)	100	100	100
1.0 Hz (%)	110 ± 14	100 ± 18	102 ± 18
1.5 Hz (%)	98 ± 20	112 ± 18	109 ± 11
2.0 Hz (%)	88 ± 10	90 ± 17	99 ± 20
2.5 Hz (%)	78 ± 9†	88 ± 15	85 ± 5
3.0 Hz (%)	76 ± 18†	81 ± 12†	80 ± 16†

* $P < 0.05$ *versus* 2.5 mM Ca^{2+}. † $P < 0.05$ *versus* 0.5 Hz.
FFR = force–frequency relation.

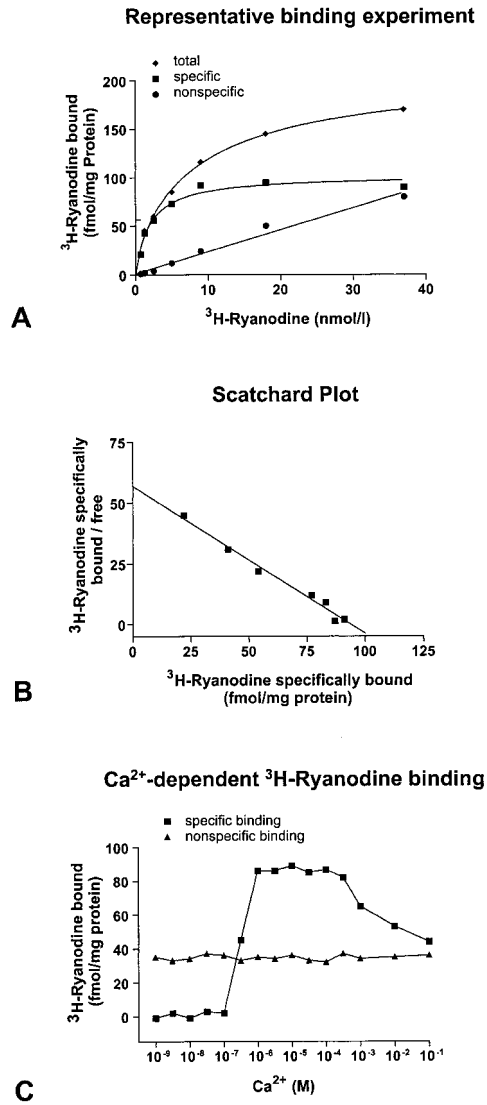

Fig. 4. (*A*) Representative saturation binding of ^{3}H-ryanodine to a homogenate of left ventricular tissue of a patient with dilated cardiomyopathy as a function of increasing ^{3}H-ryanodine concentrations. Binding was performed at 7 concentrations of ^{3}H-ryanodine ranging from 0.5 to 38 nM in the absence (total binding) and in the presence (nonspecific binding) of 10 μM unlabeled ryanodine to determine specific binding. Free Ca^{2+} concentration was 10^{-5} M. (*B*) Scatchard analysis of specific ^{3}H-ryanodine binding of the experiment shown in (*A*). The ratio of specifically bound ^{3}H-ryanodine to free ^{3}H-ryanodine is plotted as a function of specifically bound ^{3}H-ryanodine. (*C*) Bell-shaped pattern of Ca^{2+}-dependent ^{3}H-ryanodine binding to a homogenate of the same specimen. Binding was performed at a saturating concentration of ^{3}H-ryanodine (12 nM).

showed a similar pattern, and EC_{50} values of the increase in specific ^{3}H-ryanodine binding were the same in nonfailing and failing myocardium (table 4). Figure 5 shows the influence of the halogenated anesthetics (1.5 MAC) on the Ca^{2+}-dependent ^{3}H-ryanodine binding. Halothane shifted the curve toward lower Ca^{2+} concentrations. The extent of this effect was similar in failing and nonfailing myocardium. In contrast, isoflurane and sevoflurane (1.5 and 2.5 MAC) did not alter Ca^{2+}-dependent

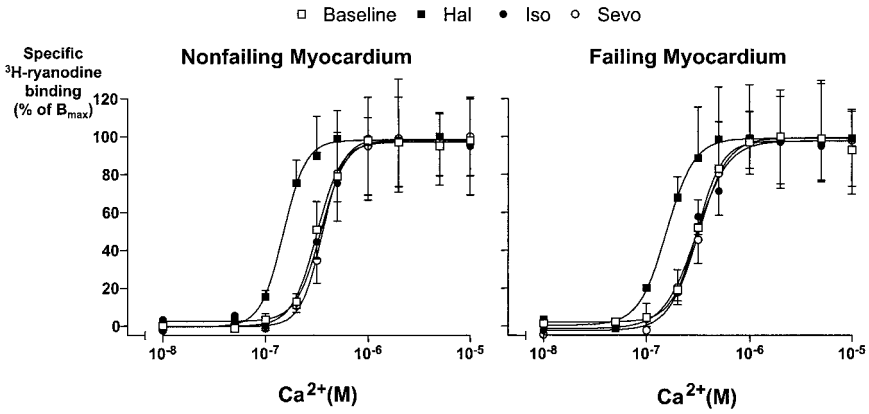

Fig. 5. Ca^{2+}-dependent ^{3}H-ryanodine binding to homogenates from nonfailing (n = 8) and failing (n = 11) myocardium at Ca^{2+} concentrations ranging from 10^{-8} to 10^{-5} M. Hal = halothane; Iso = isoflurane; Sevo = sevoflurane.

^{3}H-ryanodine binding. In addition, the maximal binding of ryanodine was not changed by either compound. Table 4 shows the statistical analysis of the binding data. Halothane at 2.5 MAC did not provoke a further shift of the binding curve to the left. Both in nonfailing and failing myocardium, the maximal effect of halothane on the SR Ca^{2+}-release channel was already reached at 1.5 MAC.

Discussion

The current study provides a direct comparison of the negative inotropic effects of halothane, isoflurane, and sevoflurane in nonfailing and failing ventricular myocardium at different stimulation frequencies. We demonstrated that in both failing and nonfailing myocardium, halothane exerts the most pronounced negative inotropic effect. In failing myocardium, halothane and ryanodine restored the positive shape of the FFR, whereas isoflurane and sevoflurane did not change FFR. Only halothane activated the myocardial Ca^{2+}-release channel of the SR, which offers an explanation for the differential effect of volatile anesthetics on the FFR and for the more pronounced negative inotropic effect of halothane.

Interaction of Volatile Anesthetics with the Sarcoplasmic Reticulum Ca^{2+}-Release Channel

As recently reported,[18] Ca^{2+}-dependent specific ^{3}H-ryanodine binding can be used as a probe for the activation of the human cardiac SR Ca^{2+}-release channel. The plant alkaloid ryanodine only binds to the open state of the channel.[22] Accordingly, a good correlation between channel activation by Ca^{2+} (*i.e.*, opening of the channel) and increased specific ^{3}H-ryanodine binding at submicromolar Ca^{2+} concentrations has been reported during various conditions.[22–24] Although ^{3}H-ryanodine binding studies are an indirect method to assess channel activation, there is increasing evidence that Ca^{2+}-dependent ^{3}H-ryanodine binding reflects Ca^{2+}-induced opening of the channel.[22]

As expected, specific ^{3}H-ryanodine binding was absent at nanomolar Ca^{2+} concentrations, indicating that the channel is closed during these conditions. At submicromolar Ca^{2+} concentrations, ^{3}H-ryanodine binding increased reflecting enhanced open probability of the channel. The Ca^{2+} concentration eliciting the half-maximal ^{3}H-ryanodine binding can be used as a measure of the Ca^{2+} sensitivity of the channel.[21] The increase of Ca^{2+}-dependent ^{3}H-ryanodine binding could be observed at lower Ca^{2+} concentrations when 1.5 MAC

Table 4. Effect of Volatile Anesthetics on Ca^{2+}-dependent ^{3}H-ryanodine Binding

		Halothane		Isoflurane		Sevoflurane	
	Baseline	1.5 MAC	2.5 MAC	1.5 MAC	2.5 MAC	1.5 MAC	2.5 MAC
Nonfailing myocardium (n = 8)							
B_{max} (fmol/mg)	86 ± 11	92 ± 14	87 ± 25	80 ± 19	90 ± 17	88 ± 19	93 ± 22
EC_{50} (μM)	0.32 (0.29–0.38)	0.15* (0.12–0.20)	0.15* (0.14–0.19)	0.33 (0.28–0.36)	0.34 (0.29–0.39)	0.36 (0.34–0.40)	0.34 (0.31–0.39)
Failing myocardium (n = 11)							
B_{max} (fmol/mg)	91 ± 30	79 ± 33	85 ± 19	83 ± 24	90 ± 24	87 ± 18	83 ± 18
EC_{50} (μM)	0.30 (0.28–0.33)	0.16* (0.14–0.19)	0.16* (0.13–0.20)	0.30 (0.28–0.35)	0.32 (0.29–0.37)	0.32 (0.29–0.36)	0.33 (0.29–0.38)

Maximal specific binding (B_{max}) and EC_{50} values of Ca^{2+}-dependent ^{3}H-ryanodine binding. B_{max} are given as mean ± SD, and EC_{50} values are given with 95% confidence intervals.

* $P < 0.05$ *versus* baseline.

MAC = minimum alveolar concentration.

halothane was present, indicating that halothane increased the Ca^{2+} sensitivity. Halothane at 3.0 MAC did not provoke a further leftward shift of the Ca^{2+}-dependant ^{3}H-ryanodine binding curve, indicating that relatively low halothane concentrations already elicit the maximal effect. In contrast, isoflurane and sevoflurane did not change the Ca^{2+} sensitivity of the SR Ca^{2+}-release channel.

The increased Ca^{2+} sensitivity of the channel in the presence of halothane will increase the average open probability of the channel[25] as a response to physiologic variations in intracellular Ca^{2+} concentrations. It has been suggested that this effect leads to a decrease in the ability of the SR to store and accumulate Ca^{2+} and thus to a Ca^{2+} depletion of the SR.[3]

Comparison of the Negative Inotropic Effect of Volatile Anesthetics

Numerous studies in various mammalian species demonstrated a pronounced, concentration-dependent, and reversible cardiodepressant effect of all halogenated anesthetics.[5,26,27] Because important physiologic differences exist between animal myocardium and human cardiac tissue,[28] especially with regard to the FFR,[29] there is a need for studies on the inotropic effect of the anesthetics in human myocardium. Only very recently was an extensive work on the differential negative inotropic effect of the most frequently used volatile anesthetics on isolated atrial human myocardium published.[13] It showed that the negative inotropic effect of halothane was more pronounced than that of isoflurane, sevoflurane, and desflurane. Some properties of electrical and mechanical function in ventricular myocardium, however, might differ from the behavior of atrial myocardium. Action potential duration[30] and contraction times[31] have been shown to be shorter in atrial myocardium, and isometric force development is lower than in ventricular myocardium.[31] Moreover, altered Ca^{2+} homeostasis in failing human myocardium[32] might have an influence on the negative inotropic mechanism of volatile anesthetics. Our data demonstrate that, in failing and nonfailing human ventricular myocardium, the negative inotropic effect of halothane is more pronounced than that of isoflurane and sevoflurane, which depress myocardial contractility to a similar extent. Interestingly, the slope of the concentration–response curve of halothane was steepest at low concentrations up to 1.5 MAC, resulting in a higher negative inotropic potency (lower EC_{40} value). At concentrations greater than this, contractile force declined parallel with the negative inotropic effect of isoflurane and sevoflurane. Because 1.5 MAC halothane exerts the maximal effect on the Ca^{2+} sensitivity of the SR Ca^{2+}-release channel, these data suggest that the initial steep decline of contractile force is mainly caused by activation of the SR Ca^{2+}-release channel and subsequent Ca^{2+} depletion of the SR. At concentrations greater than 1.5 MAC, no further activation of the SR Ca^{2+}-release channel occurs, and all anesthetics reduce force of contraction with a similar slope of the concentration–response curve, probably by inhibition of the Ca^{2+} inward current *via* the L-type Ca^{2+} channel.[33] Isoflurane and sevoflurane do not interact with the SR Ca^{2+}-release channel and therefore do not show the steep decrease in contractile force at low concentrations of the anesthetics.

Effect of Volatile Anesthetics on Force–Frequency Relation

Schmidt *et al.*[12] reported that halothane restores the positive FFR in human ventricular myocardium and proposed that prevention of diastolic Ca^{2+} overload by the antagonistic effects of halothane on L-type Ca^{2+} channels or activation of the SR Ca^{2+}-release channel might have caused the restoration of the FFR. Moreover, a decrease of the Ca^{2+} sensitivity of myofilaments might contribute to the phenomenon, but the exact molecular mechanism remained unclear. In concordance with the findings of Schmidt *et al.*,[12] we also found that the positive shape of the FFR is restored by halothane in failing human myocardium. The comparison with other volatile anesthetics and with the effect of verapamil and ryanodine allow further conclusions to be drawn on the intracellular mechanism. With respect to inhibition of the Ca^{2+} inward current *via* the L-type Ca^{2+} channel, no major differences have been reported between halothane, isoflurane, and sevoflurane.[33] Because only halothane restored the positive shape of the FFR in failing human myocardium, the interaction of the anesthetics with L-type Ca^{2+} channels cannot account for this effect of halothane. Accordingly, the Ca^{2+} antagonist verapamil did not influence the shape of the FFR in failing myocardium. Similarly, the low availability of Ca^{2+} itself cannot account for the positive shape of the FFR in the presence of halothane. At low Ca^{2+} concentrations in the bathing solution (1.5 or 0.5 mM), the FFR was still negative in failing myocardium.

A decrease in Ca^{2+} sensitivity of the myofilaments also cannot explain why halothane, but not isoflurane, alters the FFR in failing myocardium. Halothane and isoflurane depressed the Ca^{2+} sensitivity of human ventricular myofilaments to a similar extent.[4]

A more promising explanation for the restoration of the positive FFR by halothane is its interaction with the SR Ca^{2+}-release channel of the SR. As demonstrated by our ^{3}H-ryanodine radioligand studies, only halothane, but not isoflurane or sevoflurane, increased the Ca^{2+} sensitivity of the Ca^{2+}-release channel, leading to an increased open probability of the channel and Ca^{2+} depletion of the SR. The Ca^{2+} depletion of the SR lowers contractile force, particularly at low stimulation frequencies at which time for Ca^{2+} reuptake into the SR is not limited, and thus the contribution of intracellular Ca^{2+}

cycling *via* the SR to contractile force is high. Because the activity of the SR Ca^{2+}–adenosine triphosphatase is reduced in failing human myocardium,[34] the contribution of SR Ca^{2+} cycling to contractile force becomes less important at higher stimulation frequencies with decreased time for reuptake of Ca^{2+} into the SR.[35] This could explain why, in failing human myocardium, halothane decreased contractile force preferentially at low stimulation frequencies, resulting in the restoration of the FFR. The hypothesis is strongly supported by our observation that ryanodine also restored the positive shape of the FFR in failing myocardium. Ryanodine keeps the SR Ca^{2+}-release channel in a 40% subconductance state and thereby blocks the repetitive reuptake and release of Ca^{2+} by the SR, resulting in Ca^{2+} depletion of the intracellular Ca^{2+} stores.[22] The similarity of halothane and ryanodine with respect to their interaction with the SR and their effect on the FFR in failing myocardium strongly suggests that halothane restores the FFR by increasing the open probability of the SR Ca^{2+}-release channel, thereby depleting the sarcoplasmic reticular Ca^{2+} stores, leading to a cardiodepressant effect particularly at low stimulation frequencies. Because reduction of the heart rate has been suggested to improve myocardial contractility in patients with heart failure,[10] knowledge about the influence of halogenated anesthetics on the FFR might help to improve the clinical outcome of anesthetized patients with depressed myocardial function.

Although the negative inotropic effect of halothane was also more pronounced than that of isoflurane and sevoflurane in nonfailing human myocardium, the positive shape of the FFR was not altered by any anesthetic. Because the activity of the SR Ca^{2+}–adenosine triphosphatase was not depressed, intracellular Ca^{2+} cycling *via* the SR strongly contributed to the active force development at all stimulation frequencies. Thus, inhibitors of the sarcoplasmic reticular function are expected to reduce contractile force at all rates. Indeed, our data demonstrate that both ryanodine and halothane depress contractile force to a similar extent at all stimulation frequencies. Accordingly, in nonfailing myocardium, the positive shape of the FFR was maintained in the presence of all drugs.

Limitations

Extrapolation of our data to the situation *in vivo* should be performed with caution. Although changes in the shape of the FFR *in vitro* go hand-in-hand with respective alterations of the FFR *in* vivo,[7] the effect of volatile anesthetics on frequency-dependent force development might be influenced by a number of additional factors beyond changes of intrinsic myocardial contractility. Changes of cardiac performance during the administration of anesthetics may also depend on modifications of the physiologic variations in sympathetic and parasympathetic tone, venous return, and vascular tone.

Moreover, the number of specimens from nonfailing hearts was small because of the limited access to appropriate tissue samples. However, in the absence of volatile anesthetics, baseline force of contraction and the shape of the FFR was very similar to results previously reported in similar tissue samples,[8] and the variability within our population of muscle preparations was not extraordinarily high. Thus, we suggest that the data obtained from the limited number of specimens investigated here represent the typical contractile behavior of nonfailing myocardium studied during similar conditions.

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