Effects of Volatile Anesthetics on Mechanical Properties of Rat Cardiac Skinned Fibers

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Volatile anesthetics were demonstrated to decrease calcium sensitivity and maximal developed force of detergent-treated rat cardiac skinned fibers. To further investigate the possible mechanisms involved in the decrease of force production, stiffness measurements were performed at defined levels of activation with the use of quick length changes of 0.3 to 4% of initial muscle length in the absence and in the presence of 2 MAC of halothane, enflurane, or isoflurane. The results of various series of experiments suggest that these anesthetics have multiple sites of action on cardiac myofibrillar proteins: 1) they decreased active stiffness indicating a decreased number of attached force-generating cross-bridges; 2) they increased the stiffness/force ratio suggesting that the individual force developed by each cross-bridge was decreased during anesthetic exposure; and 3) they increased the time constant of force recovery, which is consistent with the decreased rate of ATP hydrolysis described by others. These changes in cross-bridges kinetics and efficiency may result from conformational changes in all the protein systems involved in force production, and especially actin–myosin attachment and detachment. However, the changes observed were small despite a relatively high concentration of anesthetics; therefore, they will probably participate only to a moderate extent in the overall negative inotropic effect of these agents. (Key words: Anesthetics, volatile; enflurane; halothane; isoflurane. Heart; contractile proteins; cross-bridges; stiffness. Sites of anesthetic action.)

Most volatile anesthetics are potent myocardial depressants. The halogenated agents used in clinical practice, halothane, enflurane, and isoflurane, were demonstrated to decrease developed force or shortening of isolated ferret papillary muscles in a dose-dependent fashion.1,2 This depressant effect is thought to be mainly related to effects on plasma membrane and sarcoplasmic reticulum, both actions leading to decrease the amount of free intracellular calcium available for contractile activation.3 A decreased intracellular calcium concentration would allow less force-generating cross-bridges to be attached at any one moment, with therefore less force to be developed. Indeed, using sigmoidal length perturbations, Shibata et al.4 observed that the number of cross-bridges involved in force production decreased during exposure to volatile anesthetics in intact rabbit papillary muscles, as attested by a decrease in the plateau of the dynamic stiffness versus frequency of perturbation curve in the presence of anesthetics during Ba2+ contracture. This effect was suppressed by increasing the bathing Ba2+ concentration; therefore, it seems to reflect mainly the lower affinity of troponin C for Ba2+ in the presence of anesthetics. Indeed during Ba2+ contracture maximal activation of contractile proteins is not achieved. However, in detergent-treated rat cardiac fibers, in which all functional membranes are chemically destroyed,5 as well as in mechanically disrupted rabbit cardiac fibers,6 and in tetanized intact rabbit papillary muscles, in which the function of sarcoplasmic reticulum is suppressed by ryanodine,7 in addition to effects on Ca2+ sensitivity, volatile anesthetics decreased maximal Ca2+-activated force, an effect that is dose-dependent and reversible. This effect occurred at clinical anesthetic concentrations and therefore may participate to the overall negative inotropic effect of these agents. The decreased maximal force observed in these models is not related to changes in calcium concentration because this latter is maintained at a high level sufficient to saturate the binding sites of troponin C (pCa 4.5); yet, it reflects a change in one or more of the three components responsible for force production: 1) the number of force generating cross-bridges; 2) the individual force a cross-bridge is able to generate; and 3) the rate of cycling of these cross-bridges.8–13 Stiffness, represented as the ratio of force changes over length changes, has been demonstrated to be proportional to the number of cross-bridges attached at any one moment, whereas stiffness normalized for the same developed force or the stiffness over force ratio would indicate the mechanical properties of individual cross-bridges.14–18 To further investigate the respective part of the mechanisms involved in the decrease of maximal activated tension, stiffness changes and cross-bridge kinetics of detergent-treated rat cardiac fibers were studied during anesthetic exposure using quick stretches and quick releases in the range of 0.3–4% of the initial length of the preparation. The results of the present study favor the hypothesis that the anesthetics have a multisite effect on myofibrillar proteins.
Methods

MUSCLE PREPARATION

After approval from the Institutional Animal Care Committee (INSERM, Paris), hearts were rapidly removed from rats (160–220 g body weight) that had been anesthetized with ethyl carbamate (20%) 1 ml/100 g body weight. Subendocardial left ventricular fibers were excited at room temperature in a zero-calcium Krebs solution. The muscles were skinned for 60 min in a relaxing solution containing the nonionic detergent triton X-100 (1% v/v). After skinning the fibers were mounted between two stainless steel hooks, and the sarcomere length was adjusted to 2.1–2.2 μm using laser diffraction (laser He-Ne 10 mW, Spectra Physics, Inc., Mountain View, California). The length and diameter of ventricular fibers were determined optically with a binocular microscope and a micrometer. The mean length (±SD) was 1,365 ± 160 μm and the mean diameter was 250 ± 20 μm.

SOLUTIONS

The composition of the solutions used was calculated using the computer program of Fabiato and Fabiato and the binding constants of Fabiato. Imidazole (30 mM) and acetic acid were used as a buffer to adjust solutions to pH 7.1. Ionic strength was adjusted to 0.16 M with K acetate. All solutions were freshly prepared from frozen stock solutions. Two different solutions were prepared: a relaxing solution (A) and an activating solution (B). They were calculated so that the following composition was maintained constant: free Mg2+ 3.16 mM, MgATP 3.16 mM, creatine phosphate 12 mM, Na+ 30.6 mM EGTA 10 mM, and dithiothreitol 0.3 mM. In the relaxing solution pCa was set at 9, and in the activating solution pCa was set at 4.5 (pCa = -log10 [free Ca2+]). Solutions of intermediate Ca2+ concentrations were obtained by mixing solutions A and B of extreme Ca2+ concentrations.

ANESTHETIC EQUILIBRATION

For each experimental condition the solutions were equilibrated in separate chambers by continuous bubbling for 15 min with halothane, enflurane, or isoflurane, as previously described. The anesthetic concentrations used were 2% halothane, 4.2% enflurane, and 3% isoflurane. These concentrations are roughly equivalent to 2 MAC of each agent in rats at 37°C. These concentrations were chosen because they were demonstrated to produce a significant decrease in maximal calcium-activated force ranging between 10% and 20%, whereas a concentration of 1 MAC or less would produce changes of less than 10% of maximal developed tension. The anesthetic concentrations obtained in the solutions were measured by gas liquid chromatography with the head space technique. The anesthetic concentrations measured in the experimental conditions after 15 min of continuous bubbling were 1.26 ± 0.12 mM for halothane, 2.18 ± 0.08 mM for enflurane, and 1.64 ± 0.02 mM for isoflurane at 22°C.

MECHANICAL APPARATUS

Details of the mechanical apparatus were previously described. Briefly, force was measured with an AE801 transducer (AME, Horten, Norway) glued to one hook of the experimental setup. The other hook was glued to a lever connected to the moving coil of a conventional loudspeaker (Pioneer TS 130A). The length changes were monitored through a position detector (Hamamatsu S1543), which served as a feedback control for the vibrator. This permitted 80% of the length changes to be complete in less than 1.0 ms. Both were coupled to a digital storage oscilloscope (OS4020, Gould, Inc., Cleveland, Ohio) and to a computer (Compaq Deskpro 286). Tension was also continuously recorded on a paper recorder at a speed of 0.5 mm/min (Ifèlec IF 4000, Paris, France).

Muscules were immersed in 2.5-ml chambers arranged around a disk, immersed in a temperature-controlled bath, positioned on a magnetic stirrer. Each solution was well stirred at high speed (>1,000 rpm). All experiments were performed at 22°C.

DATA ANALYSIS

Quick length changes of 0.3–4% of initial muscle length were applied in the relaxing and activating solutions. They were performed when tension had reached a plateau after a change in solution. Transient tension responses were digitized by a DT2801 AD card (Data Translation), and responses to stretches were analyzed on-line with the use of a program written in Pascal on the Compaq Deskpro 286 computer. Only responses to stretches were used for calculations to avoid interferences of internal shortening of sarcomeres that would occur during releases. Each value of each variable used for calculations is the mean of 5–7 determinations following stretches of different amplitudes.

During quick stretches two distinct phases in tension changes were measured (fig. IA). The spike of tension (F1) in phase with length change characterizes the elastic phase. It is rapidly followed by a rapid decrease in force where F2 represents the maximal recovery of tension. Stiffness is the extreme tension reached during stretch (mN·mm⁻²) divided by the length change at the sarcomere level (microns). Passive stiffness was measured in the relaxing solution at the beginning of each experiment. Active stiffness (linked to actively cycling cross-bridges) was calculated as the difference between total stiffness measured in the activating solution minus passive stiffness.
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Fig. 1. Forces traces recorded during a quick stretch of 2.8% of the initial length. A (left). Trace obtained in control conditions. F0 represents the maximal active developed force (activating solution B, pCa 4.5); F1 represents the force developed during a quick stretch of 2.8%; this value is used to calculate stiffness (force changes over length changes); F2 represents recovery of tension following quick stretch experiments. All values are measured from the resting tension (relaxing solution A, pCa 9) B (right). Same force trace as shown in figure 1A (indicated by F0 and F1) and force trace obtained in the presence of 2% halothane in the same fiber for the same length change (2.8%). Maximal active force (F1h) and maximal force during stretch (F1h) are indicated.

because at low Ca\(^{2+}\) concentrations (inferior to the threshold of contraction) and high ionic strength, all myosin heads are in the detached state in rat cardiac skinned fibers or quiescent intact muscle.\(^{24}\) The term of stiffness when used in the present paper will refer only to active stiffness unless otherwise indicated. For each fiber tension responses have been normalized to the maximal tension obtained in control activating solution (B) at the beginning of the experimental procedure (F0), as shown in figure 2, to take into account changes in maximal activated tension with time and anesthetic exposure; “normalized stiffness” (S\(_{Nx}\)) represents the mean value of 5–7 measurements obtained during stretches of various amplitudes, using the following formula:

\[
S_{Nx} = \frac{S_x \times F_0}{F_0 x}
\]

where Sx represent active stiffness for a specific length change (Sx = δF/δL), F0x the corresponding level of maximal activated force in the absence of length change, and F0 the level of maximal Ca\(^{2+}\) developed force at the beginning of the experimental procedure in a particular fiber. The term “normalized stiffness” will refer to this mode of calculation. The time constant for tension recovery (τ in milliseconds) was calculated by least square regression analysis according to a single exponential model between 50% and 80% of recovery using stretches of more than 2% of the initial muscle length. This range of values was chosen to suppress the rapid component of the recovery phase so that only the slow component of cross-bridge kinetics was considered. The validity of this mode of calculation was assessed by determining the best fit of force traces decay using a computer program especially designed for analysis of multieponential functions based on the mathematical method of Padé-Laplace.\(^{25,26}\) Thirty-eight traces recorded in the activating control solutions during quick stretches of 2–4% amplitude were analyzed. In 30 of 38 traces recovery was best fitted with a biexponential model, and a single exponential decay was only observed in the remaining eight traces. The mean value of the time constant measured in experimental conditions agreed closely with the slower phase of the biexponential curve or the time constant of the single exponential curve, mean values (±SEM) of the time constants being respectively 11.80 ± 0.55 ms in experimental conditions and 12.88 ± 0.70 ms using the multiexponential analysis. The percent of tension recovery (R%) was calculated as the ratio between recovery from F1 to F2 to the extent of tension change (F1 - F0) at maximal force (F0):

\[
R\% = \frac{F_1 - F_2}{F_1 - F_0} \times 100
\]

EXPERIMENTAL PROTOCOL

Measurements of active stiffness were performed on seven fibers with each of the three anesthetics used in a random order. Stiffness measurements were performed at a steady state level of activation, first in the control

Fig. 2. Normalized stiffness. Tension changes following length changes expressed as percent of the Initial length of the preparation. Length changes were performed after active developed force had reached a plateau in the activating solution B free of anesthetic in control conditions or equilibrated with 2% halothane in this particular experiment. In the absence of anesthetic, both passive (F1r) and active (F1) forces and force at recovery (F0) are represented. Force traces in the presence of 2% halothane (F1h and F2h) were normalized for tension in the absence of length changes obtained in control conditions (F0). Normalized stiffness increased significantly during anesthetic exposure.
activating solution free of anesthetics and then in the activating solution previously equilibrated with one of the three agents. After both measurements had been obtained, the muscle was immersed again in the relaxing solution for at least 15 min before the next series of measurements was started. Effects of anesthetics were immediately and completely reversible when switching from the activating solution equilibrated with anesthetics to the activating solution free of anesthetics, as previously shown. Measurements obtained during anesthetic exposure were always compared with those obtained during the immediately preceding control period. Therefore, time-dependent changes of the preparation were considered throughout the study. Because no differences between agents were observed (analysis of variance, $P > 0.5$), the results of experiments using equipotent anesthetic concentrations were pooled together for further data analysis, including those of three additional fibers in which (for technical reasons) only one or two of the three anesthetics had been tested. Twenty-six sets of data were analyzed. Passive stiffness in the relaxing solution without and with anesthetics was measured in six additional fibers.

In a second series of experiments, the fibers ($n = 11$) were immersed in a solution free of anesthetics, the pCa of which was adjusted to produce a stable level of developed force identical to the one obtained in the activating solution (pCa 4.5) equilibrated with anesthetics. This allowed us to compare stiffness changes at an identical level of developed tension but different level of activation.

In a third series of experiments the fibers ($n = 5$) were immersed in solutions of increasing Ca$^{2+}$ concentrations. Quick stretches and releases were obtained at each Ca$^{2+}$ concentration in the absence of anesthetics first and in the same solution previously equilibrated with 2 MAC multiples of halothane or enflurane. The fibers were allowed to relax for 15 min between two sets of experiments. Only one agent was tested on each fiber in this particular series of experiments. Only solutions with a pCa of 5.75 or less were used because rat ventricular muscle is working at a higher [Ca$^{2+}$] range in the tension/pCa relation than the cardiac muscles of other species.

**TABLE 1. Effects of Anesthetics on Passive Stiffness**

<table>
<thead>
<tr>
<th>Passive stiffness</th>
<th>Control</th>
<th>Anesthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force</td>
<td>158 ± 19</td>
<td>199 ± 21</td>
</tr>
<tr>
<td>(mN·mm$^{-2}$)</td>
<td>3.7 ± 0.9</td>
<td>3.3 ± 0.6</td>
</tr>
<tr>
<td>Stiffness/force</td>
<td>42.3 ± 10.6</td>
<td>48.1 ± 10.7</td>
</tr>
</tbody>
</table>

Passive stiffness obtained in the relaxing solution (pCa = 9), rested force, and ratio of passive stiffness/force in control conditions and during anesthetic exposure ($n = 6$). Data are mean ± SEM.

**Statistical Analysis**

Differences between anesthetics effects were analyzed by repeated analysis of variance (ANOVA). For each anesthetic data obtained in control solutions were compared with those obtained in solutions with anesthetic by Student's $t$ test for paired data. A $P$ value of 0.05 or less was considered statistically significant. All results are expressed as mean ± SEM.

**Results**

**Passive Stiffness**

In the absence of anesthetics, passive stiffness represented about 30% of the total stiffness, whereas force represented about 13% of the total (active plus passive) maximal developed force. The passive stiffness measured in the relaxing solution remained unchanged by the anesthetics. Because the rested force was also unchanged, the ratio of passive stiffness over resting force remained stable (table 1). Passive stiffness was always measured at the beginning of each series of experiments, and this value was subtracted from total stiffness.

**Stiffness Measurements at Maximal Activation**

Quick length changes of 0.3–4% of the initial muscle length were performed in the activating solution after a steady state level of force had been achieved with control solutions first and subsequently in solutions equilibrated with one of the anesthetics. Within these limits stiffness is independent of the extent of length changes. Tension changes recorded after stretches of identical size in the same fiber in control conditions and after exposure to 2% halothane are represented in figure 1B. Tension changes plotted against length changes are represented in figure 2. Forces measured in the absence of anesthetics in the relaxing solution (F$_{1r}$) and the activating solutions (F$_{1}$) during stretches of various amplitudes and force at recovery (F$_{2}$) are shown. In the absence and in the presence of anesthetics, stiffness was normalized for the maximal tension measured in control solution (B) at the beginning of the experimental procedure (F$_{0}$) in the absence of length changes as shown in figure 2, normalized stiffness (which represents the slope of F$_{1}$ curves) increased during anesthetic exposure. Tension changes over length changes were linear during stretches experiments but not during release experiments; thus, the latter were not used to calculate the F$_{1}$ slopes.

Results of force, stiffness, and stiffness/force ratio measured in the seven fibers in which all the three anesthetics had been tested are shown in table 2. In these experiments time-dependent changes in the characteristics...
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TABLE 2. Stiffness and Force Changes Obtained in the 7 Fibers in Which the 3 Anesthetics Were Used in a Random Order

<table>
<thead>
<tr>
<th></th>
<th>C$_H$</th>
<th>Halothane</th>
<th>C$_E$</th>
<th>Enflurane</th>
<th>C$_I$</th>
<th>Isoflurane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force (mN·mm$^{-2}$)</td>
<td>22.8 ± 2.3</td>
<td>20.5 ± 2.2</td>
<td>25.6 ± 3.1</td>
<td>22.3 ± 2.8†</td>
<td>23.4 ± 3.6</td>
<td>20.8 ± 3.2†</td>
</tr>
<tr>
<td>Stiffness (mN·mm$^{-2}$·μm$^{-1}$)</td>
<td>428 ± 69</td>
<td>421 ± 65</td>
<td>466 ± 74</td>
<td>448 ± 68</td>
<td>444 ± 85</td>
<td>438 ± 82</td>
</tr>
<tr>
<td>Stiffness/force (μm$^{-1}$)</td>
<td>18.8 ± 1.6</td>
<td>20.5 ± 1.7*</td>
<td>18.2 ± 1.4</td>
<td>20.1 ± 1.3†</td>
<td>19.0 ± 1.5</td>
<td>21.0 ± 1.5*</td>
</tr>
<tr>
<td>Normalized stiffness (mN·mm$^{-2}$·μm$^{-1}$)</td>
<td>501 ± 81</td>
<td>547 ± 91*</td>
<td>504 ± 77</td>
<td>555 ± 79†</td>
<td>519 ± 96</td>
<td>575 ± 99*</td>
</tr>
<tr>
<td>Time constant (ms)</td>
<td>13.7 ± 1.1</td>
<td>15.4 ± 1.0*</td>
<td>13.4 ± 0.9</td>
<td>14.8 ± 0.8*</td>
<td>13.7 ± 1.2</td>
<td>15.1 ± 1.3*</td>
</tr>
<tr>
<td>Tension recovery (%)</td>
<td>76.1 ± 4.2</td>
<td>71.4 ± 4.3†</td>
<td>77.8 ± 9.5</td>
<td>73.1 ± 4.1</td>
<td>74.2 ± 5.1</td>
<td>71.0 ± 5.1*</td>
</tr>
</tbody>
</table>

Values measured during anesthetic exposure were compared with their respective controls (C$_H$, C$_E$, C$_I$) by paired t test (*P < 0.05; †P < 0.01). N = 7 for each group. 

‡ Normalized stiffness is calculated in reference to the maximal activated force obtained at the beginning of the experimental protocol in each fiber.

of the preparation were observed, mainly a moderate decrease in maximal tension, which represented less than 20%. This problem was solved by the design of our study, in which anesthetics were studied in a random order and measurements during anesthetic exposure were compared with those obtained in the immediately preceding control period. Mean maximal Ca$^{2+}$ developed force obtained in control conditions before anesthetic exposure was not different for the three agents (table 2; ANOVA, P > 0.5). No differences were observed in control conditions for the other variables shown in table 2. Each of the three agents produced a significant decrease in maximal force and a significant increase in both stiffness/force ratio and normalized stiffness, whereas stiffness itself was unchanged (table 2). The effects of the three agents were not different (ANOVA P > 0.5). On recovery from length changes, time constants (τ) of tension recovery increased significantly with each anesthetic, whereas the extent of tension recovery (R%) decreased significantly with both halothane and isoflurane.

When all data (including the five additional experiments) were pooled together, in addition to the changes previously described, stiffness decreased also significantly in the presence of anesthetics (table 3).

Stiffness Changes at Different Levels of Activation

To avoid possible interference of the level of isometric force, the fibers were immersed in solutions of an intermediate pCa so that the force developed was identical to that achieved in the activating solution equilibrated with the anesthetics. A mean pCa value of 5.44 ± 0.14 was necessary to achieve the same level of developed force. Eleven sets of data were obtained (table 4). In the absence of anesthetics, stiffness decreased in solution of intermediate pCa compared with that obtained with the activating solution. However, when stiffness values were normalized for tension (normalized stiffness), the data obtained in both experimental conditions were identical, as expected if the force developed by each individual cross-bridge was unchanged. The ratio of stiffness/force remained also constant. The percentage of tension recovery increased significantly at lower force, whereas the time constant of recovery decreased significantly.

In the presence of anesthetics, maximal tension decreased compared with that obtained in the control activating solution and was identical to the one obtained with a solution of a mean pCa of 5.44 ± 0.14. At an identical level of developed force, stiffness was again significantly higher in the presence of anesthetic, as well as the time constant of recovery, whereas the percentage of tension recovery decreased (table 4). The ratio of stiffness/force and normalized stiffness increased significantly as well.

Stiffness changes at increasing Ca$^{2+}$ concentrations were obtained in five fibers. Experiments were successively performed at pCa 5.75, 5.625, 5.5, and 4.5. At each pCa force and stiffness were significantly lower in the presence of anesthetics, whereas the stiffness/force ratio increased significantly at maximal activation (table 5). However, the

TABLE 3. Stiffness and Force Changes at Maximal Activation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Anesthetics</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force (mN·mm$^{-2}$)</td>
<td>23.9 ± 1.4</td>
<td>21.1 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stiffness (mN·mm$^{-2}$·μm$^{-1}$)</td>
<td>450 ± 35</td>
<td>435 ± 35</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Stiffness/force (μm$^{-1}$)</td>
<td>18.8 ± 0.8</td>
<td>20.6 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normalized stiffness (mN·mm$^{-2}$·μm$^{-1}$)</td>
<td>542 ± 44</td>
<td>592 ± 46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time constant (ms)</td>
<td>14.6 ± 0.6</td>
<td>16.1 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tension recovery (%)</td>
<td>72.9 ± 2.3</td>
<td>68.1 ± 2.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Stiffness and force changes at maximal activation (pCa 4.5) in the absence of anesthetics (control) during exposure to 2 MAC of halothane, enflurane, or isoflurane. Data are mean ± SEM (n = 26). Statistical analysis was done by paired t test.
Table 4. Stiffness Changes at Various Force Levels

<table>
<thead>
<tr>
<th></th>
<th>Control (B)</th>
<th>Anesthetic (B)</th>
<th>Intermediate pCa</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCa Force</td>
<td>4.5</td>
<td>4.5</td>
<td>5.44 ± 0.4</td>
</tr>
<tr>
<td>(mN · mm⁻²)</td>
<td>24.2 ± 2.0</td>
<td>21.4 ± 1.8</td>
<td>21.5 ± 1.9</td>
</tr>
<tr>
<td>Stiffness</td>
<td>503 ± 52</td>
<td>475 ± 53*</td>
<td>452 ± 51†</td>
</tr>
<tr>
<td>(mN · mm⁻² · μm⁻¹)</td>
<td>20.8 ± 1.3</td>
<td>22.2 ± 1.5†</td>
<td>21.0 ± 1.4</td>
</tr>
<tr>
<td>Normalized stiffness</td>
<td>658 ± 61</td>
<td>700 ± 66†</td>
<td>664 ± 66</td>
</tr>
<tr>
<td>(mN · mm⁻² · μm⁻¹)</td>
<td>16.0 ± 1.0</td>
<td>17.1 ± 1.0†</td>
<td>13.9 ± 0.9§</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>68.2 ± 5.4</td>
<td>63.1 ± 3.9*</td>
<td>73.9 ± 2.9§</td>
</tr>
</tbody>
</table>

Values (±SEM) of stiffness, force, ratio of stiffness/force, time constant, and per cent of tension recovery in three experimental conditions: control (pCa 4.5); anesthetic (pCa 4.5, solutions equilibrated with 2 MAC of one anesthetic); and intermediate pCa (pCa 5.44, no anesthetic, in which developed force was identical to the one developed by fibers in the activating solution B in the presence of anesthetic).

The time constant of recovery was significantly increased at each pCa in the presence of anesthetics; this increase was the most important at the low pCa values. In both conditions (control and anesthetic exposure) there was a linear relationship between stiffness and force (fig. 3) (y = 21.9x - 59.6, r = 0.999 in control; y = 23.6x - 62.1, r = 0.996 in the presence of anesthetics). The mean slope values of this relationship were significantly higher with anesthetics compared with control conditions (mean control slopes 21.9 ± 3.1 μm⁻¹; mean anesthetic slopes 23.6 ± 3.0 μm⁻¹; paired t test, P < 0.02). Median values of coefficients of correlation of the individual slopes were 0.989 (range, 0.977–0.993) in control conditions and 0.993 (range, 0.983–0.999) during anesthetic exposure.

Discussion

This study suggests that at the concentrations studied, halothane, enfurane, and isoflurane have multiple sites of action on cardiac myofibrillar proteins. In addition to their effects on calcium sensitivity, they are likely to decrease both the force a cross-bridge is able to generate at any one moment and the number of attached cross-bridges during active contraction, and at the same time they also decrease the cycling rate of these cross-bridges.

The cyclic interaction between thin (actin) and thick (myosin) filaments causes maximal force development when the muscle is prevented from shortening. It is generally thought that: 1) the total force on any filament arises from those cross-bridges within the fields of overlap of the thick and thin filaments; 2) the cross-bridges act independently of each other; and 3) each cross-bridge acts cyclically. Measurements of stiffness provide further information on the behavior of cross-bridges during the contraction cycle. Stiffness (which is total stiffness minus passive stiffness) mainly represents the series elastic elements that reside mostly within the cross-bridges. Passive stiffness, which does not change during the time course of the experimental procedures, represents the noncontractile elements of cardiac muscle. Stiffness increases with Ca²⁺ activation, and this effect reflects the recruitment of more cross-bridges during Ca²⁺ activation and/or changes in cross-bridges turnover kinetics. At various [Ca²⁺], however, the average cross-bridge extension (force/stiffness ratio) during the power stroke and hence the force per cross-bridge remains identical. The results of our experiments done with solutions of intermediate pCa or with the activating solution but free of anesthetics agree closely with these experimental reports. In these conditions the stiffness/force ratio and normalized stiffness remained identical, indicating that the force developed by individual cross-bridges is independent of the level of activation (at least in the range of Ca²⁺ activation levels above 50%).

The lack of feedback control system to keep the length

Table 5. Stiffness and Force Changes at Different Levels of Activation

<table>
<thead>
<tr>
<th>pCa</th>
<th>Force (mN · mm⁻²)</th>
<th>Stiffness (mN · mm⁻² · μm⁻¹)</th>
<th>Stiffness/Force (μm⁻²)</th>
<th>Time Constant (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>14.5 ± 2.7</td>
<td>259 ± 48</td>
<td>17.9 ± 2.3</td>
<td>14.6 ± 1.3</td>
</tr>
<tr>
<td>A</td>
<td>9.9 ± 2.0*</td>
<td>173 ± 41*</td>
<td>17.5 ± 3.1</td>
<td>20.0 ± 1.5*</td>
</tr>
<tr>
<td>5.625</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>19.5 ± 3.1</td>
<td>366 ± 59</td>
<td>18.8 ± 2.2</td>
<td>14.0 ± 1.3</td>
</tr>
<tr>
<td>A</td>
<td>13.4 ± 1.5*</td>
<td>254 ± 47*</td>
<td>19.0 ± 3.0</td>
<td>18.1 ± 1.4*</td>
</tr>
<tr>
<td>5.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>23.4 ± 3.0</td>
<td>450 ± 70</td>
<td>19.2 ± 2.6</td>
<td>15.2 ± 1.3</td>
</tr>
<tr>
<td>A</td>
<td>18.8 ± 2.7†</td>
<td>371 ± 60*</td>
<td>19.7 ± 2.9</td>
<td>18.3 ± 1.2†</td>
</tr>
<tr>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>24.0 ± 3.1</td>
<td>468 ± 79</td>
<td>19.5 ± 2.6</td>
<td>18.2 ± 1.0</td>
</tr>
<tr>
<td>A</td>
<td>20.6 ± 2.9†</td>
<td>433 ± 84*</td>
<td>21.0 ± 3.0*</td>
<td>19.4 ± 0.8†</td>
</tr>
</tbody>
</table>

Data (±SEM) for force, stiffness, stiffness/force ratio, and time constant at four different levels of activation (pCa = -log₆ [Ca²⁺]) with control solutions (C) and with solutions of identical composition previously equilibrated with 2 MAC of anesthetics (A) (n = 5).

Significant changes at an identical level of activation (paired t test): *P < 0.01; †P < 0.001; ‡P < 0.05.
of the preparation exactly constant between stretches does not permit us to precisely assess mechanical properties of individual cross-bridges but only to assess the relative changes due to anesthetic exposure. Passive stiffness was not changed by the anesthetics, which is not surprising because passive stiffness is not related to the contractile elements. This value (obtained at the beginning of each experimental procedure) was therefore subtracted from total stiffness in each experimental condition. At maximal activation the decrease of maximal activated force and stiffness is consistent with a decreased number of attached force generating cross-bridges. This decreased number of cross-bridge interactions is quantitatively small (stiffness being decreased by less than 5%); although it reached statistical significance, it is not sufficient to account for the overall decrease of maximal developed force.

The main finding of our study is that the force developed by individual cross-bridges was decreased by the anesthetics, as attested by the increased stiffness/force ratio and normalized stiffness observed at maximal activation during anesthetic exposure. The lack of significant increase of the stiffness/force ratio at low Ca\(^{2+}\) concentrations is likely related to the importance of noncontractile compliance elements present in cardiac muscle preparation compared with those of skeletal muscle; among them, the connectin content of bovine heart myofibrils is about 18% of the total myofilibrillar protein, three times as much as in skeletal myofibrils.

The increased time constant, as observed at any Ca\(^{2+}\) level in the presence of anesthetics, is consistent with a small decreased rate of cycling of the attached cross-bridges, but our experimental conditions are not adequate to determine more precisely the mechanism(s) involved. Halothane was demonstrated to decrease maximal actomyosin ATPase activity. In view of the results of Merin et al., maximal rate of actomyosin ATPase activity would be decreased by about 10% in the conditions of our experiments (halothane concentration 1.2 mM, pCa 4.5). This would indicate a decreased rate of ATP hydrolysis, and this is consistent with the increased time constant observed in our study; this cannot, however, account for the decreased maximal tension observed. Therefore, from the present study, it can be inferred that the volatile anesthetics mainly interfere with force production at the level of each cross-bridge. It seems likely that the anesthetics mainly modify the thermodynamic efficiency of the actomyosin complex. Several biochemical models have been proposed to account for the kinetic behavior of cross-bridges, but correlation between biochemical events and force development cannot be simply described and still remains a subject of controversy.

The results of our study are apparently conflicting with those reported by Shibata et al.\(^{4}\) in intact rabbit papillary muscles at a similar temperature of 24° C. They observed that volatile anesthetics decreased the number of cross-bridge interactions without changes in cross-bridge kinetics. This effect is identical to the one produced by a sarcolemmal calcium blocker, nifedipine.\(^{42}\) However, as in both studies, the effects of volatile anesthetics were reversed by increasing the bathing Ba\(^{2+}\) concentration, maximal activation was not achieved, and these results are similar to our findings at intermediate calcium concentrations reported in table 5. Indeed, at an identical level of calcium activation, stiffness was lower in the presence of anesthetics, an effect that is mainly the consequence of decreased myofibrillar Ca\(^{2+}\) (or Ba\(^{2+}\)) sensitivity in our experiments, and that would result from both effects on sarcolemmal membrane and contractile proteins in intact muscle. No changes in cross-bridge kinetics were obvious in this latter preparation, but our changes were small and may be difficult to observe in intact preparations. In intact ferret papillary muscles, a dose-dependent decrease in maximal unloaded velocity of shortening (MUVS) was observed in the presence of volatile anesthetics, an effect that was more pronounced for halothane or enflurane than for isoflurane.\(^{4}\) This was attributed to a decreased cyclic rate of actomyosin cross-bridges. In view of both our results and those of Shibata et al.,\(^{4}\) this decrease in MUVS seems more likely to be the consequence of the decreased myofibrillar Ca\(^{2+}\) availability rather than to a direct effect of volatile anesthetics on actin–myosin interactions.

The precise mechanism of action of these anesthetics remains to be determined. Anesthetic molecules are relatively small molecules, having a molecular weight of about 200. The structural diversity of the inhaled anesthetics and their multiple effects strongly suggest that they do not interact directly with a single specific receptor. In addition to their effects on membrane systems, which can
be explained by structural or biochemical changes in lipid bilayer arrangement or function, there is strong evidence for a potential interaction with protein systems. The dose-dependent decrease in calcium sensitivity and maximal developed tension described in our model of detergent-treated rat cardiac skinned fibers can only be interpreted as resulting from interaction with protein systems. One theory postulates that anesthetic molecules may induce conformational changes in the protein molecules by combining with hydrophobic regions of these latter. In view of these mechanisms, changes in cross-bridge kinetics and efficiency may result from conformational changes in all the protein systems involved in force production and especially actin–myosin attachment and detachment. In a study performed in rabbit skeletal muscle, Leuwenkroon-Strössberg et al. reported that halothane in a concentration of 3% and 10% (v/v) reduced optical rotatory dispersion of myosin, whereas maximal myosin Ca²⁺ ATPase activity was unchanged (98% of control) at the lowest halothane concentration or slightly increased at the highest concentration (125% of control). Therefore, the authors concluded that conformational changes in myosin may occur in the presence of the drug without being necessarily correlated with changes in enzymatic activity. Although this study investigated the effects of anesthetics on skeletal myosin, the authors reported that they found similar changes in cardiac myosin. These results favor the hypothesis that the anesthetics may produce conformational changes in actin–myosin cross-bridges. Small structural changes may induce minor changes in the relative position of myosin heads and actin filaments leading to modify the free energy transduction process.

The relative contribution of our findings to the overall negative inotropic effects of volatile anesthetics is difficult to appreciate. First, our observations were done at 22°C and at a rather high anesthetic concentration (2 MAC). This concentration only reduces maximal activated force by 10–20%, but a dose-dependent decrease in maximal calcium activated tension was observed in different preparations. In living tissues of most species, even during a positive inotropic intervention, the force development is far from maximal so that the force can easily be varied by increasing or decreasing the free Ca²⁺ concentration. Therefore, for calcium concentrations likely to be reached during the time course of normal activation, the effects of volatile anesthetics on intracellular calcium content, myofibrillar calcium sensitivity, and maximal activated force may play a role. In rabbit papillary muscles studied at a more physiologic temperature of 37°C, Berman et al. found that 2% halothane decreased maximal activated force generated by myofilaments to 79% of control, whereas twitch tension was reduced to 58% of control, both experiments being performed in the presence of ryadonide to suppress the function of sarcoplasmic reticulum. However, in intact ferret papillary muscles (at a temperature of 30°C), 2 MAC isoflurane reduced twitch tension to about 25% of control, whereas 1.5 MAC halothane decreased it to less than 20%. Therefore, this effect of volatile anesthetics on maximal activated force can account for only a small fraction of the profound depression of contractility.

The effects of volatile anesthetics on contractile proteins as described in the present study and the previous one were similar for the three anesthetics at equipotent anesthetic concentrations. Therefore, this action on cardiac myofilaments cannot explain the differential effect of volatile anesthetics on myocardial contractility. This differential effect is mainly due to the lesser depression of the sarcoplasmic reticulum function caused by isoflurane compared with halothane and, to a lesser extent, to the smaller decrease of membrane currents. Interestingly, both the sarcolemmal membrane and the sarcoplasmic reticulum have a complex lipidoprotein structure, instead of being only a pure proteic model, as was our myofibrillar preparation.

In conclusion, our study demonstrates that the decrease in maximal tension as observed in rat cardiac skinned fibers during anesthetic exposure is related to small changes in cross-bridge interactions, kinetics, and efficiency. These changes were observed at high anesthetic concentrations and remained relatively small compared with the overall negative inotropic effects of volatile anesthetics.

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