Anesthesiology
2000; 92:1114–25

Comparison of Volatile Anesthetic Effects on Actin–Myosin Cross-bridge Cycling in Neonatal versus *Adult Cardiac Muscle*

Yedatore S. Prakash, Ph.D., Mark J. Cody, B.A.,† James D. Hannon, M.D.,‡ Philippe R. Housmans, M.D.,§ Gary C. Sieck, Ph.D.‖*

Background: **The neonatal myocardium is more sensitive to volatile anesthetics compared with adults. The greater myocardial sensitivity of neonates may be attributable to greater anesthetic effect on force regulation at the level of the cross-bridge. In the current study, the authors compared the effects of 1 and 2 minimum alveolar concentration (MAC) halothane and sevoflurane on cardiac muscle from 0- to 3-day-old (neonate) and 84-day-old (adult) rats.**

Methods: **Triton X-100–skinned muscle strips were maximally activated at pCa (negative logarithm of the Ca^{2+} concentration) of 4.0, and the following were measured in the presence or absence of anesthetic: Rate of force redevelopment after rapid shortening and restretching (k_{tr}) and isometric stiffness at maximal activation and in rigor. The fraction of attached cross-bridges (α_{fs}) and apparent rate constants for cross-bridge attachment (f_{app}) and detachment (g_{app}) were calculated assuming a two-state model for cross-bridge cycling. Anesthetic-induced changes in the mean stiffness per cross-bridge were also estimated from values in rigor versus maximum activation in the presence or absence of anesthetic.**

Results: **Neonatal cardiac muscle displayed significantly smaller α_{fs}, slower k_{tr}, and slower f_{app} compared with adult cardiac muscle; however, g_{app} was not significantly different. Halothane, and sevoflurane to a significantly lesser extent, decreased α_{fs}, f_{app}, and the mean force per cross-bridge and increased g_{app} to a greater extent in neonates.**

Conclusions: **These data indicate that weaker force production in neonatal cardiac muscle involves, at least in part, less efficient cross-bridge cycling kinetics. The authors conclude that the greater myocardial sensitivity of neonates to volatile anesthetics reflects, at least in part, a direct inhibition of cross-bridge cycling, especially the rates of cross-bridge attachment and detachment. (Key words: Contraction; development; halothane; heart; sevoflurane.)**

VOLATILE anesthetics such as halothane and sevoflurane are known to depress the myocardium, albeit with differing potencies.[1–4] Compared with the adult heart, the neonatal heart has been generally found to be more sensitive to volatile anesthetic depression,[5–7] with one exception.[8] A potential mechanism explored in previous studies is the interference of volatile anesthetics on the regulatory mechanisms of force generation. Volatile anesthetics such as halothane have been shown to decrease the Ca^{2+} sensitivity of force generation in both neonatal[7,8] and adult[7–10] cardiac muscles. However, the precise targets of anesthetic action are currently being evaluated. Furthermore, there is currently little data on the potential mechanisms that underlie differences in the effects of halothane *versus* sevoflurane on cardiac function.

Previous studies in different species have proposed that volatile anesthetic effects on the myocardium are mediated, at least in part, *via* actions at the level of the actin–myosin cross-bridge,[7–12] including a potential decrease in the total number of cross-bridges in the force-generating state, and/or the force produced by a single cross-bridge.[12,13] However, these hypotheses have not been directly tested, especially with newer anesthetics such as sevoflurane. Furthermore, the implications of volatile anesthetic effects on cardiac muscle observed in some studies are difficult to interpret because these effects were observed at relatively high anesthetic concentrations.

The purpose of the current study was to compare the

* Assistant Professor, Department of Anesthesiology.

† Research Technician, Department of Anesthesiology.

‡ Assistant Professor, Department of Anesthesiology.

§ Associate Professor, Department of Anesthesiology.

‖Professor, Departments of Anesthesiology, Physiology, Biophysics, and Molecular Neuroscience.

Received from the Departments of Anesthesiology, Physiology, Biophysics, and Molecular Neuroscience, Mayo Clinic and Foundation, Rochester, Minnesota. Submitted for publication June 2. 1999. Accepted for publication November 15, 1999. Supported by grants GM57816 (Dr. Prakash), GM57891 (Dr. Hannon) and GM36365 (Dr. Housmans) from the National Institutes of Health, Bethesda, Maryland, and by the Mayo Foundation, Rochester, Minnesota.

Address reprint requests to Dr. Prakash: Anesthesia Research, Mayo Clinic, Rochester, Minnesota 55905. Address electronic mail to: prakash.ys@mayo.edu

effects of clinically relevant concentrations (1 and 2 minimum alveolar concentration [MAC]) of halothane *versus* sevoflurane on different parameters of actin-myosin cross-bridge cycling kinetics in skinned ventricular muscle strips from neonate (0- to 3-day-old) *versus* adult (84-day-old or older) rats.

Methods

Tissue Preparation

All experimental procedures described in this article were reviewed and approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. Six male Sprague-Dawley rats (Harlan Inc., Madison, WI) each from three different litters (18 rats total) were used within 3 days of parturition and comprised the neonate group (body weight, 8–10 g at birth). These neonates were gender delineated based on the distance between urethra and anus. Eighteen adult male Sprague-Dawley rats from a different litter comprised the adult group (at least 84 days old; body weight, 250–275 g). Six animals at each age were used for the halothane group, and six of the remaining animals were used for the sevoflurane group. The final six animals were used to estimate myofibrillar density of cardiac muscle.

Animals were anesthetized using intramuscular ketamine (60 mg/kg) and xylazine (2.5 mg/kg). The heart was quickly excised, cleaned of blood, and placed in a balanced salt solution (130 mM NaCl, 3 mM KCl, 1.2 mM KH_2PO_4, 1.0 mM $MgSO_4$, 1.24 mM $CaCl_2$, 10 mM HEPES, 10 mM glucose, pH 7.2) vigorously bubbled with 100% O_2. During continued oxygenation, the ventricles were carefully dissected out, pinned in a Sylgard-coated (Dow Corning Corp., Midland, MI) Petri dish, and cut into thin strips (1–2 mm wide).

Estimation of Myofibrillar Density

Muscle strips were stretched and pinned on cork at an estimated optimal fiber length (L_o) and flash-frozen in melting isopentane cooled by liquid nitrogen. Cross-sections were cut at 10 μm using a cryostat (Reichert-Jung model 2000E; Cambridge Instruments Inc., Buffalo, NY; −20°C), placed on glass slides, and incubated for 2 h in a primary antibody directed against cardiac myosin (antimyosin rabbit immunoglobulin G; Sigma Immunochemicals, St. Louis, MO; 1:50 dilution in 0.1 M phosphate buffer). Control sections were incubated only in buffer. All sections were then washed with phosphate buffer and incubated further for 1 h in a rhodamine-tagged secondary antibody (donkey antirabbit IgG; Jackson Immunoresearch, West Grove, PA). Sections were visualized using confocal microscopy (model MRC500, BioRad Laboratories, Hercules, CA). A fluorescence intensity threshold was established based on the residual fluorescence of the control sections. Pixels with intensities greater than threshold were considered to represent areas of myosin staining. The cross-sectional area of individual myocytes was circumscribed using manufacturer-supplied software. The proportion of pixels above threshold was then used as an index of myofibrillar density.

Experimental Setup for Force and Stiffness Measurements

For each ventricular strip, aluminum foil T clips were attached to the ends to minimize compliance. These muscle strips were then skinned for 20 min with 1 mg/ml Triton X-100 and placed between two small stainless-steel hooks in a temperature-controlled, flow-through, 120-μl-volume acrylic chamber located on the stage of an inverted microscope (Olympus IMT-2; Olympus America Inc., Melville, NY). Extreme caution was used to ensure that the hooks inserted only into the aluminum clips and thus the fixed ends of the muscle. One hook was attached to a force transducer (AE-801; Aksjeselskapet, Horten, Norway) with a resonant frequency of 5 kHz, and the other hook was attached to a servo motor (G120DT; General Scanning, Watertown, MA) with a step time of 800 μs. Sarcomere length was adjusted to 2.2 μm based on calibrated microscopy of the sarcomeric pattern. Custom-built software (based on LabView from National Instruments, Austin, TX) and a data acquisition board (AT-MIO16-L9; National Instruments) were used to control the servo system and acquire length and force signals. Data were acquired at a frequency of 10 kHz.

The Triton X-100–skinned ventricular strips were perfused with solutions of different pCa (negative logarithm of the Ca^{2+} concentration) prepared in accordance with the procedure described by Fabiato and Fabiato[14] with stability constants listed by Godt and Lindley.[15] The solutions contained the following: 10.0 mM EGTA, 1.0 mM free Mg^{2+}, 5.0 mg adenosine triphosphate (ATP), 15.0 mM creatine phosphate, 50.0 mM imidazole, 2.0 mM dithiothreitol, and 1 mg/ml creatine phosphokinase with a total ionic strength of 150 mM. Strips were initially perfused with a relaxing solution of pCa 9.0. In preliminary studies, strips were sequentially exposed to different lower pCa (higher Ca^{2+}) for 30 s to determine the

level for maximum activation and to assess rundown of tissue. Maximal activation of the muscle was obtained at a pCa of 4.0. Exposure to pCa beyond 5.5 for greater than 45 s resulted in significant rundown of the tissue. However, exposure for 30 s allowed attainment of a stable plateau force for a particular pCa and subsequent determination of cross-bridge cycling parameters in the presence or absence of halothane.

Sarcomere lengths during relaxation and activation were maintained according to the techniques of Brenner[16] as modified by Sweeney *et al.*[17] This procedure essentially involved a repetitive sequence of rapid slackening of muscle length such that force instantaneously dropped to zero, followed by rapid restretch of the muscle to the original length. This cycle was repeated every 5 s throughout the course of the experiment. Sarcomere length was measured using video microscopy and was verified to be consistent. The average sarcomere length was found to be 2.2 ± 0.6 μm.

At the end of each experimental protocol, the diameter and depth of the muscle bundle was measured *in vitro* using a calibrated microscope. The cross-sectional area of the bundle was then estimated as the area of an ellipse with diameters equal to the width and depth.

Administration of Volatile Anesthetics

Halothane (Wyeth-Ayerst Laboratories, Philadelphia, PA) and sevoflurane (Abbott Laboratories, Deerfield, IL) were added to the aerating gas mixture *via* a calibrated on-line vaporizer. The vaporizer was set to produce aqueous concentrations of anesthetic in the pCa equivalent to 1 and 2 adult rat MAC at room temperature, concentrations that are well within the range of clinical usage. All experiments were performed at 25°C. Given the lack of previously published MAC values for neonatal rats and the need to have a basis for comparison of effects at equimolar anesthetic concentrations, the adult rat MAC values were also used in the neonates. Aqueous concentrations of halothane in the perfusion chamber (1 atm) were determined by gas chromatography from anaerobically obtained samples using an electron capture detector (Hewlett-Packard 5880A; Palo Alto, CA).[18] In the solutions used to examine the effects of halothane, concentrations were 0.22 ± 0.05 mM for 1 MAC and 0.48 ± 0.07 mM for 2 MAC. Sevoflurane concentrations in the perfusion chamber were determined using a flame ionization detector, and the concentrations were found to be 0.40 ± 0.12 mM and 0.64 ± 0.16 mM for 1 and 2 MAC, respectively.

Experimental Protocols

A number of theoretical models have been developed to explain cross-bridge cycling in striated muscle. Most of these have limitations but are convenient frameworks for understanding the effects of various perturbations on cross-bridge cycling. In the current study, we used the two-state model developed by Huxley as a convenient model to examine the effects of volatile anesthetics. According to this model, cross-bridges are either in a force-generating state (myosin attached to actin) or a non-force-generating state (myosin detached).[19,20] Contraction then involves a cyclical attachment and detachment of myosin from actin (cross-bridge cycling) and basically involves: (1) binding of ATP to the actin-myosin complex (force generating state) resulting in dissociation of the complex, (2) hydrolysis of ATP by the myosin head into adenosine 5′-diphosphate (ADP) and phosphate, (3) reattachment of actin to the myosin-ADP-phosphate complex, and (4) liberation of ADP and phosphate accompanied by a power stroke resulting in the production of force by the single cross-bridge. Due to the cyclical nature of cross-bridge attachment and detachment and a lack of synchronicity in the actin-myosin interactions across the length and width of the muscle, the steady state force produced by the muscle is proportional to the steady state fraction of cross-bridges in the force-generating state (α_{fs}). Accordingly, relaxation involves a reduction in α_{fs}. Therefore,

$$\text{isometric force} = n\bar{F}\alpha_{fs} \quad (1)$$

where n is the maximum number of cross-bridges available in parallel per half sarcomere and $\bar{F}$ is the mean force produced per cross-bridge.

Previous studies have reported that changes in steady state force closely approximate changes in steady state stiffness (*i.e.*, the change in force produced by oscillatory length perturbations of extremely small amplitude [< 1% of optimal muscle length L_o], normalized for the length change).[21,22] Oscillations are typically imposed at ~1 kHz in both skeletal and cardiac muscle. In this technique, the length perturbations are small and fast enough as to not actually disrupt cross-bridge binding, but the incremental force required of each cross-bridge

to oppose the length perturbation is a reflection of the number of attached cross-bridges. Accordingly,

$$\text{isometric stiffness} = n\bar{S}\alpha_{fs} \quad (2)$$

where $\bar{S}$ *is the mean stiffness per cross-bridge.*

As described earlier, ATP is essential for cross-bridge cycling to continue. During conditions of rigor, in which ATP is unavailable for cross-bridge detachment, $\alpha_{fs} = 1$. Therefore, the α_{fs} during other conditions, such as maximal Ca^{2+} activation (with ATP present), can be estimated by taking the ratio of stiffness during that condition to that during rigor, thus eliminating the unknowns n and $\bar{S}$ in equation 2. To determine the effects of volatile anesthetics on α_{fs}, stiffness measurements could then be made during conditions of maximal Ca^{2+} activation and rigor, both in the presence of anesthetic.

Using the techniques employed in the current study, it is not possible to directly determine $\bar{F}$ or $\bar{S}$. However, we attempted to determine the relative change in these parameters during control *versus* anesthetic conditions. The total number of cross-bridges (n) is determined by contractile protein content and is unlikely to be altered by volatile anesthetics in the short time frame of their action. Accordingly, as $\alpha_{fs} = 1$ during rigor regardless of the presence or absence of anesthetic, comparison of isometric force or stiffness during rigor in the presence and absence of anesthetic should indicate whether volatile anesthetics affect the mean force stiffness per cross-bridge. Accordingly,

$$\text{isometric force under rigor (control)} = F_{CR} = n\overline{F_C} \quad (3)$$

$$\text{isometric force under rigor (anesthetic)} = F_{AR} = n\overline{F_A} \quad (4)$$

where $n\bar{F}_C$ and $n\bar{F}_A$ are the mean forces per cross-bridge during control and anesthetic conditions, respectively. However, because both variable are unknown, only the relative change in mean force per cross-bridge can be determined from the measurement of isometric forces as

$$\frac{\overline{F_C} - \overline{F_A}}{\overline{F_C}} = \left(1 - \frac{F_{AR}}{\overline{F_{CR}}}\right) \quad (5)$$

Similarly, for stiffness,

$$\frac{\overline{S_C} - \overline{S_A}}{\overline{S_C}} = \left(1 - \frac{S_{AR}}{\overline{S_{CR}}}\right) \quad (6)$$

Effect of Volatile Anesthetics on Force and Stiffness. Isometric force and stiffness were measured in separate Triton-X-skinned strips by exposure to pCa 9.0 (relaxation) or pCa 4.0 (maximum activation). In the first half of each experiment, strips were first placed in high Ca^{2+} rigor (pCa 4.0), relaxed with pCa 9.0, and then activated with pCa 4.0. Control values of different cross-bridge cycling parameters were obtained in this phase. The strips were then relaxed with pCa 9.0, preexposed to a single anesthetic concentration (either halothane or sevoflurane; separate sets of studies for the two anesthetics), and taken through the same protocol as the control in the continued presence of anesthetic. Thus, each strip served as its own control for either anesthetic. Rundown of the tissue preparation was estimated by exposing a separate set of strips to two pairs of rigor and pCa 4.0 activations.

Stiffness was calculated from the changes in force produced by 1 kHz sinusoidal oscillations of extremely small amplitude (< 1% L_o). Three sets of oscillations were imposed for 100 ms (100 cycles) each, with intervening periods of 3 s. Force and length data were acquired using LabView and averaged across 5 cycles in the middle of each oscillation period, and stiffness was calculated. The average stiffness across three periods was then calculated. The effect of volatile anesthetics on $\bar{F}$ and $\bar{S}$ were estimated by measurements during rigor in the presence or absence of 1 or 2 MAC anesthetic, as explained earlier. Separate strips were also used for each anesthetic concentration.

Effect of Volatile Anesthetics on α_{fs}. In a single strip, the α_{fs} was measured in the presence and absence of 1 or 2 MAC halothane or sevoflurane. Separate strips were used for the two anesthetics and the two concentrations. Stiffness measurements in pCa 4.0 *versus* rigor were performed as described earlier.

Effect of Volatile Anesthetics on Cross-bridge Attachment and Detachment. By extending the Huxley model, two apparent rate constants can been used to describe cyclical cross-bridge kinetics [23]: The rate of cross-bridge attachment (f_{app}), and the rate of cross-bridge detachment (g_{app}). These parameters are related by the expression

$$\alpha_{fs} = \frac{f_{app}}{f_{app} + g_{app}} \quad (7)$$

If an activated muscle at optimal length (L_o) is rapidly released to a shorter length (*e.g.*,~ 80–85% L_o) and then rapidly restretched back to L_o, all cross-bridges are ini-

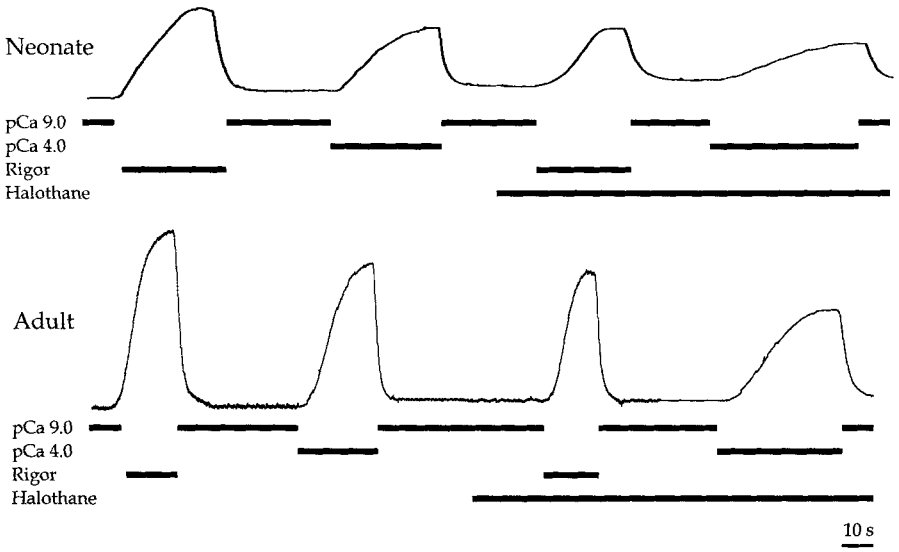

Fig. 1. Effect of halothane on force production during conditions of maximum Ca^{2+} activation and rigor in neonate *versus* adult rat cardiac muscle. A representative example for 2 minimum alveolar concentration (MAC) halothane is shown. Note the greater depressant effect of halothane on neonatal cardiac muscle.

tially broken, and force decreases abruptly to zero. As cross-bridges reform, the rate constant for force redevelopment (k_{tr}) is given by:

$$k_{tr} = f_{app} + g_{app} \quad (8)$$

Therefore,

$$\alpha_{fs} = \frac{f_{app}}{k_{tr}} \quad (9)$$

Equation 9 is valid at all values of α_{fs} except during conditions of rigor, where $\alpha_{fs} = 1$ but $k_{tr} = 0$ and $g_{app} = 0$ (*i.e.*, cross-bridges do not detach). By measuring k_{tr} and estimating α_{fs} from stiffness, the value of f_{app} and g_{app} can be derived.

In strips where α_{fs} was determined, k_{tr} was also determined in the presence or absence of 1 or 2 MAC halothane or sevoflurane. During pCa 4.0 activation, force was allowed to stabilize at its maximum value at a muscle length of L_o. Muscle length was then rapidly dropped by 20%, held at the new length for 100 ms, and then rapidly stretched back to L_o. Decrease of the force response to zero after slackening was verified *post hoc*. A curve-fitting program was used to measure k_{tr}. Separate strips were used for each anesthetic and concentration.

Statistical Analysis

Quality control of experimental data was maintained by excluding samples that displayed greater than 10% rundown in force over a 45-s period during maximum activation. All data are expressed as means ± SD. Cross-bridge cycling parameters were compared between age groups and a single anesthetic using unpaired Student *t* tests. Comparisons across age groups and anesthetics were performed using two-way analysis of variance (ANOVA) with Bonferroni corrections for multiple comparisons. All *P* values were two-sided, and $P < 0.05$ was considered significant.

There were no significant differences between the control groups for halothane *versus* sevoflurane in any of the measured or calculated parameters. Accordingly, the results of the control group were pooled in the creation of figures and tables. However, it must be emphasized that separate statistical analyses were performed for the two anesthetics using only their corresponding controls.

Results

Age-related Differences in Force

In both neonate and adult cardiac muscle bundles, exposure to pCa 4.0 resulted in maximal activation and a stable force response (figs. 1 and 2). In control neonatal cardiac muscle bundles, (*i.e.*, those not exposed to anesthetic) maximum force per cross-sectional area was ~45% lower compared with the adult muscle bundles ($P < 0.05$; n = 12; fig. 3A). However, myofibrillar density, estimated using myosin staining, was 52 ± 13% in neonates and 67 ± 12% in adults (n = 6). Therefore, even when corrected for myofibrillar density (by dividing specific force by the density fraction), maximum force per cross-sectional area was still smaller in neonates by ~30%.

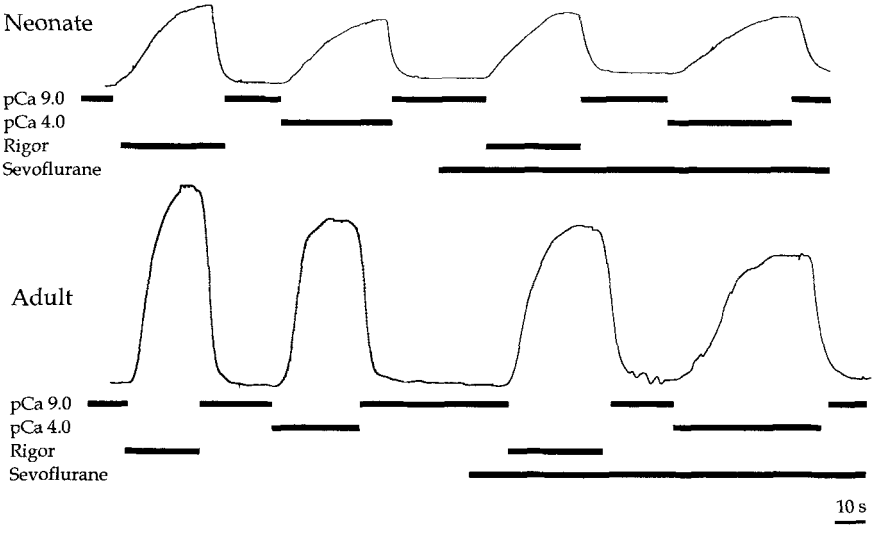

Fig. 2. Effect of sevoflurane on force production during maximum Ca^{2+} activation and rigor in neonate *versus* adult rat cardiac muscle. A representative example for 2 minimum alveolar concentration (MAC) sevoflurane is shown. Note the greater depressant effect on neonatal cardiac muscle.

Effect of Volatile Anesthetics on Isometric Force and Stiffness

Exposure to volatile anesthetics resulted in a decrease in force, with the effect being significantly more pronounced in neonates compared with adults, for both 1 and 2 MAC of either anesthetic (representative tracings in fig. 1 [halothane] and fig. 2 [sevoflurane]). Exposure to either 1 or 2 MAC anesthetic resulted in a decrease in the maximum force per cross-sectional area in both neonates and adults (figs. 3A and 3B for halothane and sevoflurane, respectively). At either anesthetic concentration, the effect on maximum force was significantly greater in neonates than in adults, with the effects of halothane being significantly more pronounced compared with sevoflurane (figs. 3C and 3D for halothane and sevoflurane, respectively; n = 6 for all groups; $P < 0.05$ for all comparisons). Furthermore, at any age, the effect of 2 MAC anesthetic was greater than that of 1 MAC. Overall, both halothane and sevoflurane decreased the maximum force per cross-sectional area to a greater extent in neonates, with halothane having a greater effect compared to sevoflurane.

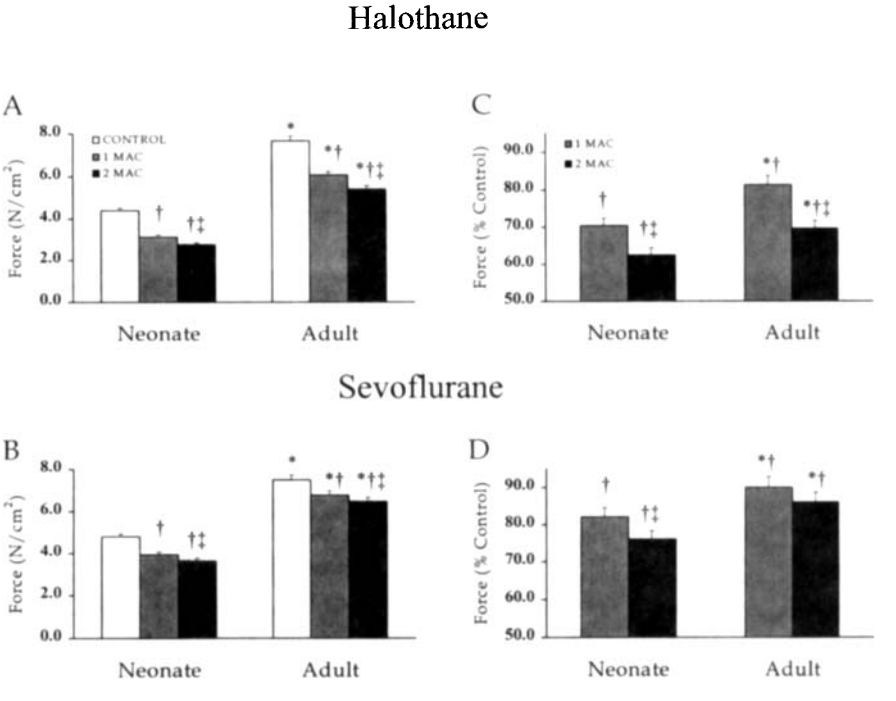

Fig. 3. Effect of halothane and sevoflurane on maximum force normalized for cross-sectional area in neonatal *versus* adult cardiac muscle. Data are expressed in absolute force (*A* and *C*), and relative to control values (*B* and *D*). *Indicates significant difference between ages ($P < 0.05$; n = 12 for controls, and n = 6 for all other groups). †Indicates a significant difference between control and halothane groups. ‡Indicates a significant difference between 1 and 2 minimum alveolar concentration (MAC) anesthetic. Note the considerably smaller effect of sevoflurane.

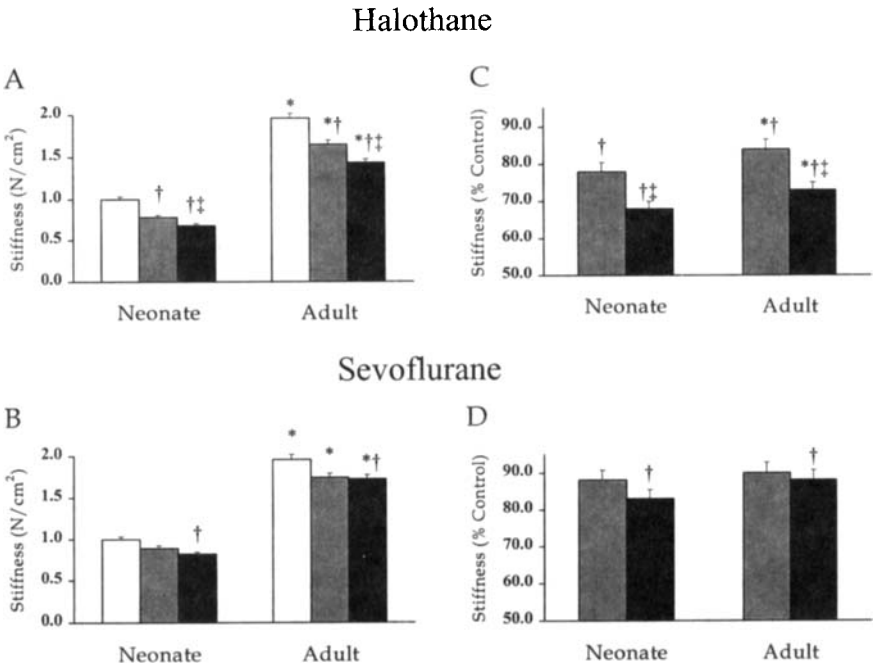

Fig. 4. Effect of halothane and sevoflurane on maximum stiffness in neonatal *versus* adult cardiac muscle. Data are expressed in absolute stiffness (*A* and *C*), and relative to control values (*B* and *D*). *Indicates a significant difference between ages ($P < 0.05$; n = 12 for controls, and n = 6 for all other groups). †Indicates a significant difference between control and halothane groups. ‡Indicates a significant difference between 1 and 2 minimum alveolar concentration (MAC) anesthetic. Note the considerably smaller effect of sevoflurane.

In both neonate and adult cardiac muscle bundles, exposure to pCa 4.0 in the absence of ATP produced a rigor force response (figs. 1 and 2). In strips that were exposed to two pairs of the activating solutions (thus serving as time controls for anesthetic exposure), the rundown in force production at pCa 4.0 was found to be ~6 and ~7% in neonates and adults respectively. Rundown with rigor solution was found to be ~7 and ~8% in neonates and adults, respectively. Exposure to either 1 or 2 MAC halothane during rigor conditions also decreased the force produced. In contrast, 1 MAC sevoflurane had no significant effect on the rigor force, whereas 2 MAC sevoflurane did decrease rigor force, but to a significantly smaller effect, compared with halothane (n = 6 for all groups; $P < 0.05$ for anesthetic comparisons).

Similar to maximum force, maximum stiffness corrected for cross-sectional area (*i.e.*, elastic modulus) was ~50% lower in control neonatal cardiac muscle compared with adult cardiac muscle. Exposure to 1 or 2 MAC halothane decreased the stiffness in muscle strips from both ages (fig. 4A), and the effect was greater in neonates and at 2 MAC (fig. 4B; n = 6 for all groups; $P < 0.05$). In comparison, 1 MAC sevoflurane had no significant effect on stiffness, whereas 2 MAC sevoflurane produced a small but significant effect in both neonates and adults that was considerably smaller than that produced by halothane (figs. 4C and 4D; n = 6 for groups; $P < 0.05$ for anesthetic comparisons).

The ratio of $\overline{F_A}$ to $\overline{F_C}$ (mean force per cross-bridge during anesthetic and control conditions, respectively) was significantly lower than 1.0 (*i.e.*, decreased) in both neonates and adults after exposure to either 1 or 2 MAC halothane ($P < 0.05$; n = 6 for all groups; fig. 5A); this effect was greater at 2 MAC in neonates only. At 1 MAC, the effect on this ratio was significantly greater in adults compared with neonates ($P < 0.05$). However, at 2 MAC, this ratio was decreased to a greater extent in neonates compared with adults. In comparison, both 1 and 2 MAC sevoflurane had no significant effect on the ratio of $\overline{F_A}$ to $\overline{F_C}$ in neonates and only a small effect in adults at 2 MAC (fig. 5B). In time controls, the ratio of $\overline{F_A}$ to $\overline{F_C}$ was found to be 0.92 ± 0.08 in neonates and 0.95 ± 0.10 in adults (n = 6 for all groups).

The ratio of $\overline{S_H}$ to $\overline{S_C}$ (mean stiffness per cross-bridge during anesthetic and control conditions, respectively) was also significantly decreased in both neonates and adults by exposure to halothane ($P < 0.05$; n = 6 for all groups; fig. 5C). This effect was actually more pronounced in adults for 1 MAC halothane and comparable between ages for 2 MAC halothane. In comparison, both 1 and 2 MAC sevoflurane had no significant effect on the ratio of $\overline{S_H}$ to $\overline{S_C}$ in neonates but did have a small but significant effect at 2 MAC sevoflurane in adults, although considerably less than that with 2 MAC halo-

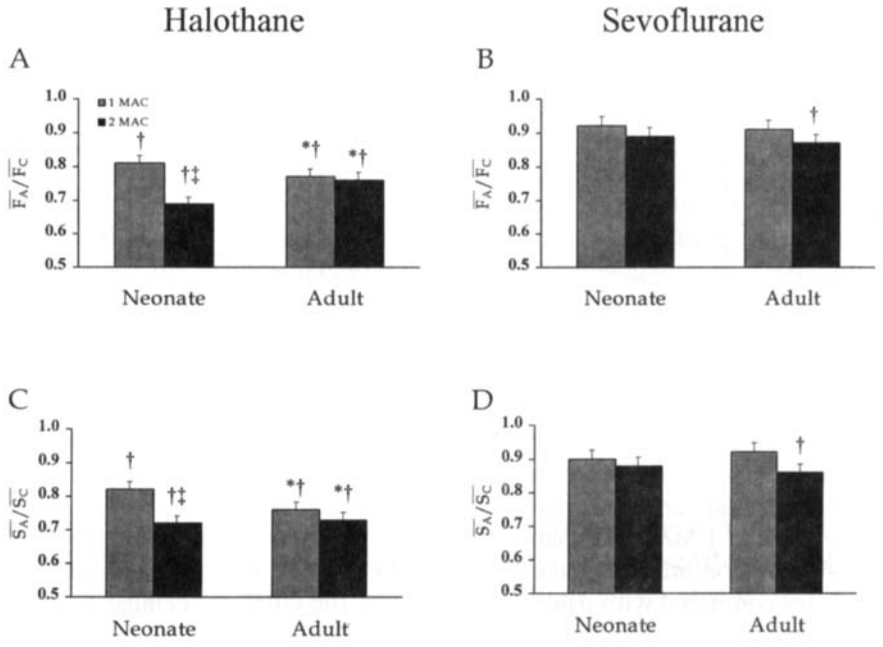

Fig. 5. Changes in mean force per cross-bridge (*A* and *B*) and mean stiffness per cross-bridge (*C* and *D*) in neonatal *versus* adult cardiac muscle with exposure to halothane (*A* and *C*) and sevoflurane (*B* and *D*). It was not possible to directly determine the absolute values for force or stiffness per cross-bridge; therefore, only the ratio of these values for anesthetic (F_A) *versus* control (F_C) are displayed. *Indicates a significant difference between ages ($P < 0.05$; n = 12 for controls, and n = 6 for all other groups). †Indicates a significant difference between control and halothane groups. ‡Indicates a significant difference between 1 and 2 minimum alveolar concentration (MAC) anesthetic.

thane (fig. 5D). In time controls, the ratio of $\overline{S_H}$ to $\overline{S_C}$ was found to be 0.94 ± 0.05 in neonates and 0.94 ± 0.10 in adults.

Effect of Volatile Anesthetics on Cross-bridge Cycling Parameters

In control tissue, the fraction of strongly bound cross-bridges (α_{fs}) was significantly smaller (by ~20%) in neonates compared with adults (table 1). Exposure to 1 MAC halothane had no significant effect on α_{fs} in either neonates or adults, whereas 2 MAC halothane significantly decreased α_{fs} in both age groups ($P < 0.05$) but to a greater extent in neonates. In contrast, only 2 MAC sevoflurane had significant effects on α_{fs} and then only in neonates (table 1). These effects on neonates were comparable to those induced by 2 MAC halothane.

The rate of force redevelopment after rapid relaxation and restretch (k_{tr}) was significantly lower (by ~30%) in neonates compared with adults (fig. 6 and table 1). Exposure to both 1 and 2 MAC halothane significantly decreased k_{tr} ($P < 0.05$) in both groups but to a greater extent in neonates. Exposure to sevoflurane also resulted

Table 1. Effect of Volatile Anesthetics on Cross-bridge Cycling Parameters

			Halothane		Sevoflurane	
Parameter	Age	Control	1 MAC	2 MAC	1 MAC	2 MAC
α_{fs}	Neonate	0.70 ± 0.07	0.69 ± 0.07	0.61 ± 0.07†‡	0.65 ± 0.07	0.61 ± 0.10†
	Adult	0.81 ± 0.08*	0.79 ± 0.08*	0.72 ± 0.10*†‡	0.77 ± 0.10*	0.76 ± 0.11*
k_{tr}	Neonate	6.51 ± 0.42	5.44 ± 0.25†	4.89 ± 0.30†‡	6.05 ± 0.25†	5.30 ± 0.27†‡
	Adult	9.48 ± 0.63*	8.55 ± 0.34*†	8.46 ± 0.27*†	9.01 ± 0.30*†	7.58 ± 0.34*†‡
f_{app}	Neonate	4.56 ± 0.52	3.60 ± 0.32†	2.61 ± 0.28†‡	3.91 ± 0.34†	3.29 ± 0.32†‡
	Adult	7.69 ± 0.43*	6.72 ± 0.34*†	6.36 ± 0.29*†‡	6.95 ± 0.32*†	5.77 ± 0.30*†‡
g_{app}	Neonate	1.90 ± 0.45	1.70 ± 0.27	2.28 ± 0.29†‡	2.15 ± 0.30	2.07 ± 0.31
	Adult	1.83 ± 0.42	1.81 ± 0.30	2.11 ± 0.32*†‡	2.08 ± 0.32	1.82 ± 0.27

Values are means ± SD.

MAC = minimum alveolar concentration; α_{fs} = fraction of attached cross-bridges; k_{tr} = rate of force redevelopment after rapid shortening and restretching; f_{app} = apparent rate constant for cross-bridge attachment; g_{app} = apparent rate constant for cross-bridge detachment.

* Significant difference between ages ($P < 0.05$; n = 12 for controls, n = 6 for all other groups).

† Significant difference between control and halothane groups.

‡ Significant difference between 1 and 2 MAC anesthetic.

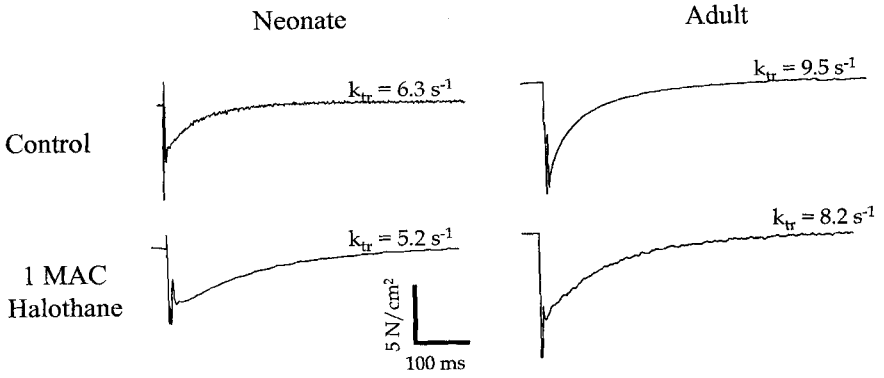

Fig. 6. Representative example of the effect of 1 minimum alveolar concentration (MAC) halothane on the rate of force redevelopment (k_{tr}) after rapid slackening and restretching. Note the slower k_{tr} of neonatal cardiac muscle and the greater effect of halothane on k_{tr} at that age.

in a significant decrease in k_{tr} (table 1). At 1 MAC, the effects of sevoflurane were comparable between neonates and adults and both were considerably less than the effects of 1 MAC halothane ($P < 0.05$). At 2 MAC, sevoflurane continued to have a smaller effect on k_{tr} in neonates compared with halothane. However, the effect in adults was greater compared with halothane.

From the control values of k_{tr} and α_{fs}, the control rates of f_{app} and g_{app} were calculated. The f_{app} was significantly smaller by ~50% in neonates compared with adults (table 1). However, the g_{app} was not significantly different between neonates and adults. Exposure to 1 MAC halothane resulted in a comparable decrease in f_{app} in both neonates and adults, whereas g_{app} was unaffected. However, 2 MAC halothane significantly decreased f_{app} ($P < 0.05$; table 1) and increased g_{app} ($P < 0.05$) to a greater extent in neonates. In comparison with halothane, 1 MAC sevoflurane produced smaller decreases in f_{app} in both neonates and adults; these effects were comparable between the two ages (table 1). Exposure to 2 MAC sevoflurane produced a greater decline in f_{app}, but only in adults. Exposure to 1 or 2 MAC sevoflurane had no significant effect on g_{app} in either neonates or adults.

Discussion

Clinically relevant concentrations of halothane and sevoflurane generally affect force production to a greater extent in neonatal cardiac muscle compared with the adult. The depressant effects of sevoflurane on cardiac muscle were found to be generally smaller than those induced by halothane. For both anesthetics, these effects appear to be mediated, at least in part, by a direct inhibition of cross-bridge cycling, especially the rates of cross-bridge attachment and detachment. Since MAC multiples are generally higher in neonates, extrapolation of the current results using equivalent concentrations to MAC values would only suggest even greater actual myocardial depression in this age group. The results further suggest anesthetic differences in both potency and intracellular targets.

Studies were conducted at a sarcomere length of ~2.2 μm, corresponding to ~25% above slack length. This length approximated the plateau of the force–length relation in both neonates and adults[24] and reflected a more physiologic condition. The Ca^{2+} sensitivity of myofibrillar proteins is also known to be dependent on sarcomere length.[25,26] Accordingly, we attempted to both standardize and optimize the conditions for age and anesthetic comparisons.

The lower force production by the neonatal cardiac muscle is attributable, at least in part, to the lower concentration of myofilaments, as reported by Reiser *et al.*[27] and in the current study. Another contributing factor may be age-related differences in the geometry of myocytes, which would influence the actual number of cross-bridges. However, the contribution of this factor is likely to have been systematic and constant in both control and volatile anesthetic conditions within the same muscle sample.

Neonatal and adult rodent hearts predominantly express the V_1 *versus* the V_3 myosin heavy chain isoforms, respectively,[28] which differ in their capacities for force generation, shortening velocity, and actomyosin ATPase activity,[29–31] with the V_3 isoform being slower but more energy efficient. Furthermore, there are age-related differences in isoform expression of other thick and thin filament proteins.[28] Accordingly, the relative expression of different myosin heavy chain isoforms may underlie, at least in part, the age-related differences in cross-bridge cycling kinetics and force generation. These issues are of

significance not only in comparing volatile anesthetic effects of different age groups but also in evaluating anesthetic effects on adult hearts in pathologic conditions in which neonatal protein isoforms are reexpressed.

According to the model employed in the current study, the lower force in neonates is attributable to any of the following conditions: (1) a smaller total maximum number of cross-bridges (n), (2) a lower mean force per cross-bridge ($\bar{F}$), and (3) a smaller fraction of strongly bound cross-bridges (α_{fs}). The total number of cross-bridges is reflected by the myofibrillar density and is known to be lower in neonates than in adults. Using the current techniques, it was not possible to estimate $\bar{F}$. Therefore, we focused on estimation of α_{fs} using sinusoidal analysis, a procedure previously employed in skeletal muscle.[21,22] Using this index, we found that the α_{fs} in neonates is significantly smaller than that in adults, suggesting an additional mechanism for lower force production. The α_{fs} reflects cross-bridge attachment and detachment, represented by the corresponding rates f_{app} and g_{app}. We found f_{app} to be significantly slower in the neonate; this was also reflected by the slower rate of force redevelopment (k_{tr}). These data are consistent with the slower shortening velocity of neonatal cardiac muscle reported by other groups.[29,30] However, g_{app} was not significantly different between ages. According to the model of Brenner *et al.*,[23] differences in both ATPase activity and shortening velocity should parallel differences in g_{app}. The lack of age-related difference in g_{app} observed in our study appears to be in conflict with the higher ATPase activities[31] and shortening velocities[29,30] of adult cardiac muscle compared with neonate cardiac muscle. This discrepancy may be more due to the limitation of the two-state cross-bridge model, in that the model does not consider the possibility that cross-bridge detachment may actually involve two rate constants, only one of which is estimated by sinusoidal analysis, but does not necessarily reflect ATPase activity.[32] Regardless, the lower ATPase activity in the neonate suggests a lower actual rate of cross-bridge detachment and thus a greater proportion of time spent in the attached state, which would lead to a greater efficiency of ATP utilization. Overall, these data suggest that the lower force generated by the neonatal muscle is more a reflection of the slower f_{app} and lower α_{fs} than the result of cross-bridge detachment.

Halothane decreased maximum force (normalized for cross-sectional area) to a greater extent in neonates, which is generally consistent with previous reports in several species.[5–8] The effect of halothane is potentially attributable to the three factors described earlier for age-related differences. However, given the time frame of anesthetic action, it is unlikely that the total maximum number of cross-bridges (n), reflecting protein content, would be affected. Because muscles were preexposed to anesthetic, the possibility exists that the total number of cross-bridges were already decreased. However, the stiffness measurements were comparable in pCa 9.0 during control and anesthetic conditions (data not shown), suggesting that n was unaffected. In contrast, halothane does appear to decrease the fraction of strongly bound cross-bridges (α_{fs}), and thus the number of cross-bridges in the force-generating state ($n \cdot \alpha_{fs}$). These findings are a direct demonstration of anesthetic effects at the level of the cross-bridge and support the suggestions made by Murat *et al.*[13] in a previous study in skinned rat myocardium.

The considerably smaller effect of sevoflurane on force production compared with halothane is consistent with clinical and research literature on this issue.[33,34] However, compared with the clinical situation, the depressant effect of sevoflurane *in vitro* appears to be more pronounced in both neonates and adults. This most likely reflects the lack of compensatory neural and humoral mechanisms *in vitro*. As with halothane, the effects of sevoflurane may be due to an effect on $\bar{F}$ or α_{fs}. However, we found no effects of sevoflurane on α_{fs} except at high concentrations, and then only in the neonate. This dissimilarity emphasizes the point that the two volatile anesthetics may differ in their intracellular targets.

Halothane decreased the ratio of $\overline{F_A}$ to $\overline{F_C}$ (mean force per cross-bridge during anesthetic and control conditions, respectively) to a greater extent in the neonate compared with the adult. An inherent assumption in this technique is that the total number of cross-bridges (n) remains unchanged during rigor over time or in the presence of anesthetics (*i.e.*, there is no dropout of cross-bridges). In time controls, we found this dropout to be less than 5% as reflected by changes in the respective ratios, suggesting that this assumption is not strictly true. Nonetheless, this dropout was considerably less than the decrease in ratio observed with halothane, suggesting that the technique provides at least a qualitative measure of the effects of volatile anesthetic on $\bar{F}$, supporting the suggestion by Murat *et al.* of a decrease in $\bar{F}$.[13] In a subset of muscle samples, we added anesthetic after the muscle had been placed in rigor (data not shown). We found that halothane decreased force production even during rigor conditions. These data are also

consistent with a halothane-induced decrease in $\bar{F}$. In this regard, it is of interest that sevoflurane had a considerably smaller, and sometimes insignificant, effect on $\bar{F}$.

Previous studies in rabbit papillary muscle have shown that the plateau of dynamic stiffness *versus* frequency of length perturbations is decreased by volatile anesthetics,[13] indicating a reduction in the number of cross-bridges ($n \cdot \alpha_{fs}$). This effect has been largely attributed to a decreased affinity of Troponin C. However, it appears that volatile anesthetics may not affect the Ca^{2+} affinity of Troponin C (TnC).[35] Several other studies have used Ca^{2+} concentrations sufficiently high to saturate TnC binding sites to show that decreased force due to volatile anesthetics is attributable to effects at the level of the cross-bridge itself.[9,11,12] For example, in mechanically skinned rabbit[11] and ferret papillary muscles[10] (in which the sarcoplasmic reticulum is intact but the Ca^{2+} is chelated), volatile anesthetics have been shown to decrease the maximal Ca^{2+}-activated force, albeit to different extents. Furthermore, in Triton-X-skinned rat cardiac fibers (in which both sarcolemma and SR are destroyed), Murat *et al.*[13] found that both maximal tension and Ca^{2+} were decreased by volatile anesthetics. A recent study in Brij58-skinned human cardiac fibers also found that halothane and isoflurane decrease the maximum force in dose-dependent fashion.[12]

Halothane, and sevoflurane to a lesser extent, decreased the total stiffness in neonates to a greater extent than adults. Stiffness during maximum Ca^{2+} activation is mainly a representation of the series elastic components of the muscle and is thought to chiefly reside in the cross-bridges themselves. In this regard, a lower total stiffness in neonates likely reflects a smaller number of attached cross-bridges, a combined effect of the smaller α_{fs} and protein content. In the case of anesthetic-induced changes in stiffness, the decreased stiffness at any age likely reflects the decreased α_{fs}.

Halothane significantly decreased α_{fs} and the rate for force redevelopment (k_{tr}). Based on the analytical models described earlier, these effects corresponded to a predominant depressant effect of halothane on the rate of cross-bridge attachment, f_{app}, and an acceleration of the rate of cross-bridge detachment, g_{app}. These effects, especially f_{app}, appear to be more pronounced in the neonate, especially at higher halothane concentrations. Sevoflurane also decreased k_{tr}, albeit to a somewhat smaller extent compared with halothane, especially in neonates. A slower f_{app} and a concomitant faster g_{app} would lead to the cross-bridge being in the force-generating state for a smaller period of time, and thus a smaller number of attached cross-bridges at any given time. Accordingly, for force to be maintained, the rate of cross-bridge cycling would need to increase to compensate; this would be reflected by an increase in ATPase activity. In this regard, it is also of significance that the V_3 myosin isoform of the neonatal myocardium, where anesthetic effects on the cross-bridge appear to be greater, has a lower ATPase activity in the first place and, therefore, the proportionate compensatory increase in ATPase activity would have to be even greater. A number of studies have examined actomyosin ATPase in the presence of volatile anesthetics, albeit at relatively high concentrations, and have found either no significant changes[36] or an actual decrease[37,38] in ATPase activity. Nonetheless, it is unlikely that clinically relevant concentrations of volatile anesthetics will actually allow for an increase in ATPase activity. Therefore, the effects of halothane or sevoflurane at the level of the cross-bridge are likely to significantly contribute to the observed lower force.

In addition to the potential targets outlined earlier, age-related differences in myocardial sensitivity to volatile anesthetics may also be mediated *via* effects on other regulatory proteins involved in force production. For example, the phosphorylation level of Troponin I may differ between neonates and adults[27] and may be differentially affected by halothane. Furthermore, differences in Troponin T isoform expression[28] may also contribute to age-related differences in force production. Differential effects of halothane *versus* sevoflurane may also underlie the observed differences in the extent of cardiac depression. These potential mechanisms remain to be explored.

References

1. Rusy BF, Komai H: Anesthetic depression of myocardial contractility: A review of possible mechanisms. ANESTHESIOLOGY 1987; 67: 745–66

2. Graf BM, Vicenzi MN, Bosnjak ZJ, Stowe DF: The comparative effects of equimolar sevoflurane and isoflurane in isolated hearts. Anesth Analg 1995; 81:1026–32

3. Park WK, Pancrazio JJ, Suh CK, Lynch C III: Myocardial depressant effects of sevoflurane. Mechanical and electrophysiologic actions in vitro. ANESTHESIOLOGY 1996; 84:1166–76

4. Skeehan TM, Schuler HG, Riley JL: Comparison of the alteration of cardiac function by sevoflurane, isoflurane, and halothane in the isolated working rat heart. J Cardiothorac Vasc Anesth 1995; 9:706–12

5. Palmisano BW, Mehner RW, Stowe DF, Bosnjak ZJ, Kampine JP: Direct myocardial effects of halothane and isoflurane. Comparison between adult and infant rabbits. ANESTHESIOLOGY 1994; 81:718–29

6. Baum VC, Klitzner TS: Excitation-contraction coupling in neona-

tal myocardium: Effects of halothane and isoflurane. Dev Pharmacol Ther 1991; 16:99-107

7. Krane EJ, Su JY: Comparison of the effects of halothane on newborn and adult rabbit myocardium. Anesth Analg 1987; 66:1240-4

8. Krane EJ, Su JY: Comparison of the effects of halothane on skinned myocardial fibers from newborn and adult rabbit. I. Effects on contractile proteins. ANESTHESIOLOGY 1989; 70:76-81

9. Murat I, Ventura-Clapier R, Vassort G: Halothane, enflurane, and isoflurane decrease calcium sensitivity and maximal force in detergent-treated rat cardiac fibers. ANESTHESIOLOGY 1988; 69:892-9

10. Housmans PR, Murat I: Comparative effects of halothane, enflurane, and isoflurane at equipotent anesthetic concentrations on isolated ventricular myocardium of the ferret. I. Contractility. ANESTHESIOLOGY 1988; 69:451-63

11. Su JY, Kerrick WG: Effects of halothane on Ca^{2+}-activated tension development in mechanically disrupted rabbit myocardial fibers. Pflugers Arch 1978; 375:111-7

12. Tavernier BM, Adnet PJ, Imbenotte M, Etchrivi TS, Reyford H, Haudecoeur G, Scherpereel P, Krivosic-Horber RM: Halothane and isoflurane decrease calcium sensitivity and maximal force in human skinned cardiac fibers. ANESTHESIOLOGY 1994; 80:625-33

13. Murat I, Lechene P, Ventura-Clapier R: Effects of volatile anesthetics on mechanical properties of rat cardiac skinned fibers. ANESTHESIOLOGY 1990; 73:73-81

14. Fabiato A, Fabiato F: Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. J Physiol 1975; 249:469-95

15. Godt RE, Lindley BD: Influence of temperature upon contractile activation and isometric force production in mechanically skinned muscle fibers of the frog. J Gen Physiol 1982; 80:279-97

16. Brenner B: Technique for stabilizing the striation pattern in maximally calcium-activated skinned rabbit psoas fibers. Biophys J 1983; 41:99-102

17. Sweeney HL, Corteselli SA, Kushmerick MJ: Measurements on permeabilized skeletal muscle fibers during continuous activation. Am J Physiol 1987; 252:C575-80

18. Van Dyke RA, Wood CL: Binding of radioactivity from 14 C-labeled halothane in isolated perfused rat livers. ANESTHESIOLOGY 1973; 38:328-32

19. Huxley AF: Muscle structure and theories of contraction. Prog Biophysics Biophys Chem 1957; 7:255-318

20. Huxley AF, Simmons RM: Proposed mechanism of force generation in striated muscle. Nature 1971; 233:533-8

21. Kawai M, Brandt PW: Sinusoidal analysis: A high resolution method for correlating biochemical reactions with physiological processes in activated skeletal muscles of rabbit, frog, and crayfish. J Muscle Res Cell Motil 1980; 1:279-303

22. Ford LE, Huxley AF, Simmons RM: The relation between stiffness and filament overlap in stimulated frog muscle fibres. J Physiol 1981; 311:219-49

23. Brenner B: Kinetics of the crossbridge cycle derived from measurements of force, rate of force development and isometric ATPase. J Muscle Res Cell Motil 1986; 7:75-6

24. Friedman WF: The intrinsic physiologic properties of the developing heart. Prog Cardiovasc Dis 1972; 15:87-111

25. Kentish JC, Ter Keurs HEDJ, Ricciardi L, Bucx JJJ, Noble MIM: Comparison between the sarcomere length-force relations of intact and skinned trabeculae from rat right ventricle: Influence of calcium on these relations. Circ Res 1986; 58:755-68

26. Hibberd MG, Jewell BR: Calcium- and length-dependent force production in rat ventricular muscle. J Physiol (Lond) 1982; 329: 527-40

27. Reiser PJ, Westfall MV, Schiaffino S, Solaro RJ: Tension production and thin-filament protein isoforms in developing rat myocardium. Am J Physiol 1994; 267:H1589-96

28. Murphy AM: Contractile protein phenotypic variation during development. Cardiovasc Res 1996; 31:E25-33

29. Schwartz K, Lecarpentier Y, Martin JL, Lompre AM, Mercadier JJ, Swynghedauw B: Myosin isoenzymic distribution correlates with speed of myocardial contraction. J Mol Cell Cardiol 1981; 13:1071-5

30. Cappelli V, Bottinelli R, Poggesi C, Moggio R, Reggiani C: Shortening velocity and myosin and myofibrillar ATPase activity related to myosin isoenzyme composition during postnatal development in rat myocardium. Circ Res 1989; 65:446-57

31. Rossmanith GH, Hamilton AM, Hoh JF: Influence of myosin isoforms on tension cost and crossbridge kinetics in skinned rat cardiac muscle. Clin Exp Pharmacol Physiol 1995; 22:423-9

32. Julian FJ, Sollins KR, Sollins MR: A model for the transient and steady-state mechanical behavior of contracting muscle. Biophys J 1974; 14:546-62

33. Young CJ, Apfelbaum JL: Inhalational anesthetics: Desflurane and sevoflurane. J Clin Anesth 1995; 7:564-77

34. Ebert TJ, Harkin CP, Muzi M: Cardiovascular responses to sevoflurane: A review. Anesth Analg 1995; 81(suppl 6):S11-22

35. Blanck TJ, Chiancone E, Salviati G, Heitmiller ES, Verzili D, Luciani G, Colotti G: Halothane does not alter Ca^{2+} affinity of troponin C. ANESTHESIOLOGY 1992; 76:100-5

36. Merin RG, Kumazawa T, Honig CR: Reversible interaction between halothane and Ca^{++} on cardiac actomyosin adenosine triphosphatase: Mechanism and significance. J Pharmacol Exp Ther 1974; 190:1-14

37. Brodkin WE, Goldberg AH, Kayne HL: Depression of myofibrillar ATPase activity by halothane. Acta Anaesthesiol Scand 1967; 11:97-101

38. Merin RG, Kumazawa T, Honig CR: Halothane decreases actomyosin ATPase activity: a possible mechanism of the negative inotropic effect. Recent Adv Stud Cardiac Struct Metab 1975; 5:405-12