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Comparison of the Effects of Halothane on Skinned Myocardial Fibers from Newborn and Adult Rabbit: II. Effects on Sarcoplasmic Reticulum

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The effect of halothane on Ca^{2+} uptake or release by the sarcoplasmic reticulum (SR) was compared in the newborn and adult rabbit myocardium. The sarcolemma of right ventricular myocardium was disrupted (skinned) by homogenization. Fiber bundles were dissected from the homogenate, mounted on tension transducers, and immersed sequentially in five solutions that loaded Ca^{2+} into the SR, then in solutions containing either 2 or 25 mM caffeine to release SR-stored Ca^{2+}, resulting in transient tension development. Experimental solutions were saturated with halothane in N_2 gas during Ca^{2+} uptake by SR, Ca^{2+} release by SR, or during both SR Ca^{2+} uptake and release. Halothane (0.5–1.7%) resulted in dose-dependent depression of SR Ca^{2+} uptake in both newborn and adult skinned fibers. Less tension transient depression resulted in newborn (35%) than adult skinned fibers (49.5%, $P < 0.05$) with 0.5% halothane exposure during SR Ca^{2+} uptake. Similar depression resulted in newborn (53.7% and 73.4%) and adult fibers (65.2% and 77.9%) with 1.0% and 1.7% halothane. Halothane had little effect on SR Ca^{2+} release by 25 mM caffeine but enhanced submaximal SR Ca^{2+} release by 2 mM caffeine more in newborn than adult myocardium. Increased Ca^{2+} efflux from newborn SR may contribute to the greater sensitivity of intact newborn cardiac muscle to exposure to halothane. (Key words: Anesthesia, pediatric. Anesthetics, volatile: halothane. Heart: sarcoplasmic reticulum; skinned fibers. Ions, calcium.)

HALOTHANE is a potent negative inotropic agent, with direct action upon the heart.[1] Recently, clinical impressions that the negative inotropic action of halothane is greater in the newborn than the adult have been confirmed in isolated cardiac tissue preparations from rats by Rao *et al.*,[2] rabbits in our laboratory,[3] and cats by Wolf *et al.*[4]

Greater sensitivity of the newborn heart to halothane may be explained by an increased effect of halothane at any site or sites of the contractile process. Cardiac muscle contraction is triggered by an action potential, which leads to an increase in free myoplasmic calcium (Ca^{2+}) concentration. In mammals the increase in myoplasmic Ca^{2+} comes partially from outside the cell *via* sarcolemmic calcium channels but predominantly in the adult from release of Ca^{2+} from intracellular storage sites, particularly the sarcoplasmic reticulum (SR). Myoplasmic "activator" Ca^{2+} then activates contraction by binding to the regulatory proteins (troponin) associated with the contractile proteins actomyosin and myofibrillar ATPase. Halothane has an effect at each of these stages[5]: sarcolemmic Ca^{2+} currents,[6] SR Ca^{2+} uptake and release,[7] and Ca^{2+} activation of the contractile proteins.[8–10] An important effect of halothane on contractility of adult myocardium is probably by a reduction of the myoplasmic free Ca^{2+} concentration,[11] which may be secondary to depression of sarcolemmic Ca^{2+} currents or SR Ca^{2+} storage and release.

In attempting to identify a locus of increased sensitivity in the newborn heart, McAuliffe and Hickey[12] demonstrated that the newborn myocardial synthesis of high-energy phosphates was not depressed by halothane more than that in the adult, and our laboratory has shown that Ca^{2+} activation of the contractile proteins in skinned myocardial fibers is slightly less sensitive to depression by halothane in the newborn than in the adult rabbit.[13]

Here we report the study of halothane's effects on another intracellular site of the contractile process, the SR, which in adults is probably an important locus for halothane-induced depression.[5] We hypothesized that the lesser quantity and immature function of SR in newborn cardiac tissue would lead to greater depression of Ca^{2+} uptake and release by the SR of the newborn heart. To compare the sensitivity of cardiac SR to halothane in newborn and adult myocardium, we used a "skinned" fiber preparation,[7] which permits pharmacologic manipulation of the myoplasmic $[Ca^{2+}]$ while simultaneously measuring the physiologic response, *i.e.*, tension development.

Materials and Methods

Skinned Fiber Preparation

The experimental protocol was approved by the University of Washington Animal Experimentation Committee. The preparation of functionally skinned myocardial fiber preparations has been previously described in detail.[7,8] Newborn New Zealand white rabbits of either sex (age 1–4 days) were killed by decapitation. Young adult male New Zealand white rabbits (age 2–3 months, weight

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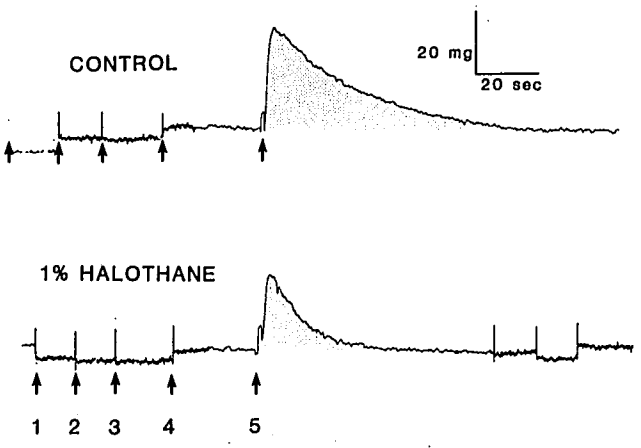

FIG. 1. A typical tracing from newborn right ventricular skinned fibers, illustrating the experimental protocol. Baseline marks on the tension tracing are artifacts created during solution changes. Solution 1 (see text and table 1 for solution composition) completes emptying of Ca^{2+} from the SR. Solution 2 washes caffeine from the fibers. Solution 3 contains Ca^{2+} ($pCa = 6.5$), which initiates SR Ca^{2+} accumulation. Solution 4 reduces [EGTA] in the fibers. The time scale changes from 6 mm/min to 1 mm/s between solutions 4 and 5; the inset scale pertains to solution 5 only. Solution 5 contains caffeine (25 mM), which releases Ca^{2+} from the SR and produces transient tension generation. The area under the tension transient (shaded) is a relative measure of the amount of Ca^{2+} released from the SR.[16] The second tension transient follows exposure of the skinned fiber to 1% halothane during the SR Ca^{2+} uptake phase (solutions 2–4). The area under the tension transient is reduced.

1.5–2.5 kg) were killed by cervical dislocation. The hearts were rapidly isolated and cooled on ice. The right ventricle walls were dissected from the remainder of the heart and cut into longitudinal strips. These strips were homogenized with a Teflon pestle at the lightest pressure in 5 ml relaxing solution (containing, in mM: $Mg^{2+} = 0.1$, $K^+ = 35$, $Na^+ = 35$, $MgATP^{2-} = 2$, creatine $phosphate^{2-} = 15$, EGTA = 7, imidazole = 70–80, and methanesulfonate = 25–40 (major anion); free $[Ca^{2+}] < 10^{-9.0}$ M). The homogenate was placed in a glass-bottomed tray and diluted with approximately 5 ml of cold relaxing solution. Bundles of myocardial fibers approximately 100 μm in width, 20–30 μm thick, and 1–2 mm in length were isolated with the aid of a stereoscopic operating microscope and clamped with stainless steel clips. One end of the fiber bundle was attached to a photodiode tension transducer similar to that of Hellam and Podolsky,[14] and the other end to a micromanipulator. The distance between the two clips was increased until slack in the fiber bundle was eliminated. Transducer signals were amplified and recorded on a Gould 2400S four-channel recorder.

BATHING SOLUTIONS

Five bathing solutions were used, which contained (mM): $Mg^{2+} = 0.1$, $K^+ = 35$, $Na^+ = 35$, $MgATP^{2-} = 2$, creatine $phosphate^{2-} = 15$, total EGTA = 0.05 or 7, caffeine = 0, 2, or 25, imidazole = 70–80, and methanesulfonic acid = 25–40 (major anion). Ca^{2+} concentration was either less than 10^{-9} M ($pCa > 9$), $10^{-6.8}$ M ($pCa = 6.8$), or $10^{-6.5}$ M ($pCa = 6.5$). Ionic strength was kept constant at 0.15 and pH at 7.00 ± 0.02 by varying the amount of imidazole methanesulfonate at 23 ± 2° C. Total $[Ca^{2+}]$ in all solutions was measured by atomic absorption spectrophotometry (Hitachi 180-70), and free $[Ca^{2+}]$ was calculated by computer program using appropriate EGTA binding constants.[15]

Solution 1 contained high concentrations of caffeine (25 mM) and EGTA (7 mM), and no Ca^{2+} ($pCa > 9$), which emptied the skinned fiber SR of Ca^{2+} and buffered intracellular Ca^{2+}, resulting in relaxation.

Solutions 2–4 are referred to as the uptake solutions, during exposure to which the skinned fiber SR was loaded with Ca^{2+}. Solution 2 contained a high concentration of EGTA (7 mM) and $pCa > 9$, which removed caffeine from the fibers. Solution 3 also contained a high concentration of EGTA, and $pCa = 6.5$, insufficient to produce contraction but sufficient to allow the skinned fiber SR to rapidly accumulate Ca^{2+}. Solution 4 contained a low concentration of EGTA (0.05 mM) and $pCa = 6.8$, which reduced [EGTA] in the fibers. The fibers were immersed in solutions 2 and 4 for 2 min each to ensure that equilibrium to the new solution was reached. Immersion in solution 3 was for 3 min for near-maximal loading of Ca^{2+} into the SR.

Solution 5 was the releasing solution, and contained caffeine (2 or 25 mM), 0.05 mM EGTA, and $pCa = 6.5$ (for 2 mM caffeine) or 6.8 (for 25 mM caffeine). Exposure to this solution released Ca^{2+} from the SR and resulted in transient tension development (fig. 1). The area under the tension transient is proportional to the quantity of Ca^{2+} released from the SR.[16] Two caffeine concentrations were used for releasing Ca^{2+}: 2 mM caffeine resulted in partial, or submaximal, release of Ca^{2+} from the SR, and 25 mM caffeine, which resulted in maximal SR Ca^{2+} release.[17]

Concentrations of halothane (Ayerst Laboratories) were controlled by directing reagent grade N_2 through a VerniTrol® vaporizer that had been previously calibrated by gas chromatography.[18] This halothane–N_2 mixture was then passed through a washing bottle containing distilled water for removal of particulate contaminants, and the gas was subsequently bubbled through the test bathing solutions. Earlier work from this laboratory demonstrated that halothane concentrations reached equilibrium in solution after 45 min of bubbling.[8]

Control bathing solutions were bubbled with 100% reagent grade N_2 alone, also after passing the gas through a separate washing bottle.

Gas flow through these solutions was stopped during immersion of the fiber bundle in the solution to avoid

TABLE 1. Composition and Function of Bathing Solutions

Solution	Composition	Duration of Immersion (min)	Function
1	EGTA = 7 mM Caffeine = 25 mM $[Ca^{2+}] = 10^{-9}$ M	2	Empty SR of Ca^{2+} in skinned fibers
2	EGTA = 7 mM $[Ca^{2+}] = 10^{-9}$ M	2	Remove caffeine from skinned fibers
3	EGTA = 7 mM $[Ca^{2+}] = 10^{-6.5}$ M	3	Load Ca^{2+} into SR
4	EGTA = 0.05 mM $[Ca^{2+}] = 10^{-6.5}$ M	2	Reduce EGTA in skinned fibers
5	EGTA = 0.05 mM $[Ca^{2+}] = 10^{-6.5}$ M Caffeine = 25 mM	q.s.	Release Ca^{2+} from SR, generate tension

Immersion in these solutions constitutes one experimental cycle and results in a tension transient. Three cycles constitute one experiment: cycles 1 and 3 are control cycles (no halothane), and cycle 2 contains halothane in solutions 2–4 (uptake), 5 (release), or 2–5 (uptake and release).

artifact. Previous work from this laboratory demonstrated that halothane loss to atmosphere from test solutions during tension measurements was approximately 0.02% per min,[8] which is insignificant compared with the range of halothane concentrations tested and the duration of tests.

EXPERIMENTAL PROTOCOL

Adult and newborn fibers were studied simultaneously in pairs to assure identical experimental conditions (table 1). Each experiment consisted of three cycles of solution changes, sequentially immersing the skinned fibers in solutions 1–5. Three experiments were made for each halothane concentration (0.5%, 1.0%, and 1.7%) to measure the effect of halothane on SR Ca^{2+} uptake, SR Ca^{2+} release, and on both SR Ca^{2+} uptake and release. Each halothane cycle was bracketed by two control cycles in which solutions 1–5 contained no halothane. In the first experimental group of three cycles, the fiber bundles were exposed to halothane during the SR Ca^{2+} loading phase only, *i.e.*, solutions 2–4 were equilibrated with halothane, and solution 5 was equilibrated with 100% N_2. In the second group of three cycles, the fiber bundle was exposed to halothane during SR Ca^{2+} release (only solution 5). In the third the fiber bundle was exposed to halothane during Ca^{2+} uptake and release (solutions 2–5).

The results of the effect of halothane on the area under the tension transient curves are expressed as the percent of the mean of the two bracketing control cycles. Each skinned fiber preparation was used for determining the effect of two halothane concentrations. Each data point represents the mean of 6–10 skinned fibers from at least three experimental animals of each age.

To measure the effect of halothane on submaximal SR Ca^{2+} release, skinned fibers in newborn–adult pairs were exposed to halothane (0.5–1.7%) during SR Ca^{2+} release only (solution 5), using 2 mM caffeine in the releasing solution; 2 mM caffeine results in partial emptying of the SR and permits the effects of halothane on Ca^{2+} release to be fully seen. Each halothane cycle was bracketed by two control cycles, the mean of which was used to calculate the effect of halothane on submaximal Ca^{2+} release.

Data were converted to a normal distribution using the arc–sine transformation prior to statistical analysis.[19] Paired and unpaired Student's t test were used to determine statistical significance. Analysis of variance and multiple corrected t tests were used to determine the differences between halothane doses. $P < 0.05$ was regarded as statistically significant.

Results

Exposure of both newborn and adult right ventricular skinned fibers to halothane (0.5–1.7%) during SR Ca^{2+} uptake resulted in reversible dose-dependent depression of the area under the caffeine-induced tension transients (fig. 2). Caffeine-induced tension transients of newborn skinned myocardial fibers exhibited 35.0% depression with a halothane concentrations of 0.5%. Exposure of newborn skinned fibers to 1.0% and 1.7% halothane resulted in further statistically significant depression of caffeine-induced tension transients of 53.7% and 73.4%, respectively.

Caffeine-induced tension transients of adult skinned fibers exposed to 0.5% halothane during SR Ca^{2+} uptake were depressed by 49.5%. The degree of depression was significantly greater in the adult skinned fibers than in the newborn skinned fibers exposed to 0.5% halothane

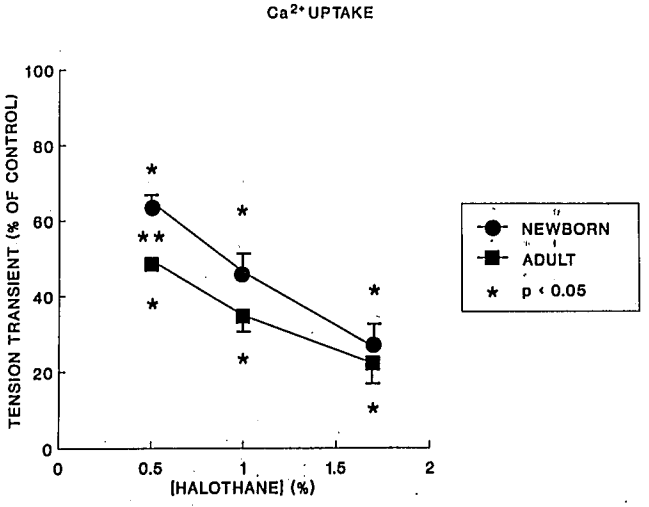

FIG. 2. Dose–response relationship of halothane exposure during SR Ca^{2+} uptake (solutions 2–4) on SR Ca^{2+} release by 25 mM caffeine (mean ± SEM). Squares = adult skinned fibers; circles = newborn skinned fibers. *$P < 0.05$ *versus* control. **$P < 0.05$, newborn *versus* adult fibers.

($P < 0.01$). Exposure to 1.0% and 1.7% halothane resulted in further statistically significant depression of 65.2% and 77.9%, respectively, which was not significantly different from the degree of depression of tension transients seen in newborn skinned fibers at these halothane concentrations.

Halothane exposure of newborn and adult cardiac skinned fibers during maximal release of Ca^{2+} by the SR (using 25 mM caffeine) was not affected by halothane between 0.5% and 1.7% (fig. 3). There was a tendency for halothane to result in slightly greater Ca^{2+} release at each

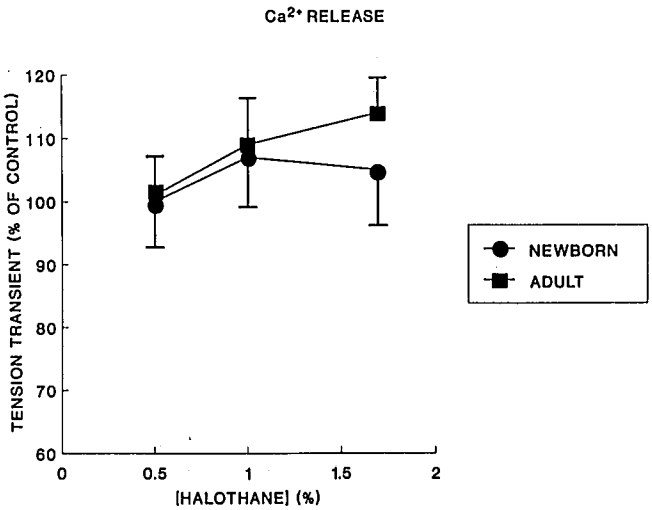

FIG. 3. Dose–response relationship of halothane exposure during SR Ca^{2+} release (solution 5) on maximal SR Ca^{2+} release by 25 mM caffeine (mean ± SEM). Squares = adult skinned fibers; circles = newborn skinned fibers.

concentration. No statistical difference was found between the response of newborn and adult skinned fibers to halothane during maximal SR Ca^{2+} release.

The results of experiments in which the skinned fibers were exposed to halothane during both SR Ca^{2+} uptake and release were qualitatively similar to exposure to halothane during SR Ca^{2+} uptake alone (fig. 4). There was a tendency for caffeine-induced tension transients of adult skinned fibers to show greater depression than those of newborn skinned fibers. This difference achieved statistical significance with 0.5% halothane ($P < 0.01$). Newborn skinned fiber tension transients were depressed by 24.9%, 59.4%, and 65.8% by 0.5%, 1.0%, and 1.7% halothane, respectively. Adult skinned fiber tension transients were depressed by 53.9%, 67.4%, and 71.0%, respectively. Analysis of variance and *t* tests showed no difference between halothane exposure during Ca^{2+} uptake alone *versus* during both uptake and release.

In skinned fibers from both newborn and adult right ventricular myocardium, halothane increased the size of tension transients following submaximal release of SR Ca^{2+} stores by 2 mM caffeine (fig. 5), and eliminated the cyclic contractures seen when 2 mM caffeine is used to induce SR Ca^{2+} release, an effect of halothane that was previously observed by Su and Kerrick.[7] Halothane augmentation of caffeine-induced tension transients was greater in the newborn than the adult at halothane concentrations of 1.0% and 1.7% ($P < 0.02$). No dose–response for halothane augmentation of Ca^{2+} release was evident in either newborn or adult skinned fibers.

Discussion

Halothane (0.5–1.7%) exposure during SR Ca^{2+} accumulation produced dose-dependent depression of caffeine-induced tension transients of right ventricular skinned fibers of newborn and adult rabbits. At the lowest halothane concentration, newborn myocardial skinned fibers were less depressed than adult skinned fibers, whereas the difference between halothane sensitivity of newborn and adult skinned fibers was not statistically significant at higher halothane concentrations.

The magnitude of depression of caffeine-induced tension transients by these clinical concentrations of halothane was of the same order of magnitude as the depression of isolated intact myocardial preparations. For example, exposure to 1% halothane depressed tension transients of newborn rabbit myocardial skinned fibers by 54% in the present study and depressed peak developed tension and maximum rate of rise of tension by approximately 70% in isolated right ventricle of newborn rabbits.[3] Similarly, adult cardiac skinned fibers exhibited 65% depression of tension transients upon exposure to 1% halothane, and adult rabbit right ventricle peak developed

tension and contractility were depressed by approximately 60% by 1% halothane.[3] Similar degrees of depression have been observed in isolated newborn and adult myocardium of cat[4] and rat.[2]

Halothane was also observed to potentiate the size of tension transients and inferentially the amount of Ca^{2+} released from the SR following submaximal Ca^{2+} release by 2 mM caffeine. We also observed that cyclic contractions of the skinned fibers from hearts of both ages were present after release by 2 mM caffeine, but not 25 mM caffeine, and that halothane eliminated these cyclic contractions. This is in agreement with the prior observations of adult rabbit papillary muscle by Su and Kerrick.[7] Halothane potentiation of Ca^{2+} release by 2 mM caffeine was greater in the newborn during exposure to 1.0% and 1.7% halothane. However, the degree of potentiation of Ca^{2+} release seen in the present experiments was 3–4 times greater than that observed by Su and Kerrick[7] for which no ready explanation is available, except to suggest that papillary muscle and right ventricular wall muscle may respond differently in this regard.

The effects of halothane on caffeine-induced tension transients are the result of one or more possible halothane effects on the SR, either depression of active Ca^{2+} accumulation, augmentation of caffeine-induced Ca^{2+} release from the SR, or increased Ca^{2+} leak from the SR throughout all or part of the cardiac cycle.

Halothane depression of tension transients was due in part to a reduction of Ca^{2+} uptake and/or and increase in Ca^{2+} leak from the SR during Ca^{2+} accumulation. This conclusion is based upon the observation that exposure of the skinned fibers to halothane during active SR Ca^{2+} accumulation and release resulted in a degree of depression similar to depression seen with exposure of the skinned fibers to halothane during SR Ca^{2+} accumulation alone, whereas exposure to halothane during maximal SR Ca^{2+} release alone resulted in an increase of caffeine-induced tension transient size. Therefore, halothane acts upon the SR by inhibiting active Ca^{2+} accumulation and/or increasing Ca^{2+} leak from the SR.

Halothane depression of SR Ca^{2+} accumulation may be explained by depression of SR Ca^{2+}–ATPase.[20] At physiologic intracellular *p*H, halothane reduces both the rate of cardiac isolated SR Ca^{2+} sequestration and the capacity of SR vesicles for Ca^{2+} sequestration.[21] It is therefore probable that reduction of the amount of Ca^{2+} available for release from intracellular storage sites by inhibition of Ca^{2+} uptake is an important mechanism of halothane depression of contractility. This effect would be expected to interact synergistically with the milder inhibitory effects of halothane on the interactions of Ca^{2+} with regulatory and contractile proteins.[8–10,13]

In addition to depression of Ca^{2+} accumulation, halothane leads to both spontaneous release of Ca^{2+} from

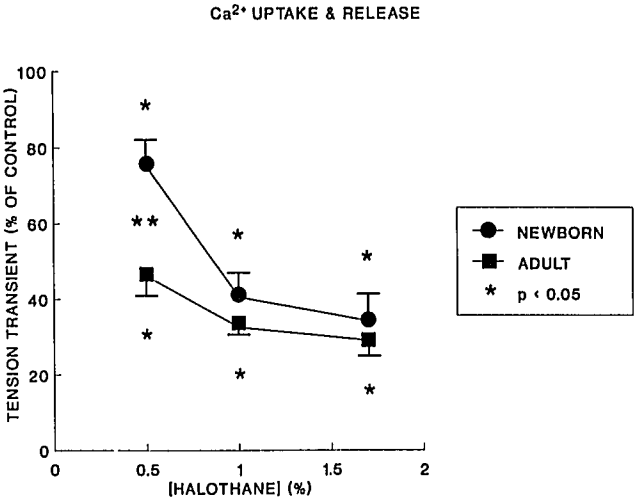

FIG. 4. Dose–response relationship of halothane exposure during SR Ca^{2+} uptake and release (solutions 2–5) on SR Ca^{2+} release by 25 mM caffeine (mean + SEM). Squares = adult skinned fibers; circles = newborn skinned fibers. *$P < 0.05$ *versus* control. **$P < 0.05$, newborn *versus* adult fibers.

quiescent myocardial cells (*i.e.*, Ca^{2+} leak)[22] and increases the effect of SR release of Ca^{2+} by caffeine.[23] It has been postulated based upon the behavior of skinned skeletal muscle that the *in vivo* Ca^{2+}-induced Ca^{2+} release mechanism and *in vitro* caffeine-induced release mechanisms are similar.[24] Therefore, halothane may potentiate Ca^{2+}-induced Ca^{2+} release *in vivo*, although there is yet no direct evidence for this. The mechanism of halothane augmentation of newborn SR Ca^{2+} release remains undefined.

Thus, the overall effect of halothane upon the SR is the result of both depression of Ca^{2+}-active transport into

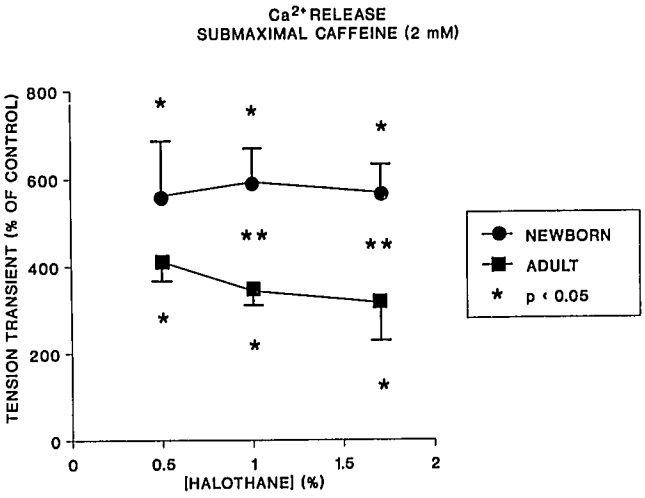

FIG. 5. Effect of halothane exposure during Ca^{2+} release (solution 5) on submaximal caffeine (2 mM)-induced Ca^{2+} release. Squares = adult skinned fibers; circles = newborn skinned fibers. *$P < 0.05$ *versus* control. **$P < 0.05$, newborn *versus* adult fibers.

the SR, and enhanced efflux of Ca^{2+} from the SR either by increased Ca^{2+} leak or by augmented Ca^{2+} release; the latter occurs in adult myocardium as Ca^{2+} release through SR Ca^{2+} release channels independently of the quantity of Ca^{2+} stored in the SR. If depression of Ca^{2+} active transport (*e.g.*, SR Ca^{2+} ATPase) were equally depressed in newborn and adult myocardium, then we would anticipate that the greater effect of halothane on caffeine-induced Ca^{2+} release would lead the newborn skinned fibers to demonstrate greater depression when tested during the uptake and release phases, a consequence of greater SR Ca^{2+} loss. This was not observed. To explain the lesser inhibition of tension transients seen when halothane was administered during SR Ca^{2+} uptake in the newborn skinned fibers, we suggest that newborn SR Ca^{2+} active transport may be less depressed by halothane than adult SR Ca^{2+} active transport, or that SR Ca^{2+} leak during Ca^{2+} uptake is less in the newborn. The latter explanation implies that halothane stimulation of SR Ca^{2+} leak and SR Ca^{2+} release occur by different mechanisms because they are affected in different directions in the newborn.

Other explanations for greater newborn cardiac sensitivity to halothane cannot be excluded. Ionic Ca^{2+} that activates cardiac contraction is derived from two sources, the extracellular stores *via* sarcolemmic calcium channels[6] and the intracellular stores, predominantly the sarcoplasmic reticulum.[7,20,21,25] Halothane depresses contractility in adult myocardium both by inhibiting SR Ca^{2+} storage, thus making less available for release for subsequent contractions and by reducing Ca^{2+} influx through the sarcolemma.[6,26–28]

Most of activator calcium in the adult mammalian heart is released from the SR. However, newborn rabbit ventricular myocardium does not contain T-tubules for the first eight postnatal days of life,[29] and the amount of SR per gram of cardiac muscle in the newborn is significantly less than in the adult.[30] Furthermore, Nishioka *et al.*[31] demonstrated that newborn rabbit myocardium is more responsive to isoproterenol and high extracellular Ca^{2+} concentration, which reflect trans-sarcolemmic Ca^{2+} current, and Seguchi *et al.*[32] found that newborn rabbit cardiac contractility is less sensitive than adult myocardium to the effects of ryanodine, which inhibits SR function by binding to Ca^{2+} release channels in the terminal cisternae of cardiac SR. This evidence also implies a less important role for the SR in newborn myocardial contraction and suggests that the newborn is more dependent upon Ca^{2+} derived from extracellular stores across the sarcolemma for activation of contraction than the adult. Halothane depression of sarcolemmic Ca^{2+} current may therefore have greater importance in the newborn than the adult.

Although the newborn cardiac SR may contribute relatively less activator Ca^{2+} than adult SR, our study demonstrates that newborn cardiac SR is indeed capable of sequestering Ca^{2+}, which can be released by caffeine. This is consistent with the findings of Nishioka *et al.*,[31] who demonstrated a greater positive inotropic response to paired electrical stimulation in newborn than adult myocardium.[31] The positive inotropic effect of paired electrical stimulation is believed to depend mainly upon Ca^{2+} derived from SR stores.[5] These data suggest that the Ca^{2+} sequestered by newborn cardiac SR may not be physiologically released because of an as yet undefined reason, such as absence of, or immaturity of T-tubules, or of a chemical intermediary that triggers SR Ca^{2+} release.

Finally, the *in vivo* physiologic signal that leads to SR Ca^{2+} release during excitation–contraction (which is undefined) may be another site at which halothane exerts a greater negative inotropic effect in newborn myocardium, either by directly interfering with an intermediary chemical stimulus or by reducing Ca^{2+}-induced SR Ca^{2+} release by virtue of an inhibitory effect on sarcolemmic Ca^{2+} currents.

In summary, caffeine-induced tension transients in newborn rabbit myocardial skinned fibers were less depressed than in adult skinned fibers by exposure to less than 1 MAC concentration of halothane during SR Ca^{2+} uptake and were equally depressed by exposure to greater than 1 MAC halothane concentrations. Tension transients were not affected by exposure of the skinned fibers of newborn and adult rabbit myocardium to halothane during maximal SR Ca^{2+} release but were increased threefold to fourfold during submaximal SR Ca^{2+} release, with greater halothane effect seen in the newborn.

We interpret these findings to indicate that whereas SR Ca^{2+} uptake is depressed less in newborn than adult myocardium by clinical concentrations of halothane, the newborn SR is more sensitive to halothane potentiation of caffeine-induced Ca^{2+} release from the SR, which may lead to a reduction of intracellular Ca^{2+} stores in the newborn and thus contribute to the sensitivity of newborn myocardium to halothane. Other possible sites of newborn sensitivity include sarcolemmic Ca^{2+} currents or the *in vivo* trigger for SR Ca^{2+} release.

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