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Left Ventricular Hypertrophy in Rabbits Does Not Exaggerate the Effects of Halothane on the Intracellular Components of Cardiac Contraction

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Inhalational anesthetics and ventricular hypertrophy have adverse effects on cardiac muscle contraction. The effects of 1, 2, and 3% halothane on the contractile protein and sarcoplasmic reticulum, but not the sarcolemma, were examined in normal left ventricular tissue from rabbits that underwent a sham surgical procedure (n = 5) and in left ventricular hypertrophied tissue from surgically induced aortic coarctation (n = 7). Muscle samples were mechanically "skinned" to disrupt the sarcolemma. Fiber bundles were mounted in photodiode transducers and bathed in a series of solutions designed to examine the contractile protein [Ca^{2+}]-tension responses or to examine Ca^{2+} storage by and release from the sarcoplasmic reticulum. Hill equation analysis of the [Ca^{2+}]-tension relationship of the contractile protein was performed. Compared to normal muscle, hypertrophied muscle was associated with an 8.2% decrease in the [Ca^{2+}] necessary for 50% maximum tension (more sensitive to Ca^{2+}) ($P < 0.001$) and an increase in the slope constant of 23% ($P < 0.001$). In normal and hypertrophied tissue, each 1% of halothane incrementally decreased the contractile protein response to maximal [Ca^{2+}] by 5% ($P < 0.01$), increased the [Ca^{2+}] at 50% maximum tension by 5% ($P < 0.01$), and had no effect on the slope of the Hill equation. Halothane also inhibited Ca^{2+} storage by the sarcoplasmic reticulum. In normal muscle, 1, 2, and 3% halothane decreased the stored Ca^{2+} to 42, 22, and 9%, respectively, of Ca^{2+} storage without halothane ($P < 0.001$). However, hypertrophied muscle demonstrated slightly less depression ($P < 0.05$ by analysis of variance). Halothane enhanced release of the available Ca^{2+} in the sarcoplasmic reticulum equally in both muscle types. It was concluded that hypertrophy does not accentuate the depressant effects of halothane on the Ca^{2+} sensitivity of the contractile protein or on the Ca^{2+} storage by the sarcoplasmic reticulum. (Key words: Anesthetics, volatile: halothane. Heart: contractile proteins; hypertrophy; sarcoplasmic reticulum.)

INHALATIONAL ANESTHETICS depress myocardial function in normal and hypertrophied muscle.[1] The mechanisms underlying anesthetic depression have been extensively investigated in normal muscle and appear to involve the sarcolemma, contractile protein, and sarcoplasmic reticulum.[2] It is not known if these cellular components in hypertrophied muscle are affected by inhalational anesthetics in the same manner and degree observed in normal muscle.

Hypertrophy leads to changes at the cellular level. In some species, hypertrophy causes a shift in the myosin heavy chain from isozyme I to isozyme III.[3–5] The percentage shift in the isozyme correlates with the percentage decrease in the velocity of contraction and the degree of hypertrophy observed in hypertrophied muscle.[4–6] The sarcoplasmic reticulum appears to have a diminished rate of Ca^{2+} uptake in hypertrophied tissue.[7,8] Because these or other changes could alter the response to inhalational anesthetics, we postulated that the contractile protein and sarcoplasmic reticulum of hypertrophied muscle might respond differently to halothane than would those of normal muscle.

This hypothesis was tested on a skinned fiber preparation obtained by mechanical disruption of the sarcolemma, in which individual organelle function was monitored by tension development.[9,10] Halothane was selected for investigation because its mechanism of action has been extensively studied. In addition, halothane, unlike isoflurane, exerts a significant influence on sarcoplasmic reticulum function.[10,11] Thus, the skinned fiber preparation was used to compare the effects of halothane on normal and hypertrophied muscle with respect to the Ca^{2+} responsiveness of the contractile protein and with respect to the storage and release of Ca^{2+} by the sarcoplasmic reticulum.

Materials and Methods

The study was approved by the Animal Care Committee of the Department of Animal Medicine, and care of the animals followed institutional guidelines.

Left Ventricular Hypertrophy by Aortic Coarctation

Male New Zealand white rabbits, 1.4–1.8 kg, underwent a sterile thoracotomy under halothane anesthesia for banding of the descending aorta or sham aortic banding.[12] The descending aorta was isolated midway between the great vessels and the diaphragm. Umbilical tape secured with 4-0 silk suture was used to reduce external aortic diameter by approximately 50%. Mean aortic pres-

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sure before and after narrowing the aorta was measured *via* a 25-G needle inserted proximal to the site of constriction. Ideally, the proximal aortic pressure increased by 20% with constriction. Sham surgery was performed in the same manner, except that the umbilical tape was merely passed around the aorta and removed.

The animals were given chloramphenicol 50 mg/kg intramuscularly preoperatively and every morning for 2 more days. Administration of buprenorphine 0.03 mg intramuscularly every 12 h for 2 days was begun on emergence from anesthesia. The animals were watched carefully for infection, wound care, and feeding and drinking habits. Recovery was uneventful, and all animals were active and eating well by 3 days after the operation.

Experimental Procedure

Four months after operation, each animal was killed with a Captive Bolt® pistol (Koch Industries, Kansas City, MO), and the heart was rapidly excised and cooled. Left ventricular weight (including septum), right ventricular free wall weight, total heart weight, lung weight, and liver weight were obtained (wet weights). The lungs were inspected for atelectasis, the liver inspected for mottling, and the abdominal cavity inspected for ascitic fluid. A sample of papillary muscle or trabeculae carneae was obtained and homogenized to achieve mechanical disruption of the sarcolemma.[9,10,13] Small bundles of fibers approximately 100 μm wide, 20 μm thick, and 1 mm long were mounted in a photodiode tension transducer.[14] Fiber bundles were stretched 10% above slack length. Each fiber bundle was exposed to one of two series of bathing solutions: one to examine the effect of halothane on the responsiveness of the contractile protein to various [Ca^{2+}] and the other to examine the effect of halothane on the storage and subsequent release of Ca^{2+} from the sarcoplasmic reticulum. The experiments were performed at room temperature.

Basic Bathing Solutions

Solutions were prepared as previously described.[9,10] The solutions used to study the contractile protein function contained (millimolar concentrations) 1 Mg^{2+}, 70 (K^+ + Na^+), 2 $MgATP^{2-}$, 7 EGTA, 15 creatine phosphate, 70–80 imidazole and propionate (anion), and various [Ca^{2+}]. The solutions used to study sarcoplasmic reticulum function differed only in the use of caffeine to release the stored Ca^{2+}, 0.05 EGTA just before and during Ca^{2+} release, [Ca^{2+}] 3×10^{-7} M or $<10^{-8}$ M, methanesulfonate (anion) and 0.1 mM Mg^{2+}. The fiber bundles were allotted exactly 3 min in the solution used to load the sarcoplasmic reticulum with Ca^{2+}.

Both sets of bathing solutions were prepared in duplicate. N_2 gas was bubbled through the control solutions. The test solutions had halothane 1, 2, and 3% added to the N_2 carrier gas for at least 40 min before experimental use to achieve equilibration. Thus, the partial pressure of the halothane in the test solutions was expressed as a percentage of atmospheric pressure at sea level (760 mmHg) and equalled 7.6 mmHg per 1% gas concentration. Partial pressure in the gas and solution phases has been confirmed by gas chromatography.[15] Given a water–gas partition coefficient of 1.7 for halothane at 20° C,[16] the halothane concentrations in the solutions were calculated to be approximately 0.7, 1.4, and 2.1 mM at gas concentrations of 1, 2, and 3%, respectively. The bubbling of halothane–N_2 through a given solution was temporarily interrupted for no longer than 10 min when the fiber was immersed in the solution. Halothane evaporation has been determined to be approximately 0.2% (at 3% halothane) during this time period.[9] The order of the halothane concentrations varied among the experiments.

Effect of Halothane on Ca^{2+} Activation of the Contractile Protein

Various [Ca^{2+}], expressed as $pCa = -\log [Ca^{2+}]$ (molar), were used to activate or relax the contractile protein. Between relaxations ($pCa > 8$), one fiber bundle from each rabbit was exposed to contracting solutions of pCa = 5.6, 5.4, and 3.8, while another bundle was used to measure the response at pCa = 5.4, 5.0, and 3.8 (fig. 1). After initial exposure to solutions without halothane, the sequence was repeated with solutions containing halothane. Sequences with and without halothane were alternated until the fiber bundle had been exposed to all three halothane concentrations.

Maximum tension (pCa = 3.8) in the presence of halothane was expressed as a percentage of the average response of the bracketing control responses (pCa = 3.8, no halothane). Analysis of variance was used to determine if the results were affected by halothane concentration or muscle type. The location of differences between control responses and responses with halothane and the location of differences between normal and hypertrophied muscle at the various halothane concentrations were accomplished with the Student-Newman-Keuls multiple-range test.[17]

Tension development at submaximum [Ca^{2+}] with and without halothane was expressed as a percentage of the response to pCa = 3.8 (100% response) with and without halothane, respectively. Given this normalization process, the comparisons of the pCa–tension relationship elicited differences in the slope constant and the [Ca^{2+}] required to develop 50% maximum tension ([Ca^{2+}]$_{50}$). The normalized submaximum data initially were examined with analysis of variance to determine if there were differences in the results attributable to the main effects of hypertro-

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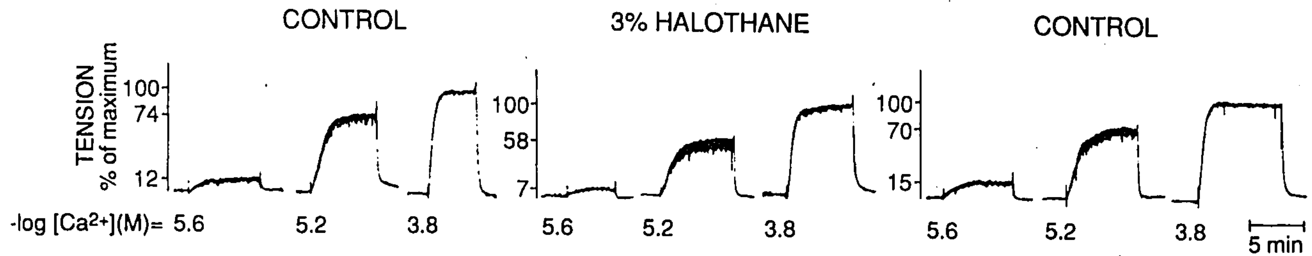

FIG. 1. The experimental protocol for the effect of halothane on the contractile protein response to various [Ca^{2+}] is shown. The tracings show a fiber bundle repetitively exposed to sequences of two submaximum [Ca^{2+}] (*p*Ca = 5.6, 5.2 in this example) and the maximum [Ca^{2+}] (*p*Ca = 3.8). Once each steady-state tension had been achieved, the fiber bundle was relaxed with a solution of *p*Ca > 8. The response to submaximum [Ca^{2+}] was expressed as a percentage of that sequence's response to *p*Ca = 3.8. After an initial exposure to a sequence of solutions without halothane (control), the fiber bundle was alternated between sequences with or without halothane until the fiber bundle had been exposed to all three halothane concentrations. In the example, only the 3% halothane exposure and the preceding and subsequent control sequences are shown.

phy, halothane, and *p*Ca and to test whether there were any interactions among the main effects. The data were fit to the Hill equation using the statistical package Allfit.[18] The Hill equation used was

$$\% \text{ tension} = \frac{100}{1 + \frac{[Ca^{2+}]_{50}{}^{n}}{[Ca^{2+}]^{n}}}$$

where $[Ca^{2+}]_{50}$ is the [Ca^{2+}] necessary for a 50% maximum tension and n is the slope of the [Ca^{2+}]–tension curve. The response to infinite [Ca^{2+}] was fixed at 100% and the response to 0 [Ca^{2+}] was fixed at 0%. The program Allfit is capable of simultaneous analysis of multiple curves and determination of the statistical likelihood that individual curves have different values for the Hill equation constants. The goodness of fit, when several curves are required to have the same value for a given Hill equation constant, can be compared with the goodness of fit when each curve is permitted a best-fit value for that constant. The program uses an F-test to determine if the change in the goodness of fit is statistically significant.[19] If the F-test is significant (at $P < 0.05$), the hypothesis that the curves are identical must be rejected, and the curves are required to have separate values for the Hill equation constant.

Effect of Halothane on Ca^{2+} Storage and Release from the Sarcoplasmic Reticulum

The second part of the study examined the effect of halothane on the ability of the sarcoplasmic reticulum to store and release Ca^{2+}. The amount of Ca^{2+} released by the sarcoplasmic reticulum was estimated by the area of the tension transient over time during exposure to caffeine.[20] A high caffeine concentration (25 mM) was used to release almost all of the available Ca^{2+}, thereby allowing estimation of the Ca^{2+} content of the sarcoplasmic reticulum.[21] A low caffeine concentration (2 mM) was used to achieve submaximum release of Ca^{2+}. Halothane was added to some or all of the bathing solutions to examine the effect of halothane on the storage of Ca^{2+} by the sarcoplasmic reticulum, the effect of halothane on the release of Ca^{2+} from the sarcoplasmic reticulum, or both. When 25 mM caffeine was used to release Ca^{2+}, halothane was added in random order to the solutions involved with loading Ca^{2+} into the sarcoplasmic reticulum (uptake phase), to the solution that contained caffeine to release the Ca^{2+} (release phase), or to the uptake and release phases. The Ca^{2+} content of the sarcoplasmic reticulum in the presence of halothane was quantified as the area of the caffeine-induced tension transient expressed as a percentage of the area in the absence of halothane (control). As illustrated in figure 2, each tension transient with halothane was preceded and followed by a transient without halothane, and the average of the bracketing transients served as the control.

A different fiber bundle from each rabbit was used to test the response to halothane in the release phase when only 2 mM caffeine was present in the releasing solution (fig. 2). Control tension transients with 2 mM caffeine without halothane were obtained before and after the transient with halothane. Unlike all other tension transients, which had a single peak with a smooth return to baseline, the 2 mM caffeine transients without halothane had multiple peaks. Even though some activity in the 2 mM caffeine contractions occasionally persisted beyond the typical transient duration of 90 s, integration of tension over time was stopped at 90 s.

Analysis of variance was used to define the effects of halothane concentration and hypertrophy on the results and to test for an interaction between the effects of halothane concentration and muscle type. The location of differences between control responses and responses with halothane and differences between normal and hypertrophied muscle was determined with the Student-Newman-Keuls multiple-range test.[17]

All computations were performed on a minicomputer with Allfit or a prepackaged statistical program. A mini-

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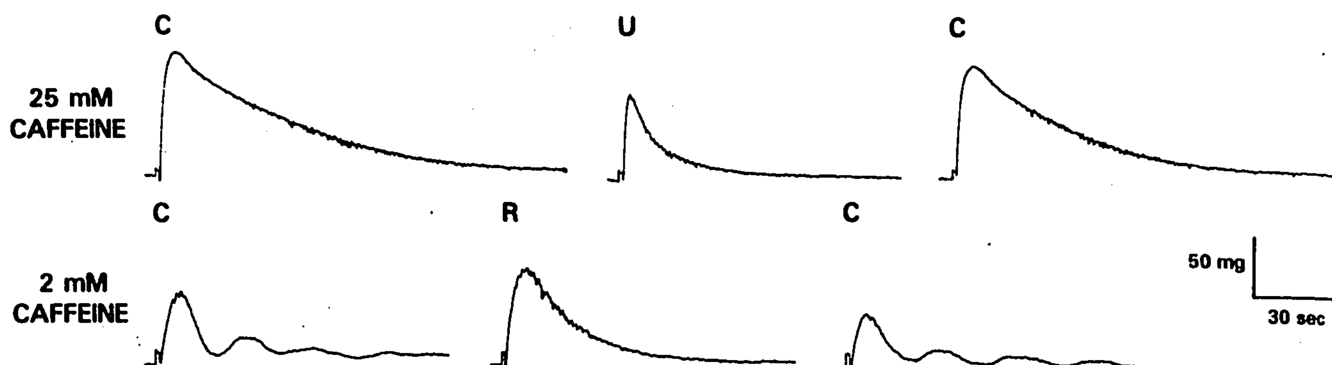

FIG. 2. The experimental protocol for the study of Ca^{2+} storage and release from the sarcoplasmic reticulum is shown. After loading the sarcoplasmic reticulum with Ca^{2+}, tension transients were induced with 25 mM caffeine (*top tracings*) or with 2 mM caffeine (*bottom tracings*). Control cycles (C, no halothane) bracketed each test cycle. Test cycles had halothane added to the solutions involved with Ca^{2+} storage (U, uptake phase) or the solutions with caffeine for Ca^{2+} release (R, release phase) or both phases (uptake and release phase, not illustrated). The area of the tension transient for a test cycle was expressed as a percentage of the mean area of the adjacent control cycles.

mum significance level of 0.05 was applied. Allfit was obtained from the National Institutes of Child Health and Human Development at the National Institutes of Health (Bethesda, MD).

Results

Post mortem examination revealed minimal lung atelectasis in all but one rabbit that had been otherwise clinically healthy. Average lung and liver weights were 16 and 87 g, respectively, and the weights were not significantly different between rabbits subjected to aortic banding and those that underwent the sham surgical procedure. None of the rabbits had ascites or enlarged, mottled livers. Compared to the rabbits that underwent sham surgery, rabbits subjected to aortic banding had left ventricular hypertrophy (table 1). Samples from three of the hypertrophied ventricles were sent for histologic evaluation. All revealed cellular enlargement and increased numbers of nuclei per cell, which is consistent with hypertrophy. The right ventricular free-wall weight tended to be greater in rabbits with hypertrophied left ventricles than in rabbits subjected to a sham surgical procedure, which may indicate some degree of left ventricular decompensation during the recovery period.

EFFECT OF HALOTHANE ON THE CONTRACTILE PROTEIN RESPONSE TO Ca^{2+}

In the study of the contractile protein, the contribution to contraction of the sarcolemma was eliminated by mechanical disruption of the sarcolemma, and the contribution of the sarcoplasmic reticulum to contraction was eliminated by controlling the free $[Ca^{2+}]$ at the contractile protein. At all $[Ca^{2+}]$, halothane depressed the tension. These effects could be summarized as a decrease in the maximum tension produced by the contractile protein and a decrease in the sensitivity of the contractile protein to Ca^{2+}. The effect of halothane on tension when the contractile protein was exposed to a saturating $[Ca^{2+}]$ (pCa = 3.8) is shown in figure 3. Analysis of variance indicated that the results were significantly affected by halothane concentration but not by muscle type. Each 1% concentration of halothane was associated with approximately a 5% reduction in maximum tension from control concentrations.

The submaximum tensions produced by the contractile protein in response to pCa = 5.6, 5.4, 5.2, and 5.0 with and without halothane were examined initially by analysis of variance. The results indicated that tension was affected by each of the three main effects: $[Ca^{2+}]$, muscle type (normal *vs.* hypertrophied), and halothane concentration

TABLE 1. Body and Ventricular Weights (±SD)

	Body Weight (kg)	Left Ventricular Weight (g)	Right Ventricular Weight (g)	Left Ventricular Weight / Body Weight (g/kg)
Normal (n = 5)	3.01 ± 0.27	3.75 ± 0.42	0.89 ± 0.10	1.25 ± 0.16
Hypertrophy (n = 7)	3.06 ± 0.42	5.57 ± 1.09	1.10 ± 0.20	1.85 ± 0.43
Normal *versus* hypertrophy	P = NS	$P < 0.01$	P = NS	$P < 0.02$

NS = difference not significant.

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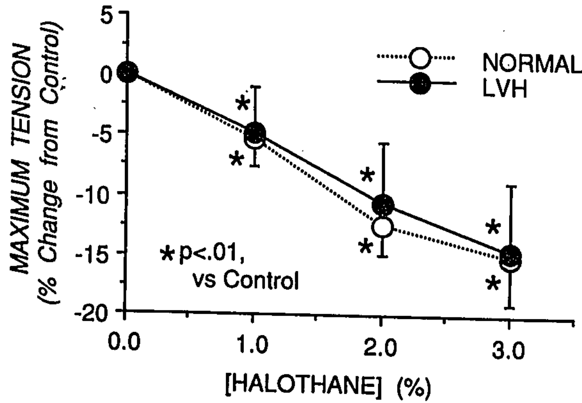

FIG. 3. Halothane decreased the Ca^{2+}-activated tension produced by the contractile protein of skinned fiber bundles under conditions of a controlled, maximally activating Ca^{2+} concentration (pCa = 3.8). Results at 1, 2, and 3% halothane are expressed as a percentage change from the control (0% halothane) maximum tension. Each point represents the mean ±SD of the results from 10 fiber bundles from five animals that underwent sham surgery (NORMAL) or from 13 fiber bundles from seven animals with left ventricular hypertrophy (LVH). Each percent halothane decreased the maximum tension by approximately 5%. No significant differences were found between the response of sham and hypertrophied muscle.

($P < 0.001$). Analysis of variance also indicated that there was an interaction between the effects of muscle type and $[Ca^{2+}]$ ($P < 0.001$). Examination of the data showed that at low $[Ca^{2+}]$ (pCa = 5.6), hypertrophied muscle was less responsive than normal muscle, but at high $[Ca^{2+}]$ (pCa = 5.2 or 5.0), hypertrophied muscle was more responsive. The $[Ca^{2+}]$-tension curves for the two muscle types crossed each other at some low value of $[Ca^{2+}]$.

The effects of $[Ca^{2+}]$, muscle type, halothane concentration, and $[Ca^{2+}]$–muscle type interaction were confirmed and quantified by Hill equation analysis. Table 2 lists the values for slope constant and $[Ca^{2+}]_{50}$ for each of the eight combinations of muscle type and halothane concentration. F-test analysis revealed that halothane did not affect the slope constant, but the two muscle types did have significantly different slopes. Further F-test analysis with a common value of $[Ca^{2+}]_{50}$ either for each muscle type or for each halothane concentration significantly worsened the goodness of fit in comparison with separate values for $[Ca^{2+}]_{50}$. The final results from the F-test analysis also are listed in table 2. The average values for the data and the curves defined by the Hill equation constants listed in the final analysis are plotted in figure 4 for 0 and 3% halothane. The final values for the $[Ca^{2+}]_{50}$ in table 2 indicate that hypertrophy decreased $[Ca^{2+}]_{50}$ by an average of 0.44×10^{-6} M. Regression analysis of $[Ca^{2+}]_{50}$ *versus* halothane concentration for each muscle type yielded an average increase in $[Ca^{2+}]_{50}$ of 0.27×10^{-6} M per 1% change in halothane ($P < 0.01$). In comparison with the $[Ca^{2+}]_{50}$ of 5.37×10^{-6} M for normal muscle at 0% halothane, the $[Ca^{2+}]_{50}$ in hypertrophied muscle therefore was decreased by 8.2% (more sensitive to Ca^{2+}), and halothane increased the $[Ca^{2+}]_{50}$ by 5% per 1% halothane (less sensitive to Ca^{2+}).

The interaction between muscle type and $[Ca^{2+}]$ was manifested in the Hill equation analysis by a change in slope constant. Hypertrophied muscle was associated with a steeper slope and a lower $[Ca^{2+}]_{50}$ than was normal muscle. This nonparallel shift caused the curves for normal and hypertrophied muscle to cross each other, as shown in figure 4. Using the values for the slope constant and $[Ca^{2+}]_{50}$ for 0% halothane listed in table 2, the crossover point was calculated to be $[Ca^{2+}] = 3.45 \times 10^{-6}$ M (pCa = 5.46). Tension is 28% of maximum at this $[Ca^{2+}]$.

EFFECT OF HALOTHANE ON THE SARCOPLASMIC RETICULUM

When halothane was present only in the release phase with 25 mM caffeine, the area of the tension transient

TABLE 2. Hill Equation Analysis of $[Ca^{2+}]$–Tension Results for Left Ventricular Tissue

Condition	Halothane (%)	Number of Points	Initial Anlaysis		Final Analysis (pooled data)	
			Slope	$[Ca+]_{50}$ (μM)	Slope	$[Ca^{2+}]_{50}$ (μM)
Normal (4 rabbits)	0	45	2.00 ± 0.69	5.37 ± 0.77	2.15 ± 0.73	5.37 ± 0.73
	1	15	2.27 ± 0.76	5.54 ± 0.73		5.55 ± 0.76
	2	15	2.14 ± 0.75	6.35 ± 0.88		6.35 ± 0.88
	3	15	2.53 ± 0.84	6.05 ± 0.75		6.10 ± 0.84
Hypertrophy (4 rabbits)	0	48	2.60 ± 0.84	4.94 ± 0.61	2.65 ± 0.86	4.94 ± 0.60
	1	16	2.74 ± 0.88	5.36 ± 0.64		5.37 ± 0.66
	2	16	2.64 ± 0.86	5.60 ± 0.69		5.60 ± 0.68
	3	16	2.76 ± 0.92	5.68 ± 0.68		5.68 ± 0.70

The slope (n) and $[Ca^{2+}]_{50}$ of the Hill equation,

$$\text{percent tension} = \frac{100}{1 + \frac{[Ca^{2+}]_{50}{}^n}{[Ca^{2+}]^n}}$$

are listed (±standard deviation) as obtained from normal rabbits or those with left ventricular hypertrophy. Further analysis found that pooling of the slopes by muscle type was statistically permissible, but attempts to pool $[Ca^{2+}]_{50}$ values resulted in significantly worse statistical fits.

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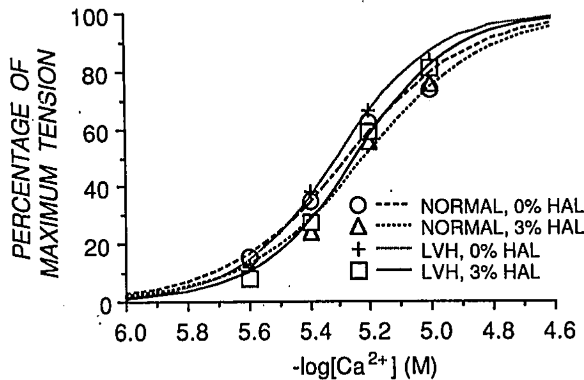

FIG. 4. The pCa–tension curves are plotted as defined by the Hill equation values taken from the final analysis in table 2. The data points represent the mean value of all points for the given muscle type and halothane (HAL) concentration. Hypertrophy is associated with an increase in slope and a decrease in $[Ca^{2+}]_{50}$ of the Hill equation, whereas halothane is associated with no change in slope but an increase in the $[Ca^{2+}]_{50}$.

was, on average, 9.3% greater than the area in the absence of halothane. Analysis of variance failed to detect any differences among the responses of the three halothane concentrations or between the two muscle types.

The ability of halothane to enhance Ca^{2+} release was better illustrated when halothane was added to a lower concentration (2 mM) of caffeine (fig. 5). Without exception, the area of the transient increased in the presence of halothane. On average, halothane was associated with a greater than 90% increase in area, in contrast to the 9% increase observed with 25 mM caffeine. However, the magnitude of that increase varied considerably and produced a wide range of standard deviations, as shown in figure 5. For this reason, analysis of variance was performed on the log transform of the data.[17] In five of the six combinations of muscle type and halothane concentration, the comparison between the halothane-enhanced tension transient and control (100%) achieved statistical significance. Analysis of variance failed to detect any differences among the three halothane concentrations or between normal and hypertrophied tissue.

The addition of halothane to the solutions used in the Ca^{2+}-uptake phase markedly reduced the magnitude of the tension transient in comparison with the magnitude of the tension transient without halothane (fig. 6). Analysis of variance indicated that the response to halothane was affected by the halothane concentration ($P < 0.001$) and by the muscle type ($P < 0.01$) and that there was no interaction between halothane concentration and muscle type. The difference in response between normal and hypertrophied muscle achieved statistical significance only at 3% halothane ($P < 0.05$).

When halothane was added to both the uptake and release phases, the results were virtually identical to those when halothane was added to the uptake phase alone. Analysis of variance revealed that the results were affected by halothane concentration ($P < 0.001$) and muscle type ($P < 0.01$) and that there was no interaction between those two effects. The difference in response between normal and hypertrophied muscle achieved statistical significance with 3% halothane ($P < 0.05$). These results reflect the major impact that halothane has on the Ca^{2+} content of the sarcoplasmic reticulum and the lack of effect on Ca^{2+} release when 25 mM caffeine is used to stimulate that release.

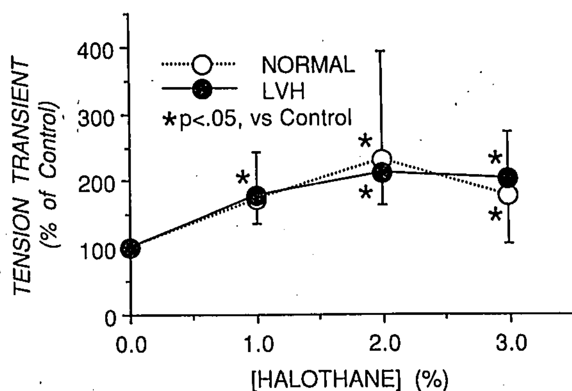

FIG. 5. The effect of halothane, in the release phase, on 2 mM caffeine-induced tension transients of skinned fiber bundles is shown. The values are expressed as a percentage of the control response (0% halothane) ± SD from five animals that underwent sham surgery (NORMAL) or seven animals with left ventricular hypertrophy (LVH). No differences were detected between the responses of normal and hypertrophied muscle.

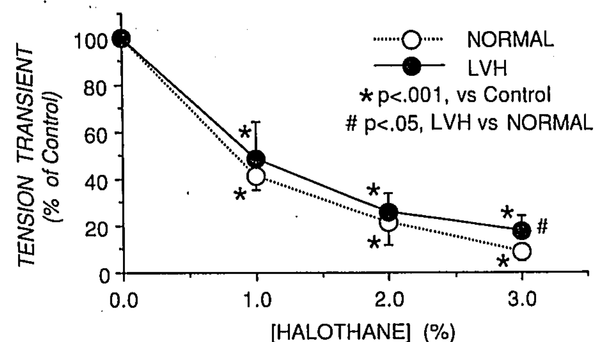

FIG. 6. The effect of halothane, in the uptake phase, on 25 mM caffeine-induced tension transient of skinned fiber bundles is shown. The values are expressed as a percentage of the control response (0% halothane) ± SD, n = 5 (NORMAL), n = 7 (LVH). Halothane depressed the area of the tension transient in a dose-dependent fashion. Analysis of variance revealed that the hypertrophied muscle was less prone to halothane depression than normal muscle ($P < 0.01$). The individual comparisons of the results at each halothane concentration achieved statistical significance only at 3% halothane.

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Discussion

In normal muscle, halothane decreased the Ca^{2+}-activated tension produced by the contractile protein, even when the $[Ca^{2+}]$ was carefully controlled. The effect of halothane on the contractile protein was similar in normal and hypertrophied myocardium. The decrease in tension by halothane could be summarized as a decrease in the maximum response and an increase in the $[Ca^{2+}]_{50}$ of the Hill equation, phenomena that have been described previously.[9,22] The net combination of these effects varies with the $[Ca^{2+}]$ at the contractile protein. At high $[Ca^{2+}]$, 1% halothane would decrease the contractile protein response by as little as 5%, but at low $[Ca^{2+}]$ the decrease could be as much as 15%. The effects of halothane on the contractile protein alone are modest compared with the overall effects of halothane on intact muscle. In normal papillary muscle, 1% halothane decreases force development by approximately 55%.[1] The greater effect on intact muscle presumably is attributable to the influence of halothane on intracellular $[Ca^{2+}]$, specifically the decrease in Ca^{2+} flux across the sarcolemma and the decrease in Ca^{2+} storage and release by the sarcoplasmic reticulum.

Not previously reported was the increase in the Ca^{2+} sensitivity of the contractile protein observed in hypertrophied muscle. Earlier studies, performed on left ventricles of guinea pigs and rats, demonstrated no change in Ca^{2+} sensitivity.[23–25] In right ventricular hypertrophy, a trend toward increased sensitivity to Ca^{2+} has been observed in rats and rabbits; however, statistical significance was not achieved until the development of heart failure.[24,26]

A value greater than 1.0 for the slope constant of the Hill equation suggests the presence of more than one interacting Ca^{2+} binding site on the C subunit of troponin.[27] However, there is evidence that only one of the three Ca^{2+} binding sites on troponin C is involved in myofibrillar adenosinetriphosphatase (ATPase) activation.[28] The current study is the first to report an increase in the slope constant in the presence of hypertrophy. If this increase in the slope constant does not represent increased numbers of binding sites, it may represent increased interaction of actin and myosin or of the various components of the thin filament.[25]

The decrease in $[Ca^{2+}]_{50}$ and the increase in slope constant in hypertrophied tissue is derived from the observation that at low $[Ca^{2+}]$, hypertrophied tissue was less sensitive to Ca^{2+} than was normal muscle, but at high $[Ca^{2+}]$, hypertrophied muscle was more sensitive to Ca^{2+} than was normal muscle. These changes in Ca^{2+} sensitivity and slope constant may represent changes in the kinetics of Ca^{2+}–troponin C binding in hypertrophied tissue. During cardiac contraction, if the myocardium operates at the low end of the $[Ca^{2+}]$–tension curve, our results would suggest that hypertrophied muscle would be less responsive to Ca^{2+} than would normal muscle. However, if intracellular $[Ca^{2+}]$ activate the contractile protein to a tension greater than the point of crossover (approximately 28% of maximum tension), hypertrophied muscle would be more responsive. Little is known about the degree of activation of the contractile protein during intact muscle contraction, but Fabiato[29] has inferred that a single twitch achieves approximately 20% of maximum contractile protein tension. With inotropic stimulation, twitch tension can achieve 50–60% of maximum.

The study was performed on rabbits, and the results may not apply directly to other species. In rats and young rabbits, hypertrophy has been associated with a shift from the V_1 to the V_3 isoenzyme of myosin[4–6]; this contrasts with findings in humans, who have primarily the V_3 isoenzyme and demonstrate no further shift in heart failure.[3,30] The rabbits in the current study were 6 months old at the time of experimentation. Rabbits of this age appear to have only V_3 and thus are more like humans.[31,32] The observation that halothane did not affect the contractile protein of hypertrophied muscle any differently than that of normal muscle is consistent with the lack of a shift in isoenzymes.

The marked decrease in the Ca^{2+} content of the sarcoplasmic reticulum in the presence of halothane is in agreement with earlier studies of nonhypertrophied rabbit myocardium either in the same preparation as that used in the current study or in a preparation of isolated sarcoplasmic reticulum.[10,33] The decrease in Ca^{2+} content could be attributable to an impaired rate of Ca^{2+} uptake or to enhanced Ca^{2+} leakage from the sarcoplasmic reticulum, and evidence exists for both mechanisms. In isolated sarcoplasmic reticulum vesicles, halothane inhibits ATPase activity and the rate of Ca^{2+} uptake.[34,35] The finding of enhanced Ca^{2+} release by halothane in a submaximum caffeine-releasing solution is consistent with results of other studies that used the same or different preparations.[10,36,37] Enhanced Ca^{2+} release may account for enhanced leakage of Ca^{2+} from the sarcoplasmic reticulum and result in decreased Ca^{2+} storage. In hypertrophied myocardium, the resistance to the halothane inhibition of Ca^{2+} storage can be interpreted as less inhibition of sarcoplasmic reticulum ATPase activity or decreased enhancement of Ca^{2+} release. The lack of a difference in halothane-enhanced Ca^{2+} release between hypertrophied and normal muscle would argue against the latter of the two proposed mechanisms.

The area of the tension transient reflects the amount of Ca^{2+} released by the sarcoplasmic reticulum and the responsiveness of the contractile protein to that Ca^{2+}. Halothane reduces the contractile protein response, a phenomenon that must contribute to the reduction in the area of the tension transient. However, the contribution

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probably is small because the change in the maximum tension of the contractile protein and the shift in the $[Ca^{2+}]_{50}$ were small. Thus, it appears that most of the reduction in the area of the tension transient is attributable to a halothane-mediated reduction in the Ca^{2+} content of the sarcoplasmic reticulum. The degree of depression, approximately 50% per 1% change in halothane, is similar to the effect of halothane on tension development in intact muscle. The resistance to halothane depression in the sarcoplasmic reticulum of hypertrophied muscle in comparison to that of normal muscle probably is not due to the effect of halothane on the contractile protein because halothane appears to have similar effects on the contractile protein of the two muscle types.

Using techniques similar to ours, Kimura *et al.*[23] found that the sarcoplasmic reticulum from hypertrophied rat left ventricles accumulated Ca^{2+} more slowly than did sarcoplasmic reticulum from normal ventricles. In our preparation, the 3 min allotted for Ca^{2+} accumulation was not sufficient to maximize the Ca^{2+} storage of the sarcoplasmic reticulum in normal muscle‡ and presumably was less sufficient for hypertrophied muscle. This difference in loading rates cannot explain halothane's more-depressed Ca^{2+} storage in normal muscle than in hypertrophied muscle because each fiber bundle served as its own control and the loading time was held constant. What cannot be determined from the current study is whether hypertrophy led to a change in the way halothane affects the rate of Ca^{2+} accumulation, the steady-state amount of stored Ca^{2+}, or both. It would be interesting to examine the relative effects of halothane using a variety of loading times.

Halothane causes marked depression in intact papillary muscle.[38] The mechanism of depression in intact muscle probably is attributable to the combined effects of halothane on the sarcolemma, the sarcoplasmic reticulum, and the contractile protein. The current study suggests that myocardial depression is more dependent on the effects of halothane on the sarcoplasmic reticulum than on the contractile protein. The influence of halothane on the sarcolemma was not examined, but it appears that Ca^{2+} flux across the sarcolemma is significantly affected by halothane.[2] The current study found that halothane depressed the Ca^{2+} storage of the sarcoplasmic reticulum in both muscle types, but the depression was less in hypertrophied muscle. Thus, in the intact muscle preparation, hypertrophied tissue would be expected to exhibit less depression from halothane than normal tissue; however, Shimosato *et al.* observed a similar degree of depression.[1] Resistance to halothane depression of the sarcoplasmic reticulum may be too small to be readily apparent in intact muscle preparations. It also is possible that other cellular components that contribute to contraction, *e.g.*, the sarcolemma, may be more affected by halothane in hypertrophied tissue and cancel the effects of hypertrophy on the sarcoplasmic reticulum. Although it appears that hypertrophied muscle is proportionally no more susceptible to halothane depression than normal muscle, hypertrophy may impose a functional deficit in addition to the depression induced by halothane.[1] Overt heart failure appears to result in even more severe impairment of baseline function.[6,24]

In summary, the effect of halothane on the contractile protein and the sarcoplasmic reticulum was examined in mechanically skinned fiber bundles from normal and hypertrophied rabbit left ventricular tissue. Halothane depressed contractile protein responsiveness to Ca^{2+} equally in the two muscle types. The Ca^{2+} storage in the sarcoplasmic reticulum was decreased by halothane but less so in hypertrophied muscle than in normal muscle. We conclude that, at these intracellular sites important to muscle contraction, hypertrophied muscle from left ventricles not in congestive failure is not more sensitive to halothane than normal tissue.

‡ Unpublished observation.

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