

# Intracellular Mechanisms of Halothane's Effect on Isolated Aortic Strips of the Rabbit

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The intracellular mechanisms of halothane action were examined in vascular smooth muscle from the aorta. Medial layers of the aorta from rabbits were mounted on photodiode tension transducers, stretched to 20 mg resting tension, and "skinned" with saponin. The skinned fiber preparations were then immersed in bathing solutions to study the effects of halothane (0.5-2%) on  $Ca^{2+}$  activation of the contractile proteins, and  $Ca^{2+}$  uptake and release from the sarcoplasmic reticulum (SR) using caffeine-induced tension transients. For comparison, isolated intact aortic rings were mounted on Blinks' dual tissue bath and attached to force transducers. The preparations were contracted with either 40 mM KCl, or norepinephrine (NE) followed by acetylcholine (ACh)- or sodium nitroprusside (SNP)-induced relaxation. At steady state contraction or relaxation, the effects of halothane (1-3%) were studied. The steady state tension during halothane was expressed as a percentage of the steady state tension before administration of halothane. In the isolated intact aortic rings, halothane (1-3%) produced biphasic effects on KCl-induced tension, *i.e.*, an initially slight increase followed by decreases, independent of endothelium. Halothane markedly increased tension in the ACh- or SNP-relaxed state. The effects were dose-dependent. In the skinned aortic strips, halothane slightly decreased maximum  $Ca^{2+}$ -activated tension development of the contractile proteins. Halothane decreased  $Ca^{2+}$  accumulation in the SR and increased  $Ca^{2+}$  release from the SR in a dose-dependent manner. The halothane-induced increases in  $Ca^{2+}$  release from the SR were blocked by ryanodine, an SR  $Ca^{2+}$  release channel blocker. It is concluded that halothane directly causes vascular contraction or relaxation, depending on the condition, and that halothane's effects on the SR may play a role. (Key words: Acetylcholine. Anesthetics, volatile: halothane. Caffeine, Ions: calcium. Muscle: contractile proteins; sarcoplasmic reticulum; smooth. Sympathetic nervous system: norepinephrine.)

HALOTHANE induces direct vascular smooth muscle relaxation<sup>1-3</sup> and decreases in myocardial contractility.<sup>4</sup> The mechanisms of halothane-induced depression of myocardial contractility have been investigated.<sup>5</sup> Yet, little is known about the mechanisms of the vasodilatory action of halothane.

It is known that halothane causes direct relaxation of phenylephrine-induced contraction in isolated rat aorta<sup>1</sup> but attenuates endothelium-mediated vasodilation.<sup>6</sup>

However, halothane effects are independent of endothelium in some preparations.<sup>6</sup>

Accordingly, the purposes of this study were to elucidate how halothane affects vascular smooth muscle and to understand the above-noted seemingly contradictory effects of halothane. We hypothesized that halothane acts similarly on vascular smooth muscle and on cardiac muscle. We have shown that in skinned myocardial cells, halothane enhances submaximal caffeine-induced calcium (Ca) release from the sarcoplasmic reticulum (SR), decreases Ca accumulation in the SR, and only slightly affects the Ca activation of contractile proteins.<sup>7,8</sup> Thus, the decreased Ca accumulation and/or the increased Ca release from the SR<sup>8</sup> may result in elevated free Ca in the cytoplasm, which accounts for the attenuation of endothelium-mediated vasodilation of halothane.<sup>6</sup> Ultimately, the Ca store in the SR would be reduced, which could account for the direct vasodilatory effect of halothane.<sup>1-3</sup>

We tested the above hypothesis by comparing the response to halothane of chemically skinned aortic strips from the rabbit with that of isolated intact aortic rings from the same animal species.

## Materials and Methods

### ISOLATED INTACT AORTIC RINGS

The method of preparing isolated intact aortic rings was described by Furchgott and Zawadzki.<sup>9</sup> New Zealand male white rabbits (2-2.5 kg) were killed by a captive bolt pistol, followed by exsanguination. Ascending and descending aorta were rapidly and carefully isolated and placed in a petri dish containing modified Krebs solution bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub> at room temperature. Connective and fat tissues were trimmed free from surfaces of the aorta. Aortic rings of 2.5-3.0 mm width were cut and mounted on Blinks' dual tissue baths containing 50 ml of modified Krebs solution saturated with 95% O<sub>2</sub>/5% CO<sub>2</sub>, pH 7.4 ± 0.02 at 37 ± 0.1° C.<sup>10</sup> The baseline tension was set at 2 g, and the rings were equilibrated for at least 2 h. To test the role of endothelium in halothane effect, aortic rings with endothelium either intact or rubbed, were suspended side by side in the same tissue bath. The aortic rings were tested with acetylcholine (ACh)-induced relaxation of norepinephrine (NE)-activated contraction as an indication of the presence of endothelium.<sup>9</sup> The tension was recorded with a Gould 2400S four-channel recorder with appropriate amplifiers.

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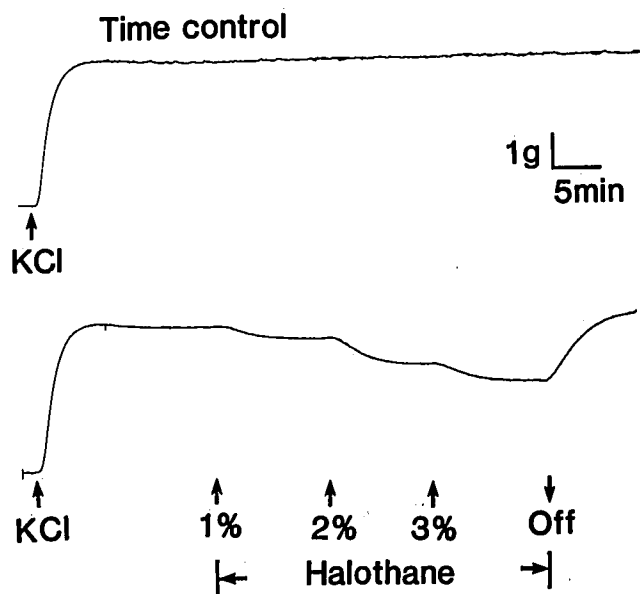


FIG. 1. Tracings showing the time course of 40 mM KCl-induced tension development (upper tracing) and the effects of cumulative concentrations of halothane (1%, 2%, and 3%) (lower tracing) on isolated intact aortic rings with or without endothelium intact. The tracings are representative of 6–16 preparations.

**Bathing solutions.** Modified Krebs solution contained (in mM) 121 NaCl, 1.2 MgSO<sub>4</sub>, 2.4 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 11 glucose, 24.8 NaHCO<sub>3</sub>, 2.5 CaCl<sub>2</sub>, and 0.03 Na<sub>2</sub>EDTA constantly bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub> to achieve pH 7.4 at 37 ± 0.1° C.<sup>9,10</sup> Halothane was delivered through a vaporizer (Foregger Fluomatic™, Smithtown, New York) and diluted with a 2 l/min flow rate of 95% O<sub>2</sub>/5% CO<sub>2</sub>. The partial pressures of halothane in the bathing solution (expressed as a percentage of one atmosphere) were assayed by a gas chromatograph.<sup>11</sup>

**Experimental procedure.** At the end of equilibration, the aortic rings were activated with 40 mM potassium chloride (KCl). When the tension development reached maximal steady state, the preparations were tested either with cumulative doses of halothane or no halothane, at 10-min intervals or until a new steady state was reached (10–15 min) (lower tracing, fig. 1). Reversibility was tested by terminating the halothane administration. The test results were expressed as a percentage of maximal steady state tension before administration of halothane, which had been corrected to the tension corresponding in time to the specific halothane concentration in the control preparations (absence of halothane). The control preparations showed a small change in tension of up to an average of 6% at the end of the study (60 min) (upper tracing, fig. 1). We found that the absence or presence of endothelium did not affect the test and time-control results. To test the integrity of the endothelium,<sup>9</sup> the aortic ring was activated by 0.1 μM NE (to steady state), followed by 0.1

μM ACh, resulting in relaxation. However, with the rubbed (to disrupt endothelium) aortic ring, either no change or a slight increase in tension was observed by administering 0.1 μM ACh.

#### CHEMICALLY SKINNED AORTIC STRIP

Only ascending aorta was used in this study. Aortic rings of 250–300 μm width were cut from ascending aorta and placed in relaxing solution (7 mM EGTA and no added Ca in basic bathing solution described below). An approximate 30–50 μm thickness of intimal and medial layers was dissected from each ring. A longitudinal strip (1.5–2.0 mm) was cut and mounted on two pairs of forceps, with one end attached to a photodiode tension transducer, as described by Hellam and Podolsky.<sup>12</sup> The resting tension was adjusted to achieve 20 mg. The aortic strip was then immersed in skinning solution (relaxing solution containing 0.5 mg/ml saponin) for 5 min and then washed in relaxing solution. Saponin makes the plasma membrane porous by binding with cholesterol. Because the SR membrane contains lower cholesterol concentrations,<sup>13</sup> the SR remains functional. The electronmicrograph of the aortic strips showed that the skinned smooth muscle cells were irregular in shape (right panel, fig. 2) compared with their shapes before saponin treatment (left panel, fig. 2). The isometric tension of the skinned aortic strip was then recorded on a four-channel 2400S Gould recorder equipped with appropriate amplifiers.

**Calcium accumulation in and release from the SR demonstrated with caffeine-induced tension transient.** Because saponin preferentially binds to cholesterol-rich sarcolemma, the SR remains functional. We were able to load Ca into the SR and after loading to release the Ca from the SR using caffeine. The loading-releasing cycle could be repeated in the same preparation. Caffeine has been shown to release Ca<sup>2+</sup> from loaded isolated heavy skeletal SR.<sup>14</sup> The rate and amount of Ca released from the SR<sup>14</sup> is comparable to that of twitch tension in isolated intact muscle preparation. Thus, caffeine was used as a tool to study the Ca loading and releasing mechanisms in the SR.

The basic protocol for studying caffeine-induced tension transient in skinned muscle fiber preparation was modified from studies on striated muscle.<sup>8</sup> Briefly, each cycle consisted of sequential immersion of the preparation in four different solutions, after an initial emptying of Ca from the SR with 25 mM caffeine in relaxing solution. The ionic composition of the four solutions was the same as that of basic bathing solution except that free magnesium was 0.1 mM and concentrations of EGTA and Ca varied as described below for each solution. The four solutions used were as follows: solution 1 (no added calcium, 7 mM EGTA) to wash away caffeine in the fibers;



FIG. 2. Electron micrographs showing smooth muscle cells (arrows) from aortic strip before (A, normal) and after (B, skinned) treated with saponin. Normal smooth muscle cells (left panel) are spindle-shaped, with distinct sarcolemma, whereas skinned smooth muscle cells (right panel) are irregular, indicating that sarcolemma is disrupted.

solution 2 (pCa 6.5, 7 mM EGTA) to rapidly load Ca into the SR; solution 3 (pCa 6.5, 0.05 mM EGTA) to reduce EGTA in the fibers; and solution 4 (pCa 6.5, 0.05 mM EGTA, and 25 mM caffeine) to induce Ca release from the SR with 25 mM caffeine, resulting in a tension transient (fig. 3). The area of the tension transient (shaded area, fig. 3) was used as an estimate of the amount of Ca released from the SR.<sup>15</sup> The duration of immersion in each solution was 5 min, except in solution 4 where immersion continued until a steady state tension transient was reached (returning to initial baseline) (shown in shaded area, second interception during relaxation, fig. 3) or for a fixed period (4 min) in the ryanodine and halothane interaction study.

Three conditions were studied under which halothane was present in the bathing solution: solutions 1 through 3, called the uptake phase; solution 4 only, called the release phase; and solutions 1 through 4 called the uptake and release phases. Each study consisted of a control (saturated with 100% N<sub>2</sub>), followed by the test (saturated with halothane-N<sub>2</sub> mixture at one of the above three conditions), and finally a control again (fig. 4). The test results were compared with a mean of two bracketing controls before and after the test by Student's *t* test for paired data.<sup>16</sup>  $P < 0.05$  was regarded as a statistically significant difference from the control.

*Ca-activated tension development of the contractile protein.* In addition to the basic bathing solution described herein, the bathing solution contained 1 mM free Mg, and various

free Ca concentrations (pCa =  $-\log [Ca^{2+}]$  (M) from  $>8$ , 5.6–3.8), buffered with 7 mM EGTA and propionate as a major anion.

High EGTA (7 mM) was used to buffer the free Ca concentrations for direct activation of the contractile proteins. Preliminary study on saponin-treated aortic strips showed that halothane increased submaximum Ca<sup>2+</sup>-activated tension (data not shown), which was due to halothane-induced Ca release from the SR (figs. 4 and 5). Thus, in this study, the skinned (saponin) aortic strips

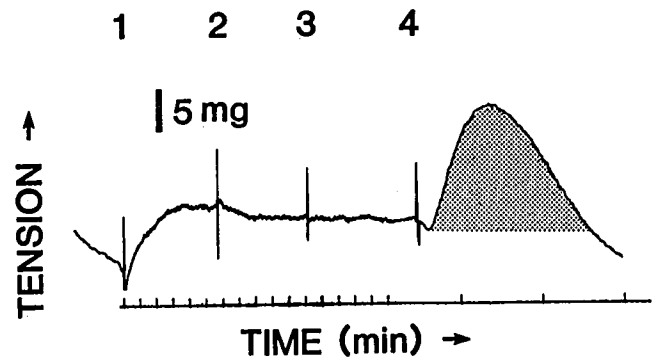


FIG. 3. A typical tracing for caffeine-induced tension transient in chemically skinned aortic strip; 1–4 are solution artifacts. Four different solutions were used to load Ca<sup>2+</sup> into the SR, and 25 mM caffeine was used to release Ca<sup>2+</sup> from the SR, resulting in a tension transient. The area of the tension transient (shaded) was analyzed as the amount of Ca<sup>2+</sup> release from the SR.

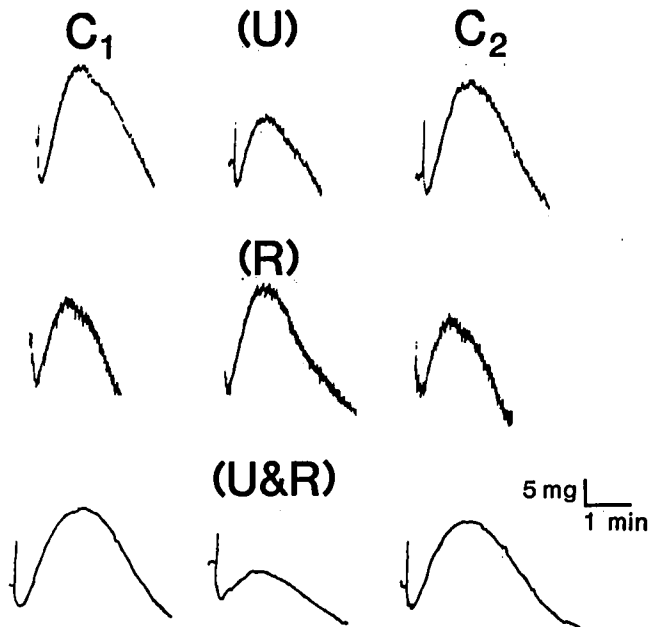


FIG. 4. Tracings showing the effects of halothane (1%) in the uptake phase (U), in the release phase (R), and in the uptake and release phases (U & R) on caffeine-induced tension transient in skinned aortic strips. C<sub>1</sub> = controls before (C<sub>1</sub>) and after (C<sub>2</sub>) halothane.

were further treated with detergent, 1% Triton X-100, for 10 min to solubilize the SR membranes.

Each preparation was activated with submaximum or

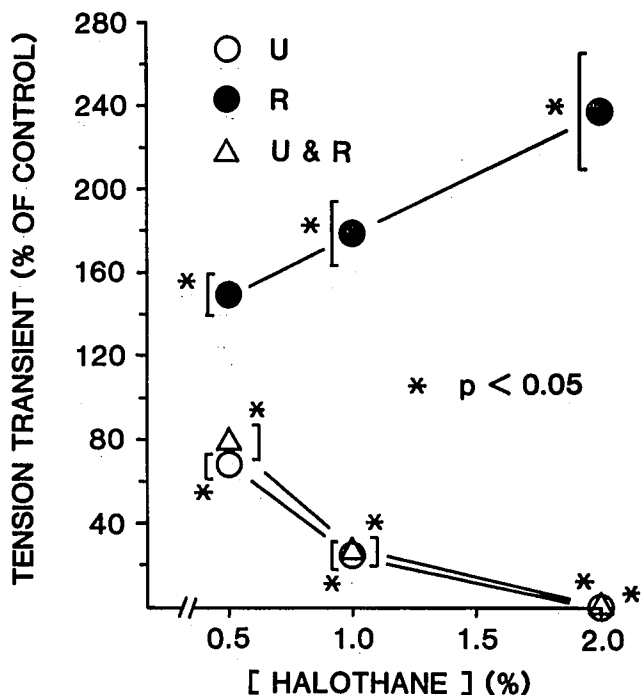


FIG. 5. Dose-response curves of halothane, in the uptake phase (U), release phase (R), and uptake and release phases (U & R) on caffeine-induced tension transients in skinned aortic strips. Values are mean  $\pm$  SEM (n = 3-9). \*P < 0.05 compared with control by Student's *t* test for paired data.

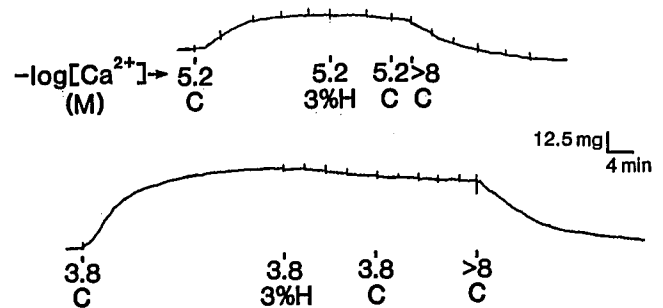


FIG. 6. Tracings showing the effect of 3% halothane on submaximum (pCa 5.2) and maximum (pCa 3.8) Ca<sup>2+</sup>-activated tension development of the contractile proteins in skinned aortic strips. C = controls (no halothane); H = 3% halothane; the tracings are representative of 3-4 preparations.

maximum Ca<sup>2+</sup> concentration until steady state tension development was achieved. Each experiment consisted of immersion of the preparation in a control solution (saturated with 100% N<sub>2</sub>) containing submaximum or maximum Ca<sup>2+</sup> concentrations, followed by a test solution (saturated with halothane-N<sub>2</sub> mixture) with the same Ca<sup>2+</sup> concentration, and, finally, in the control solution once again (fig. 6). The height of the steady state tension development was measured. The test result was compared with that of bracketing controls.

**Basic bathing solution.** The basic bathing solution was a modification of that used with skinned striated muscle<sup>7,8</sup> and contained 35 mM K<sup>+</sup>, 35 mM Na<sup>+</sup>, 15 mM creatine phosphate, 2 mM MgATP<sup>2-</sup>, 1 (for contractile proteins) or 0.1 (for SR) mM Mg<sup>2+</sup>, various free Ca concentrations (pCa = -log [Ca<sup>2+</sup>] (M)); EGTA total and major anion (propionate for CP or methanesulfonate for SR), depending on the specific study (described below); and a pH of 7.00  $\pm$  0.02 with 50 mM PIPES at 23  $\pm$  2° C to achieve the ionic strength of 0.15. The method of preparing the solution has been described in detail.<sup>17</sup> The total Ca concentration in the solution was assayed by an atomic absorption spectrophotometer (Hitachi 180-70, Zeeman effect). Halothane was delivered through a Verni-Trol® Vaporizing System (Ohio Medical Products, Madison, Wisconsin). The partial pressures of halothane in the bathing solutions (expressed as a percentage of one atmosphere) were assayed by a gas chromatograph.<sup>11</sup>

## MATERIALS

All bathing solutions were made with distilled and deionized water (Ca<sup>2+</sup>-free water). All the chemicals for making bathing solutions were reagent grade. Saponin was purchased from ICN (Lot # 13399) (Cleveland, Ohio). Halothane was purchased from Ayerst Co. (New York, New York). NE bitartrate and ACh chloride were purchased from Sigma Co. (St. Louis, Missouri). Ryanodine

(Lot 501) was supplied by Dr. Carl Hoffman of Merck, Sharp & Dohme Research Laboratories (Rahway, New Jersey).

### Results

#### EFFECTS OF HALOTHANE ON 40 mM KCl-INDUCED TENSION DEVELOPMENT IN ISOLATED INTACT AORTIC RINGS

After 2 h of equilibration, the isolated intact aortic rings (from ascending or descending aorta) were activated by 40 mM KCl, demonstrated with a tension development that nearly reached the maximum at about 10 min, followed by a small but significant increase of  $5.8 \pm 2.3\%$  ( $n = 8$ ) of the maximum tension after 40–50 min (time control, upper tracing, fig. 1). Thus, the test results were corrected with the mean of the controls at temporal changes. In the test preparations, halothane (1–3%) produced biphasic effects, with an initial small increase followed by decreases in the tension in a dose-dependent manner (lower tracing, fig. 1). The ascending aortic strips (3%, 9%, 18% decreases from control) were less depressed than were descending aortic strips (6%, 18%, 30% decreases from control) by 1%, 2%, and 3% halothane, respectively (fig. 7). Both were significantly decreased compared with controls. The effects were reversible (lower tracings, fig. 1).

#### EFFECTS OF HALOTHANE ON $Ca^{2+}$ ACCUMULATION IN AND RELEASE FROM THE SR, USING CAFFEINE-INDUCED TENSION TRANSIENT IN SKINNED AORTIC STRIPS

Halothane (0.5%, 1%, and 2%) present in the loading solutions (the uptake phase) decreased (30%, 70%, and 100%, respectively), and in the releasing solution (the release phase), increased (40%, 70%, and 140%, respectively) the caffeine-induced tension transients (figs. 4 and 5). Halothane, in the loading and releasing solutions (the uptake and release phase), decreased to a similar degree as in the loading solution (the uptake phase) the caffeine-induced tension transients. The effects were reversible ( $C_2$ , fig. 4) and were dose-dependent (fig. 5).

#### INTERACTION BETWEEN RYANODINE AND HALOTHANE IN THE RELEASE PHASE ON CAFFEINE-INDUCED TENSION TRANSIENT IN SKINNED AORTIC STRIPS

Ryanodine was used to test whether halothane-enhanced, caffeine-induced Ca release from the SR (fig. 5) is through the same Ca release channels as those containing high affinity binding sites for ryanodine. The use-dependent effect of ryanodine on the SR  $Ca^{2+}$  chan-

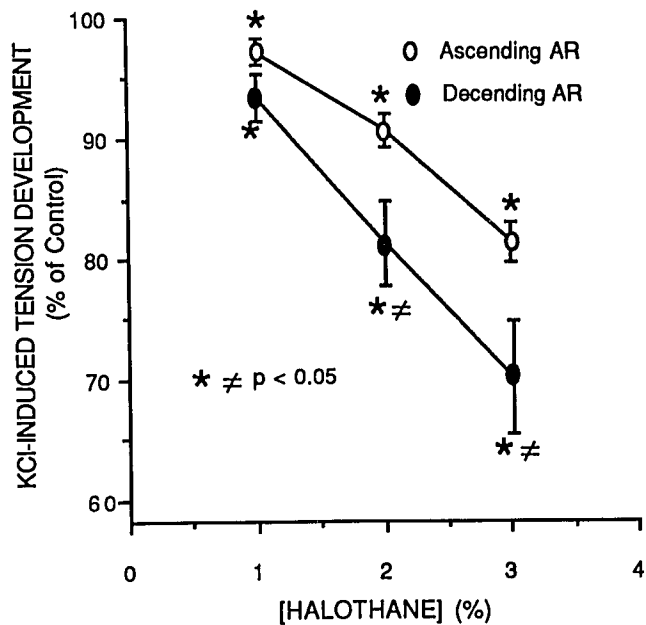


FIG. 7. Dose-response relation of halothane on KCl-induced tension development of isolated intact aortic rings (AR). Values are mean  $\pm$  SEM ( $n = 10-12$ ). \* $P < 0.05$  compared with control by Student's  $t$  test for paired data, ‡ $P < 0.05$ , ascending aortic rings versus descending aortic rings by Student's  $t$  test for nonpaired data.

nels has been demonstrated.<sup>18-20</sup> Ryanodine has been shown<sup>18-20</sup> to inhibit in a dose-dependent manner the second control ( $C_2$ ) caffeine-induced tension transients in skinned striated muscle, as well as in skinned aortic strips (Su, unpublished data), by binding to the SR Ca release channels.<sup>21</sup> The action of ryanodine alone requires time and/or channel opening to see its effect, which remains after "washout" due to high affinity. We studied the interaction between 3% halothane and 50 nM ryanodine in the release phase (solution 4 for 4 min).

As shown in figure 8, halothane (3%) alone increased the caffeine-induced tension transient ( $316 \pm 78\%$  of  $C_1$  control,  $n = 3$ ). Following halothane washout,  $C_2$  returned to control ( $85 \pm 7\%$  of  $C_1$  control,  $n = 3$ ). Ryanodine by itself did not initially alter the caffeine-induced transient ( $91 \pm 18\%$  of  $C_1$ ,  $n = 5$ ). After washout the prior ryanodine exposure depressed the repeat  $C_2$  control ( $66 \pm 12\%$  of  $C_1$  control,  $n = 5$ ). However, the combination of halothane and ryanodine markedly enhanced the depression of second control ( $C_2$ , lower row, fig. 8;  $8\% \pm 3 [5]$  of  $C_1$ ).

#### EFFECTS OF HALOTHANE ON $Ca^{2+}$ -ACTIVATED TENSION DEVELOPMENT OF THE CONTRACTILE PROTEINS IN SKINNED AORTIC STRIPS

The preparation was immersed in a glass vial containing either a submaximum calcium (pCa 5.2) (upper tracing, fig. 6) or the maximum Ca (pCa 3.8) (lower tracing, fig.

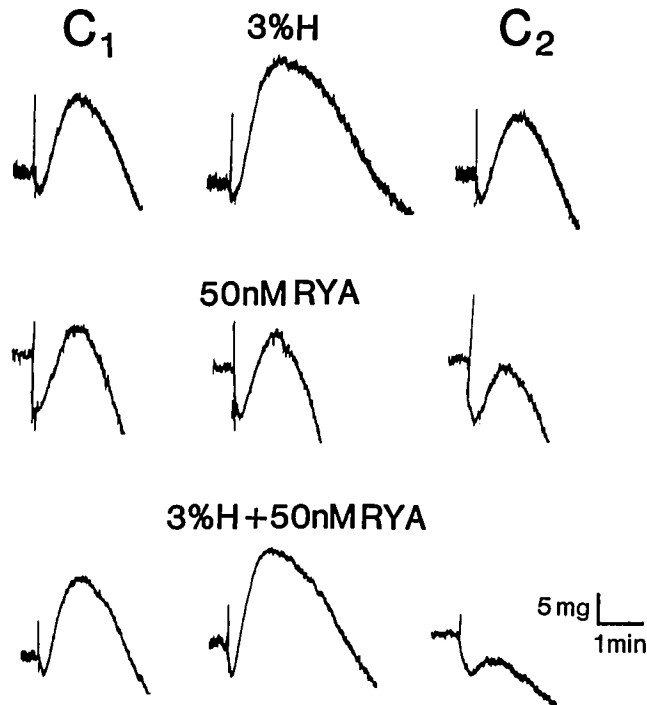


FIG. 8. Tracings showing the effects of 3% halothane (upper tracing), 50 nM ryanodine (middle tracing), and a combination of halothane and ryanodine (lower tracing) in the release phase on the caffeine-induced tension transient of skinned aortic strips.  $C_1$  = controls before ( $C_1$ ) and after ( $C_2$ ) the test. The tracings are representative of 3–5 preparations.

6) that was exchanged at 3-min intervals with a fresh saturated vial until the activated tension reached a new steady state (fig. 6). Halothane did not significantly change the submaximum, and slightly decreased the maximum,  $Ca^{2+}$ -activated tension development of the contractile proteins (fig. 6).

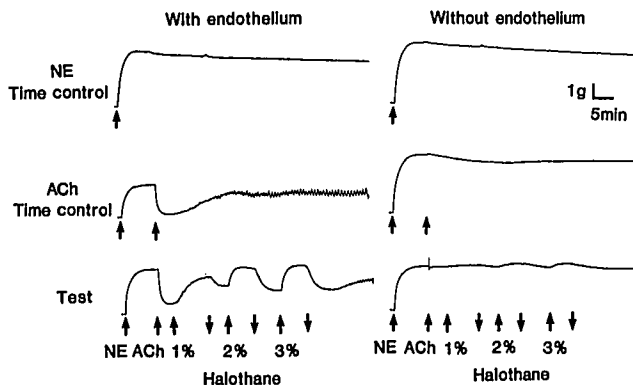


FIG. 9. Tracings showing the effects of halothane (1–3%) on NE ( $0.1 \mu M$ )-induced tension development followed by administration of ACh ( $0.1 \mu M$ ) with (left column) and without (right column) endothelium in isolated intact aortic rings.  $\uparrow$  indicates administration of the drug;  $\downarrow$  indicates termination of halothane. The tracings are representative of 4–10 preparations.

#### EFFECTS OF HALOTHANE ON NE-INDUCED TENSION DEVELOPMENT IN THE PRESENCE OF ACh OR SODIUM NITROPRUSSIDE (SNP) IN ISOLATED INTACT AORTIC RINGS WITH ENDOTHELIUM INTACT OR DISRUPTED

The halothane-induced Ca release from the SR demonstrated with increased caffeine-induced tension transient (filled circles, fig. 5) is correlated with the initial small increase in tension development in isolated intact aortic rings (fig. 1). This suggests an underlying subcellular mechanism of action of halothane. When vascular smooth muscle is in a relaxed state, halothane could cause contraction by the  $Ca^{2+}$  released from the SR. We used ACh to induce relaxation of NE-induced tension development in isolated intact aortic rings (middle tracing, left column, fig. 9). In endothelium-intact aortic rings, ACh caused initial rapid relaxation of NE-induced contraction, followed by gradual recovery of the tension with a small oscillation (middle tracing, left column, fig. 9). ACh did not induce relaxation in endothelium-disrupted aortic rings (middle tracing, right column, fig. 9). Halothane was administered at maximum ACh-induced relaxation to both preparations. We found that halothane caused marked contraction in relaxed preparations (lower tracing, left column, fig. 9) but only slightly affected nonrelaxed preparations (lower tracing, right column, fig. 9). The tension relaxed when halothane was removed (lower tracing, left column, fig. 9).

To test the role of endothelium in the halothane-induced reversal of ACh relaxation, an endothelium-independent vasodilator, SNP,<sup>22</sup> was used. We found that SNP relaxed NE-induced contraction of isolated intact aortic rings in both preparation, with or without endothelium intact (fig. 10). Halothane again caused sustained contraction in these preparations in which relaxation was SNP-induced (fig. 10).

#### Discussion

This study shows that in skinned aortic smooth muscle the intracellular mechanisms of action of halothane are similar to those in cardiac and skeletal muscle,<sup>7,8,23,24</sup> *i.e.*, there is a slight decrease in the maximum  $Ca^{2+}$  activated tension development of the contractile proteins (fig. 6), an increase in  $Ca^{2+}$  release, and a decrease in  $Ca^{2+}$  accumulation in the SR (figs. 4 and 5). The increased  $Ca^{2+}$  release initiated by halothane may be through the SR Ca release channels<sup>18–20</sup> that bind ryanodine (fig. 8). In intact aortic rings, halothane caused a biphasic response, *i.e.*, there was an initial small increase followed by a decrease in KCl-induced tension (lower tracing, fig. 1; and fig. 7). Halothane markedly enhanced the tension development in relaxed preparation caused either by ACh (fig. 9) or

SNP (fig. 10). Halothane effects are reversible and are not endothelium-dependent (fig. 1).

The mechanism for  $\text{Ca}^{2+}$  activation of contractile proteins in smooth muscle<sup>25</sup> is more complicated than that in striated muscle. The slight decreases in maximum  $\text{Ca}^{2+}$ -activated tension development caused by halothane could be due to decreases in  $\text{Ca}^{2+}$ -independent myosin phosphorylation or to decreases in myosin  $\text{Mg}^{2+}$ -ATPase activity. No literature has yet addressed this question.

Halothane enhanced 25 mM caffeine-induced tension transients in skinned aortic strips (filled circles, fig. 5) as well as those of skinned fibers from skeletal muscle<sup>23,24</sup> but did not do so in skinned cardiac muscle,<sup>8</sup> suggesting that 25 mM caffeine releases only part of the calcium in the SR, as with skinned skeletal muscle.<sup>23,24</sup> This speculation is further confirmed by the larger tension transient generated by 1% Triton X-100 after caffeine treatment (data not shown) and is also not inconsistent with data of Somlyo *et al.*<sup>26</sup> However, halothane-induced decreases in  $\text{Ca}^{2+}$  accumulation in the SR (open circles, fig. 5) are similar to those observed in cardiac and fast-twitch skeletal skinned muscle fibers.<sup>8,24</sup> This may be due to the inhibition of SR-ATPase activity,<sup>27</sup> the decreased Ca uptake reported in isolated cardiac SR,<sup>28</sup> and/or due to increased SR membrane permeability as shown with increased  $\text{Ca}^{2+}$  release by halothane (filled circles, fig. 5). Whether halothane also inhibits the SR-ATPase activity of vascular smooth muscle remains to be confirmed.

The source of  $\text{Ca}^{2+}$  for physiologic contraction in smooth muscle, either from SR or through sarcolemma, is not totally clear.<sup>29</sup> Ryanodine receptor- $\text{Ca}^{2+}$  release channel in vascular smooth muscle has not been characterized. However, the presence of SR and its importance in the excitation-contraction coupling has been identified.<sup>30</sup> If the caffeine-releasable Ca pool in the SR of the skinned aortic strips is comparable to that *in vivo*, then the halothane-induced increases in Ca release and decreases in Ca accumulation observed in the skinned aortic strips (fig. 5) would play a role in halothane's effects on intact aortic rings (figs. 1, 7, 9, and 10). Based on current knowledge of smooth muscle contraction, high K causes membrane depolarization resulting in Ca influx through the sarcolemma. This Ca may induce Ca release from the SR resulting in increased free Ca in the cytoplasm. This increased free Ca would increase Ca-dependent myosin light chain phosphorylation permitting cycling of myosin cross-bridges for muscle shortening. It is currently believed that other mechanisms are involved in maintaining isometric force.<sup>25</sup> ACh has been shown to decrease this KCl-induced Ca influx with little effect on tension development. However, ACh decreases both NE-induced Ca influx and tension development.<sup>31</sup> This suggests that Ca released from intracellular store(s) (such as SR) by NE<sup>32</sup> plays an important role in ACh-induced relaxation. The

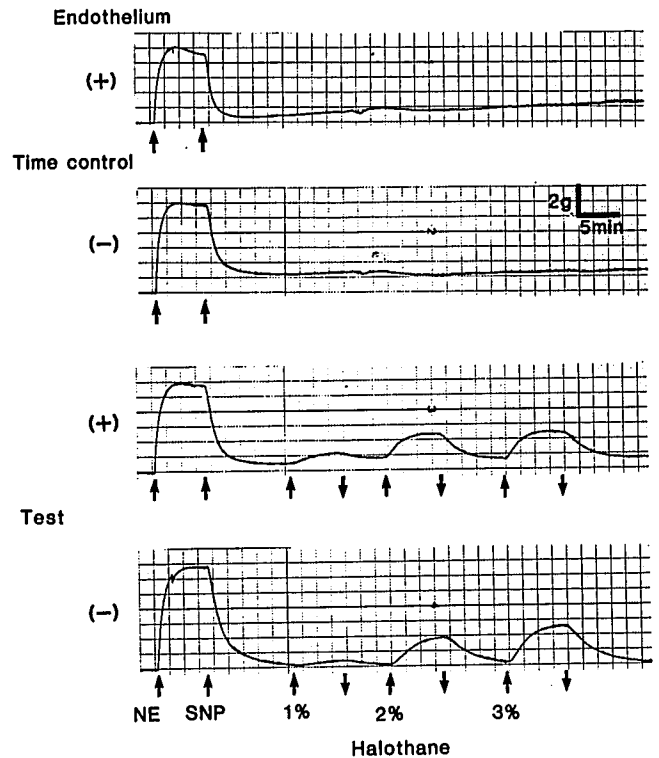


FIG. 10. Tracings showing the effects of halothane (1–3%) on NE (0.1  $\mu\text{M}$ )-induced tension development followed by the administration of SNP (0.1  $\mu\text{M}$ ) with [(+)] and without [(-)] endothelium in isolated intact aortic rings.  $\uparrow$  indicates administration of the drug,  $\downarrow$  indicates termination of the drug. The tracings are representative of four preparations.

endothelium-derived relaxing factor (EDRF) released by ACh may act by inhibiting release of Ca from or increasing Ca reuptake by the SR. The effects of halothane on intact aortic rings may be at multiple sites: Ca fluxes through the sarcolemma ( $\text{Ca}^{2+}$  influx and  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchange), the SR, and the contractile proteins.

Because halothane had little effect on the Ca activation of the contractile proteins (fig. 6), we speculate that the mechanisms of halothane actions on KCl contraction (initial increase followed by decrease) are as follows.

The initial increase in tension development of KCl contraction (fig. 1) and ACh and SNP relaxation (figs. 9 and 10) of intact aortic rings could be the net increases in Ca in the cytoplasm resulting from inhibition of Ca accumulation (open circles, fig. 5) and/or release of Ca from the SR (solid circles, fig. 5). We propose this model over other sites of action. Our speculation is further substantiated by using an endothelium-independent vasodilator, SNP.<sup>22</sup> In SNP (fig. 10)- and in ACh (fig. 9)-relaxed intact aortic rings, halothane again caused sustained tension development, indicating endothelium independency of halothane effects. It is also possible that halothane reduces production of EDRF by ACh as suggested by Mul-

doon *et al.*<sup>6</sup> Whether this halothane-induced contraction (left column, fig. 9) and attenuations<sup>6</sup> of ACh-induced relaxation are due to decreases in EDRF production remains to be confirmed. A direct measurement of EDRF, when it is identified, would resolve the speculations.

The subsequent decreases in KCl contraction by halothane could be due to the inhibition of Ca flux and/or enhancement of Na<sup>+</sup>-Ca<sup>2+</sup> exchange. However, no information is available regarding the effects of halothane on Ca fluxes through the sarcolemma. In addition, the decreased Ca accumulation in the SR by halothane (open circles, fig. 5) could also further be affected by decreased Ca influx, which would result in less Ca available to be released from the SR for subsequent contraction.

In isolated intact preparations, halothane only slightly affected the tension development when the preparations were contracted either with KCl (at 2% halothane, decrease 9% for ascending aorta and decrease 18% for descending aorta) (fig. 7) or with NE (data not shown), an effect that is not endothelium-dependent. This observation is not inconsistent with that of Muldoon *et al.*<sup>6</sup> In contrast, at 2% halothane, a 30–35% depression of maximal phenylephrine (PE)-induced contraction in isolated intact rat aorta has been observed by Sprague *et al.*<sup>1</sup> This difference could be due to variations in animal species (rabbit *vs.* rat) or different conditions (KCl and NE *vs.* PE). Whether the different degree of depression by halothane between ascending and descending aorta (fig. 7) is due to different mechanisms of action remains to be explored.

Due to its high affinity for the Ca<sup>2+</sup> release channel, sufficient ryanodine remained after washout to keep channels open and let Ca<sup>2+</sup> leak from the SR, thus decreasing the amount of Ca<sup>2+</sup> available for release from the SR. The enhancement of ryanodine's effect on caffeine transients (fig. 8) suggest that halothane alters the Ca<sup>2+</sup> release channel such that more receptors bind ryanodine. Opening of this channel by halothane would explain both the enhancement of caffeine transients by halothane and its enhanced ryanodine binding. The presence of a high affinity ryanodine receptor site in the SR of vascular smooth muscle remains to be established. Whether this halothane-induced Ca release through SR Ca release channels<sup>18–20</sup> is also responsible for the decreased calcium accumulation in the SR (fig. 5) is not clear.

In summary, halothane causes a small biphasic effect in isolated intact aortic rings; there is an initial increase, followed by a decrease of KCl-induced tension development that is not endothelium-dependent. The initial increase in tension can easily be demonstrated in relaxed preparation. In skinned aortic strips, halothane causes slight decreases in Ca activation of the contractile proteins, marked increases in Ca release, and decreases in Ca accumulation by the SR. We conclude that halothane-in-

duced increases in tension development in isolated intact aortic rings are not inconsistent with increased Ca<sup>2+</sup> release from the SR. The decreased tension development caused by halothane in the intact aortic ring preparation could be due to a combination of a slight depression of Ca activation of the contractile proteins and decreased Ca accumulation in the SR, resulting in less Ca release from the SR for muscle contraction. However, whether Ca<sup>2+</sup> influx through the sarcolemma is affected by halothane in relation to the altered vascular smooth muscle contraction remains to be tested.

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