

Anesthesiology  
81:700-709, 1994  
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## Mechanisms of Action of Enflurane on Vascular Smooth Muscle

### Comparison of Rabbit Aorta and Femoral Artery

J. Y. Su, Ph.D.,\* Y. I. Chang, B.S.,† L. J. Tang, M.S.‡

**Background:** This study was performed to elucidate the mechanisms of action of enflurane by comparing the vascular smooth muscle responses of conduit arteries of larger (aorta) and smaller (femoral artery) diameter to enflurane using isolated rings and skinned strips.

**Methods:** Isolated intact rings (endothelium denuded) of aorta and femoral artery from rabbits were activated by various concentrations of norepinephrine (NE) and the effects of enflurane were examined at the steady-state force. In a separate study, the rings were pretreated with verapamil before the NE activation and tested with enflurane. In the saponin-treated arterial strips ("skinned"), the effects of enflurane on  $Ca^{2+}$  uptake or release from the sarcoplasmic reticulum were studied using caffeine-induced tension transients.

**Results:** In isolated aortic rings, enflurane (0.9%–5%) enhanced tension development at low NE concentrations (5 and 30 nM) but depressed it at highest concentration (10  $\mu$ M). In contrast, enflurane depressed tension development in the femoral artery at all NE concentrations. Enflurane caused significant increase in the NE-activated force in rings pretreated with verapamil. In skinned strips, enflurane (1%–3%) decreased  $Ca^{2+}$  uptake (concentration resulting in 50% depression: 1.8% for aorta and 2.5% for femoral artery) and increased  $Ca^{2+}$  release from the sarcoplasmic reticulum (59%–208% for aorta and 10%–55% for femoral artery). These effects were dose-dependent. Enflurane potentiated ryanodine depression of caffeine-induced tension transients.

**Conclusions:** Enflurane has similar mechanisms of action in aorta and femoral artery: blocking  $Ca^{2+}$  influx, and causing, at least in part,  $Ca^{2+}$  release from the sarcoplasmic reticulum through the ryanodine-receptor channel. These cellular ac-

tions of enflurane account for the depression in femoral artery and enhancement in aorta of NE-activated force in isolated rings. (Key Words: Anesthetics, volatile: enflurane. Arteries: aorta; femoral artery. Organelle: sarcolemma; sarcoplasmic reticulum. Calcium channel, voltage-gated sarcolemmal calcium channel blocker: verapamil; sarcoplasmic reticulum calcium channel: ryanodine-receptor,  $IP_3$ -receptor.)

POTENT volatile anesthetics have been shown *in vivo* to constrict or dilate vascular beds.<sup>1</sup> In isolated arterial rings, halothane has been shown to have different effects among various types of conduit arteries.<sup>2</sup> Thus, the opposing effects of potent volatile anesthetics may be directly mediated by differing mechanisms of the anesthetic action, or they may result from differing responses of contractile processes in the vascular beds. However, the mechanism of action of volatile anesthetics on vascular smooth muscle contraction is not totally clear.

It is well established that the elevation of cytosolic free  $Ca^{2+}$  plays an important role in the initiation of vascular smooth muscle contraction. Two possible major sources of  $Ca^{2+}$  have been identified: (1)  $Ca^{2+}$  influx through the sarcolemma (either by voltage- or receptor-operated  $Ca^{2+}$  channels), and (2)  $Ca^{2+}$  release from the sarcoplasmic reticulum (SR).<sup>3</sup> Evidence suggests that opening of the receptor-operated channels is mediated by state of the intracellular  $Ca^{2+}$  store.<sup>4</sup> There also is evidence for a differential importance in the steps of the contractile process within resistance arterioles of different diameters and between resistance arterioles and conduit arteries. As the diameter of the resistance arterioles decreases there is a progressive increase in sensitivity to the  $Ca^{2+}$  channel blocking effect of diltiazem.<sup>5</sup> This suggests that within resistance vessels, the smaller the diameter of the arteriole, the greater the dependence of the vascular smooth muscle contraction on  $Ca^{2+}$  influx (as opposed to  $Ca^{2+}$  release from the SR). On the other hand, the aorta is about

\* Research Professor.

† Research Technologist.

‡ Visiting Scientist.

Received from the Dept. of Anesthesiology, RN-10, University of Washington, Seattle, Washington. Accepted for publication May 4, 1994. Supported by National Institutes of Health grant GM 48243 and in part by Anaquest, Murray Hill, New Jersey. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, Washington, DC, 1993.

Address reprint requests to Dr. Su: Dept. of Anesthesiology, RN-10, University of Washington, Seattle, Washington 98195.

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10,000-fold less sensitive to diltiazem than are resistance vessels,<sup>5</sup> suggesting that the conduit artery depends more on the  $\text{Ca}^{2+}$  release from the SR than on  $\text{Ca}^{2+}$  influx. This possibility is consistent with the relatively large SR content in the aorta.<sup>6</sup> Thus, it also is possible that this different response to the anesthetic is attributable to the relative importance of various steps in the contractile mechanisms of vascular smooth muscle from different vascular beds.

This study was performed to elucidate the mechanisms of action of enflurane in two conduit arteries of different diameters, the aorta *versus* the femoral artery. We hypothesized that enflurane has similar mechanisms of action in vascular smooth muscle and that the difference of response to enflurane results from the differential dependency on the SR and  $\text{Ca}^{2+}$  influx for muscle contraction in these two arterial types. We tested the hypothesis by (1) using isolated intact arterial rings without endothelium to demonstrate the direct effect of enflurane on precontracted vascular smooth muscle and (2) using skinned arterial strips to demonstrate the effects of enflurane on  $\text{Ca}^{2+}$  uptake and release from the SR using caffeine-induced tension transients.<sup>7</sup>

### Materials and Methods

Young adult New Zealand male white rabbits (2–2.5 kg) were killed by a captive bolt pistol followed by exsanguination. This method has been approved by the Animal Care Committee at the University of Washington. Ascending and descending aorta, and femoral artery were rapidly isolated and kept in Krebs solution saturated with 95%  $\text{O}_2$ –5%  $\text{CO}_2$  at room temperature (23°C) for isolated intact ring preparations or in ice for skinned strip preparations.

#### Isolated Arterial Rings

For the intact ring study, the descending aorta and femoral artery were placed in a Petri dish containing modified Krebs solution bubbled with 95%  $\text{O}_2$ –5%  $\text{CO}_2$  at room temperature. Connective and fat tissues were then trimmed free from the vessel surfaces. Aortic rings of 3.0 mm width and femoral arterial rings of 4.0 mm width were cut and the endothelium was removed by gentle rubbing. The arterial rings were then mounted side by side in one of four Blinks' dual tissue baths containing 50 ml of modified Krebs solution saturated with 95%  $\text{O}_2$ –5%  $\text{CO}_2$ ,  $\text{pH}$   $7.4 \pm 0.02$  at  $37 \pm 0.1^\circ\text{C}$ .<sup>8</sup>

The baseline tension, set at 2 g for aorta and 1 g for femoral artery, was the point at which maximum tension was achieved with submaximum or maximum norepinephrine (NE) concentrations. The rings were then allowed to equilibrate in modified Krebs solution for 1 h. Isometric force was recorded with a Gould 2400S four-channel recorder equipped with appropriate amplifiers.

**Bathing Solutions.** The modified Krebs solution contained (millimolar) 121 NaCl, 1.2  $\text{MgSO}_4$ , 2.4 KCl, 1.2  $\text{KH}_2\text{PO}_4$ , 11 glucose, 24.8  $\text{NaHCO}_3$ , 2.5  $\text{CaCl}_2$ , and 0.03  $\text{Na}_2$  ethylenediamine tetraacetic acid constantly bubbled with 95%  $\text{O}_2$ –5%  $\text{CO}_2$  to achieve a  $\text{pH}$  of  $7.4$  at  $37 \pm 0.1^\circ\text{C}$ .<sup>7</sup>

**Experimental Procedure.** At the end of the 1-h equilibration, each preparation was activated with NE at 5 nM, 30 nM, or 10  $\mu\text{M}$  until the force development reached a steady state (10–15 min). Enflurane at one of the concentrations (0.9%, or 2.4%, or 5.0%) was then delivered through a vaporizer for 10–15 min, during which time the concentration of enflurane in the tissue bath reached a steady state (fig. 1). This was followed by termination of enflurane for 15–20 min. At the end of each experiment, each arterial ring was examined for the absence of relaxation in response to an equal concentration of acetylcholine. This was done to confirm the absence of the endothelium.

The effect of enflurane on the  $\text{Ca}^{2+}$  influx through the sarcolemma was test by either removal of  $\text{Ca}^{2+}$  from the bathing medium or administration of verapamil, a voltage-gated  $\text{Ca}^{2+}$  channel blocker. Thus, in a second group of arterial rings, the experimental procedure was

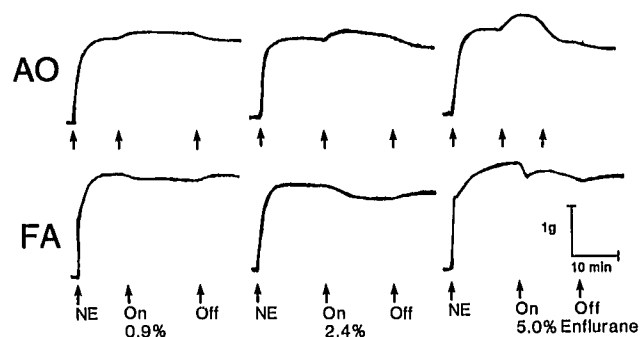


Fig. 1. Tracings showing the effect of enflurane (0.9%, 2.4%, and 5%) on 30 nM norepinephrine (NE)-activated force in isolated arterial rings from the aorta (AO) and the femoral artery (FA). Enflurane dose-dependently increased in AO (top tracings) and decreased in FA (bottom tracings) the NE-activated force. A biphasic response in FA was consistently observed at 5% enflurane (bottom tracings, right).

the same as that of the enflurane dose-response study described above, except that the arterial rings were pretreated with  $1\ \mu\text{M}$  verapamil for 30 min, and then activated by 30 nM NE. As time-controls for the first and second groups, parallel experiments were performed in a separate group of arterial rings without administration of either verapamil or enflurane, or both.

The test result was expressed as a percentage of the mean of the controls (before and after enflurane). This was then normalized with that of the time-control, and compared by Student's *t* test for unpaired data<sup>9</sup> at each enflurane concentration. Analysis of variance (StatView software program) was used to compare arterial types with respect to NE and enflurane concentrations. A  $P < 0.05$  was regarded as statistically significant.

#### Chemically Skinned Arterial Strips

Aortic or femoral arterial rings of 0.25–0.3 mm width were cut and placed in a relaxing solution (7 mM ethyleneglycol-bis- $[\beta$ -aminoethylether]-tetraacetic acid [EGTA] with no added  $\text{Ca}^{2+}$  in the basic bathing solution, as described below). From each aortic ring, a 0.03–0.05-mm-thick intimal and medial layer was dissected. Longitudinal strips of 1.5–2.0 mm were cut from the rings and mounted on two pairs of forceps, with one end attached to a photodiode tension transducer<sup>10</sup> and the other end fixed. The resting tension was adjusted to achieve 50 mg. The strips were then immersed in a skinning solution (relaxing solution containing saponin): aortic strips with 0.5 mg/ml saponin for 7 min,<sup>7</sup> femoral arterial strips with 0.3 mg/ml saponin for 5 min. At the end of the skinning protocol, the strips were immersed in fresh relaxing solution (no saponin) to wash away saponin. The isometric tension of the skinned strip was recorded on a four-channel 2400S Gould recorder equipped with appropriate amplifiers, or on a Quadra 950 (Apple Computer, Cupertino, CA) utilizing a customized Lab View software program (National Instrument, Austin, TX) and interfaced with appropriate hardware.

**Basic Bathing Solution.** The basic bathing solution<sup>7</sup> contained 35 mM  $\text{K}^+$ , 35 mM  $\text{Na}^+$ , 15 mM creatine phosphate, 2 mM Mg adenosine triphosphate, 0.1 mM  $\text{Mg}^{2+}$ , various free  $\text{Ca}^{2+}$  and total EGTA concentrations depending on the specific condition (described below), methanesulfonate (major anion), and 50 mM piperazine- $\text{N,N}'$ -bis-2-ethanesulfonic acid to achieve an ionic strength of 0.15 with a pH of  $7.00 \pm 0.02$  at  $23 \pm 2^\circ\text{C}$ .

**Experimental Procedure.** The skinned strips were first tested with 25 mM caffeine in the relaxing solution

to empty the caffeine-releasable  $\text{Ca}^{2+}$  pool, and then followed with a contracting solution ( $p\text{Ca } 5.0 + 25$  mM caffeine) to test the viability of the contractile proteins. The functionality of the SR was then demonstrated by a load-release cycle consisting of sequential immersions in four solutions (fig. 2). These four solutions contained the ionic concentration described for the basic bathing solution, except that concentrations of EGTA and  $\text{Ca}^{2+}$  varied as described below in parentheses: solution 1 (no added  $\text{Ca}^{2+}$ , 7 mM EGTA) to wash away caffeine from the vascular smooth muscle fibers; solution 2 ( $p\text{Ca } 6.5$ , 7 mM EGTA) to rapidly load  $\text{Ca}^{2+}$  into the SR; solution 3 ( $p\text{Ca } 6.5$ , 0.05 mM EGTA) to reduce EGTA in the muscle fibers; and solution 4 ( $p\text{Ca } 6.5$ , 0.05 mM EGTA, 10 mM caffeine) to induce  $\text{Ca}^{2+}$  release from the SR using 10 mM caffeine, resulting in a tension transient (fig. 2). The area of the tension transient generated with 10 mM caffeine was used as an estimate of the amount of  $\text{Ca}^{2+}$  released from the SR.<sup>11</sup> The duration of immersion in solutions 1–3 was 5 minutes. Immersion in solution 4 continued until the tension transient was completed or had returned to baseline, which occurred within 7 min in both arterial types.

For the study of the effect of enflurane on  $\text{Ca}^{2+}$  uptake or release from the SR, each strip was cycled three times through the above four solutions in the following sequence: a control cycle, a test cycle, followed by another control cycle. In the second cycle (test) enflurane was present either in solutions 1–3 (uptake phase) or solution 4 only (release phase). Results of the test (second cycle) were expressed as a percentage of the mean of the bracketing controls (first and third cycles) by analysis of variance (StatView software program, BrainPower, Calabasas, CA) with respect to enflurane

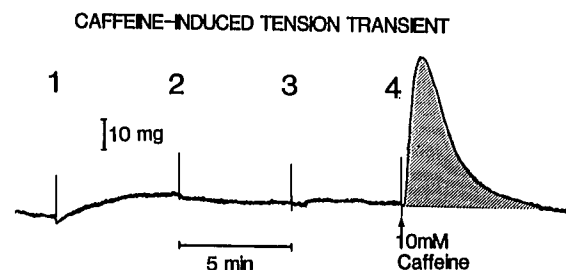


Fig. 2. A typical tracing for a 10 mM caffeine-induced tension transient in skinned aortic strips. 1–4 = solution exchange artifacts. The skinned strips were sequentially immersed in four solutions to load  $\text{Ca}^{2+}$  into (solutions 2 and 3, uptake phase) and release  $\text{Ca}^{2+}$  from (solution 4, release phase) the sarcoplasmic reticulum: a load-release cycle.

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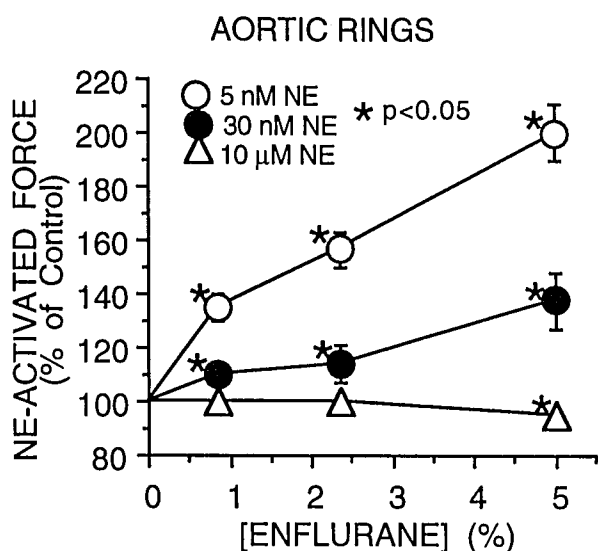


Fig. 3. Dose-response relation of enflurane concentrations (0.9%, 2.4%, and 5%) on 5 nM, 30 nM, and 10  $\mu$ M norepinephrine (NE)-activated force in isolated aortic rings. Each point represents mean  $\pm$  SEM ( $n = 8-21$ ). Enflurane, in a dose-dependent manner, increased submaximum NE-activated force (circles) as an inverse function of the NE concentration. The maximum NE (10  $\mu$ M)-activated force was decreased by 5% enflurane (triangles).

concentrations and arterial types. The slope and  $ED_{50}$  of the dose-response curves were calculated by the Flexifit program.<sup>12</sup> A value of  $P < 0.05$  was considered to be statistically significant.

For the study of the interaction of enflurane and ryanodine-receptor SR  $Ca^{2+}$ -release channels, three cycles were performed in each skinned strip. However, solution 4 of the second (conditioning) cycle contained enflurane and 10 nM ryanodine with or without 10 mM caffeine to activate the channel and allow binding of ryanodine to the receptor,<sup>13</sup> resulting in depression of caffeine-induced tension transient of the third cycle (test cycle).<sup>14,15</sup> Moreover, the duration of immersion in solution 4 was 7 min to ensure that the concentration of ryanodine and caffeine reached a steady state in both arterial types. The test result (third cycle) was compared with that of the control (first cycle) by Student's  $t$  test for paired data.<sup>9</sup> A  $P < 0.05$  was regarded as statistically significant.

Enflurane was delivered through a vaporizer (Ventrol, Ohio Medical Products) with 100%  $N_2$ . The test solutions in 2.5-ml glass vials were saturated with a specific enflurane concentration for at least 30 min. before experimentation.<sup>16</sup> One set of solutions was saturated with enflurane and  $N_2$  as test solutions, and an-

other set of solutions with 100%  $N_2$  as control solutions. The concentrations (partial pressure expressed as a percentage of one atmosphere) of enflurane in the solutions were assayed by gas chromatography.<sup>17</sup>

## Results

### *Effects of Enflurane on Isolated Arterial Rings Activated by Various Norepinephrine Concentrations*

The effect of enflurane on the NE-activated force development was examined at two low concentrations, 5 nM and 30 nM, and at the maximum concentration of 10  $\mu$ M in both arterial types. The NE-activated force development expressed as a percent of the maximum force at 30  $\mu$ M NE (mean  $\pm$  SEM [ $n$ ]) for 5 nM, 30 nM, and 10  $\mu$ M NE, respectively, was  $4.7 \pm 0.8$  (6),  $36.2 \pm 3.2$  (6), and  $100 \pm 0$  (6) for aorta  $14.4 \pm 1.6$  (10),  $54.4 \pm 4.7$  (10), and  $99.3 \pm 0.7$  (10) for femoral artery.

In aortic rings, enflurane dose-dependently increased the 30 nM NE-activated force development. This increase was a direct function of the enflurane concentration (10%, 20%, and 38% increase for 0.9%, 2.4%, and 5.0%, respectively) (figs. 1 and 3). The percent increase was greater with 5 nM NE than with 30 nM NE (5 nM NE vs. 30 nM NE at 35% vs. 10%, 57% vs. 20%, and 100% vs. 38% for 0.9%, 2.4%, and 5% enflurane, respectively, fig. 3) whereas the maximum NE (10  $\mu$ M)-activated force development was significantly decreased (5%) by 5% enflurane (fig. 3). Analysis of variance confirmed that the enflurane-induced increase in force is a function of the enflurane concentration and an inverse function of the NE concentration.

In isolated femoral arterial rings, enflurane at 0.9% and 2.4% dose-dependently decreased (6% and 11%, respectively) the 30 nM NE-activated force development (figs. 1 and 4) with a more prominent action at lower NE concentrations (fig. 4). On the other hand, 5% enflurane caused a decrease in the NE-activated force development as a direct function of the NE concentration (fig. 4). Moreover, 5% enflurane consistently caused a biphasic effect: an initial fast decrease (percentage of control =  $88.6 \pm 4.7$ ,  $n = 12$ ) followed by a slower increase (percentage of control =  $120.6 \pm 5.8$ ,  $n = 8$ ) in the 30 nM NE-activated force development of femoral arterial rings (fig. 1).

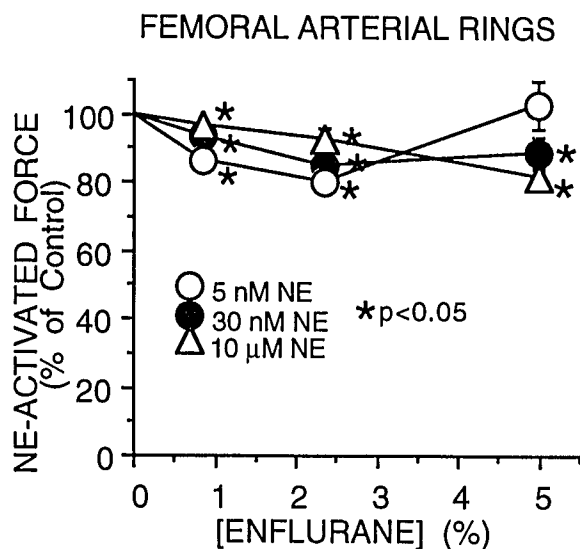


Fig. 4. Dose-response relation of enflurane concentrations (0.9%, 2.4%, and 5%) on 5 nM, 30 nM, and 10  $\mu$ M norepinephrine (NE)-activated force in isolated femoral arterial rings. Each point represents mean  $\pm$  SEM ( $n = 11-16$ ). Enflurane decreased NE-activated force. This depression was an inverse function of NE concentration at 0.9% and 2.4% enflurane and a direct function of NE concentration at 5% enflurane.

#### *Influence of Verapamil on the Enflurane Effect on Isolated Aortic and Femoral Arterial Rings Activated by Submaximum Norepinephrine*

The enflurane-induced decreases in submaximum NE-activated force development in isolated femoral arterial rings (figs. 1, 4, and 5) could be due to decreased  $Ca^{2+}$  entry through the sarcolemma, decreased  $Ca^{2+}$  release from the SR, or direct  $Ca^{2+}$ -independent depression of the contractile proteins, or a combination of these mechanisms. This study was performed to examine the first possibility.

We found that upon removal of  $Ca^{2+}$  in the bathing solution, the submaximum NE-activated force was increased by enflurane in both types of arterial rings. We further examined whether this blockade of  $Ca^{2+}$  influx by enflurane is specifically through the voltage-gated  $Ca^{2+}$  channels using verapamil and found that NE (30 nM)-activated force development was increased by enflurane in isolated femoral arterial rings (figs. 5 and 6). Although the steady-state tension developed by 30 nM NE was decreased in the presence of verapamil, enflurane caused an even greater increase in the NE-activated force development in aortic rings (open symbols, fig. 6). Analysis of variance revealed that the effect is a direct function of the enflurane concentration.

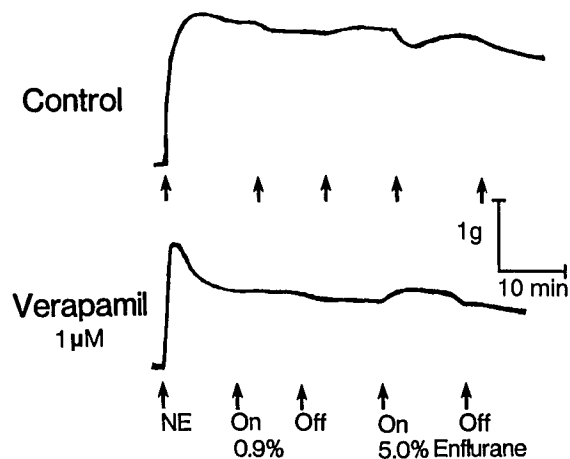


Fig. 5. Enflurane-induced decreases in submaximum NE-activated force development in isolated femoral arterial rings (top tracing). In the presence of 1  $\mu$ M verapamil, enflurane did not decrease but rather increased the 30 nM norepinephrine (NE)-activated force in isolated femoral arterial rings.

#### *Effects of Enflurane on Caffeine-induced Tension Transients in the Skinned Arterial Strips from the Aorta and the Femoral Artery*

Potent inhalation anesthetics, including halothane, enflurane, and isoflurane, have been shown in striated

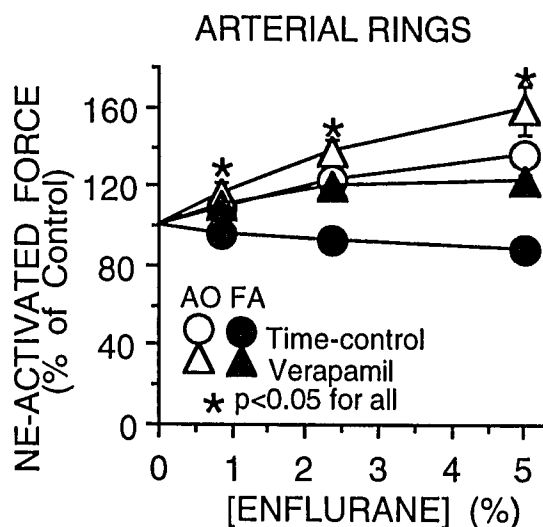


Fig. 6. Dose-response relation of enflurane concentrations on 30 nM norepinephrine (NE)-activated force in isolated arterial rings in the presence of 1  $\mu$ M verapamil. Each point represents mean  $\pm$  SEM ( $n = 7-11$ ). In the presence of verapamil, enflurane increased 30 nM NE-activated force (triangles) compared with that of the time controls (circles) in both the aorta (AO) and the femoral artery (FA).

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muscle to enhance caffeine-induced tension transients when the anesthetic was present in the release phase, and to decrease tension transients when present in the uptake phase in skinned fibers.<sup>18-20</sup> In skinned aortic strips, halothane<sup>7</sup> has also been shown to have qualitatively similar effects to those seen in striated muscle. These observations suggest that inhalation anesthetics have similar mechanisms of action on the SR. This study was done to test the hypothesis that enflurane has similar mechanisms of action on the SR as those of halothane on vascular smooth muscle of different vascular regions, specifically aorta *versus* femoral artery.

We found that enflurane (1-3%), in the release phase increased and in the uptake phase decreased caffeine-induced tension transients in skinned arterial strips from the aorta and the femoral artery (figs. 7 and 8). These effects of enflurane are dose-dependent. Analysis of variance revealed that the aorta is more sensitive than the femoral artery to enflurane's effects (59%-108% *vs.* 10%-55%, respectively, for the release phase, and 28%-77% *vs.* 12%-59%, respectively, for the up-

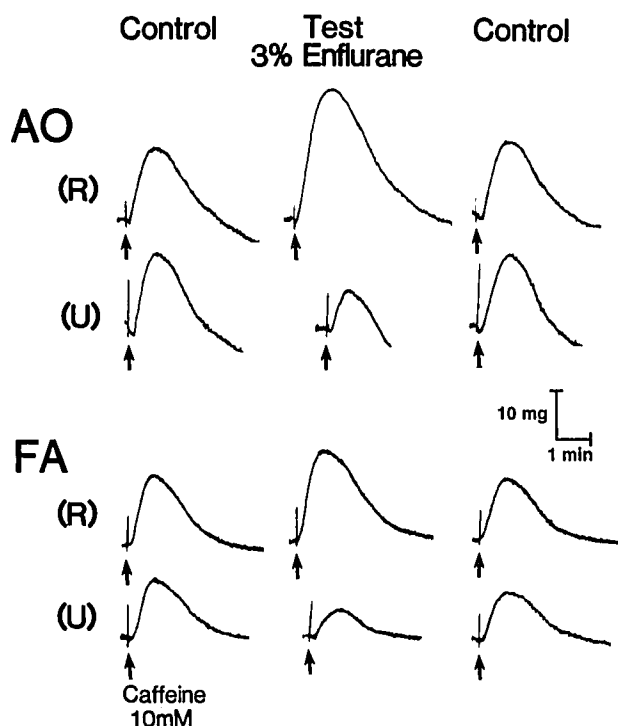


Fig. 7. Tracings showing that (compared with the mean of the two bracketing controls), 3% enflurane (Test) in the release phase (R) increased and in the uptake phase (U) decreased the caffeine-induced tension transients in skinned arterial strips from the aorta (AO) and the femoral artery (FA).

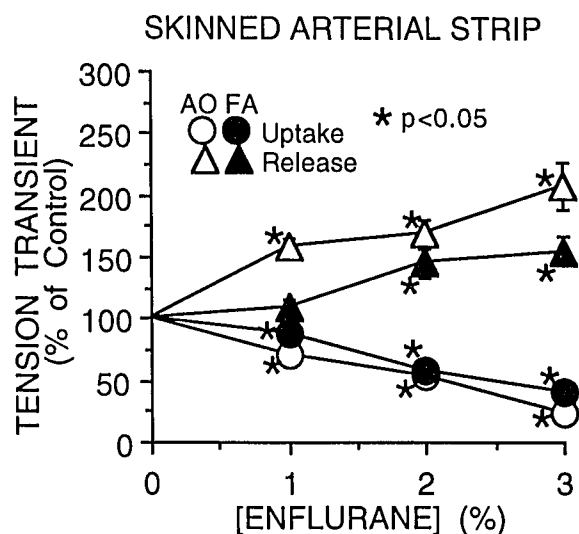


Fig. 8. Dose-response relation of enflurane concentrations (1%, 2%, and 3%) on caffeine-induced tension transients in skinned arterial strips from aorta (AO) and femoral artery (FA). Uptake = enflurane present in the uptake phase (circles); Release = enflurane present in the release phase (triangles). Each point represents mean  $\pm$  SEM ( $n = 5-9$ ). Enflurane in the uptake phase decreased and in the release phase increased the caffeine-induced tension transients of the skinned arterial strips from AO and FA. Analysis of variance revealed that enflurane caused greater effects on AO than on FA.

take phase). However, there was no interaction between arterial types and enflurane concentrations. The concentrations for enflurane in the uptake phase that induced 50% depression of the tension transient were 1.8% enflurane for the aorta and 2.5% enflurane for the femoral artery.

#### Effects of Ryanodine on Enflurane-induced Tension Transient in Skinned Arterial Strips from Aorta and Femoral Artery

Ryanodine, a plant alkaloid, has been shown to bind to a receptor in the terminal cisternae of the transverse-SR system with high specificity and affinity.<sup>21</sup> In striated muscle, the ryanodine-receptor channel plays a major role in excitation-contraction coupling.<sup>22</sup> In our laboratory, we have shown that ryanodine in the conditioning cycle (described in Materials and Methods) depresses caffeine-induced tension transients (ryanodine depression) in the test cycle of  $Ca^{2+}$  load-release cycle of the SR in skinned striated muscle fibers.<sup>1,3-15,23-25</sup> However, in smooth muscle, two SR  $Ca^{2+}$  channels have been identified: a ryanodine-receptor (caffeine as an activator) and an inositol 1,4,5-trisphosphate ( $IP_3$ ) re-

ceptor.<sup>26</sup> We have previously reported that halothane potentiates ryanodine depression of caffeine-induced tension transients,<sup>7</sup> consistent with halothane activation of the ryanodine-receptor channel. This study was undertaken to assess whether the enflurane-induced increase of caffeine-induced tension transients in skinned arterial strips (fig. 8) is also specifically mediated through the ryanodine-receptor channel.

In skinned arterial strips, in addition to having enhanced caffeine-induced tension transients (fig. 8), enflurane also directly caused tension transients with an elevated force development at the steady state (data not shown). However, these enflurane-induced tension transients decayed rapidly with time so that quantification of the data was less useful. Furthermore, ryanodine (lot 93691, Calbiochem, San Diego, CA) as high as 10  $\mu\text{M}$  was required to show significant depression of the enflurane-tension transient (data not shown). On the other hand, using 10 mM caffeine as an activator of the ryanodine-receptor SR  $\text{Ca}^{2+}$ -release channel, the 10 nM ryanodine depression (mean  $\pm$  SEM [n]) of the caffeine-induced tension transient *versus* the time control was 12% ( $83.1 \pm 5.4$  [9] *vs.* for  $93.9 \pm 1.8$  [8]) for the aorta, and 22% ( $60.8 \pm 4.3$  [14] *vs.* for  $77.8 \pm 6.1$  [8]) for the femoral artery. When the combination of enflurane and caffeine (*vs.* caffeine alone) was used to activate the ryanodine-receptor channels in the conditioning cycle (fig. 9), the ryanodine depression of the test cycle was potentiated to 59% depression ( $30.4 \pm 4.0$  [12] *vs.*  $74.6 \pm 3.5$  [10]) for the aorta, and 75% depression ( $19.1 \pm 1.4$  [15] *vs.*  $75.8 \pm 4.1$  [11]) for the femoral artery (fig. 9).

## Discussion

We found, first, that enflurane causes opposite effects in isolated arterial rings: the submaximum NE-activated force is increased in the aorta, and decreased (except at 5% enflurane, which produced a biphasic effect; initial decrease followed by increase) in the femoral artery. The enflurane effect decreases with increasing NE concentrations tested (5 nM, 30 nM, and 10  $\mu\text{M}$ ).

Second, enflurane, in a dose-dependent manner, has similar cellular mechanisms of action on the aorta and the femoral artery. In skinned arterial strips, enflurane in the release phase enhances, and in the uptake phase decreases, caffeine-induced tension transients. Ryanodine depression of caffeine-induced tension transient is potentiated by enflurane and at high ryanodine concentration, the enflurane-induced tension transients is

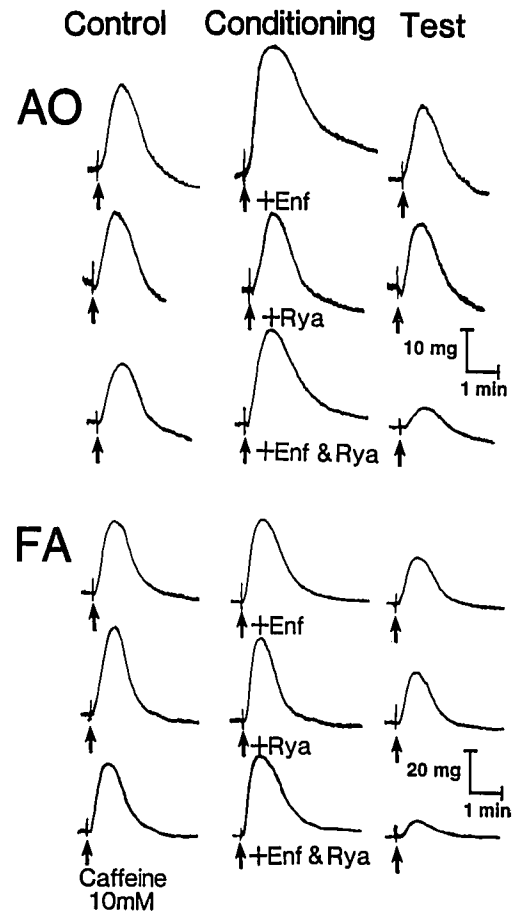


Fig. 9. Tracings showing that enflurane potentiated ryanodine-induced depression of caffeine-induced tension transients of the test cycle (third column, third row) (Test) in skinned arterial strips from the aorta (AO) and the femoral artery (FA). Enf = enflurane at 5% for AO and 3% for FA; Rya = ryanodine at 10 nM. n = 8–14 preparations.

decreased. In addition, whereas the force response of isolated arterial rings to 30 nM NE is depressed normally by enflurane, the attenuated force response in the presence of verapamil or in the absence of  $\text{Ca}^{2+}$  is actually enhanced by enflurane.

The differential response of isolated aortic and femoral arterial rings to enflurane found in this study has also been reported with halothane.<sup>2</sup> However, the increased submaximum NE-activated force development in the rabbit aorta observed in this study is in contrast to the vasodilatory effect shown in isolated, denuded rat aorta.<sup>27</sup> This difference may be due to the difference in animal species.

The enflurane-induced increase in caffeine-induced tension transients of skinned arterial strips is also ob-

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served in striated muscle,<sup>19</sup> reflecting, at least in part, an increase in  $\text{Ca}^{2+}$  release from the SR. Moreover, this enflurane effect is qualitatively similar to that of halothane and isoflurane observed in skinned striated muscle fibers<sup>18,20</sup> and skinned aortic strips.<sup>7</sup> The enflurane-induced decreases in  $\text{Ca}^{2+}$  uptake by the SR may be due to the enhanced  $\text{Ca}^{2+}$  release from the SR, resulting in less  $\text{Ca}^{2+}$  accumulation in the SR. A parallel shift between the aorta and the femoral artery in the relation of  $\text{Ca}^{2+}$  release (release phase) and  $\text{Ca}^{2+}$  uptake (uptake phase) with respect to enflurane concentrations agrees with this speculation. On the other hand, it also is possible that the decreased SR  $\text{Ca}^{2+}$  uptake by enflurane is due in part to decreased SR  $\text{Ca}^{2+}$ -adenosine triphosphatase activity, although evidence for actual enhancement of activity by enflurane has been observed in striated muscle.<sup>28,29</sup> This possibility remains to be confirmed. These effects on the SR could not be attributed to a direct effect on the contractile proteins, because neither enflurane nor halothane<sup>7</sup> affected  $\text{Ca}^{2+}$ -activated force development in the skinned strips of both arterial types (data not shown).

The enflurane-induced tension transient or enhanced caffeine-induced tension transient indicate that enflurane directly or indirectly activates the SR  $\text{Ca}^{2+}$ -release channels in the vascular smooth muscle. Ryanodine has been shown to bind specifically to a receptor in isolated SR of striated muscle<sup>21</sup> (ryanodine-receptor  $\text{Ca}^{2+}$ -release channel) in correlation with the depression of caffeine-induced tension transients in skinned fibers.<sup>13-15,23-25</sup> The enflurane activation of the channel also blocked by ryanodine suggests that enflurane activates the ryanodine-receptor SR  $\text{Ca}^{2+}$ -release channels. Whether enflurane acts directly or indirectly on the ryanodine-receptor  $\text{Ca}^{2+}$ -release channel remains to be examined. It is clear, however, that enflurane is a weak activator of the channel in the vascular smooth muscle, because the ryanodine concentration required to block enflurane-induced tension transients is about 1,000-fold greater than that required to block caffeine-induced tension transients.<sup>24</sup>

The mechanisms of NE activation of alpha-adrenergic receptor resulting in contraction of vascular smooth muscle are not totally clear. NE effect most probably is mediated through stimulation of phospholipase C- $\text{IP}_3$  and diacylglycerol pathway by releasing  $\text{Ca}^{2+}$  from the SR and  $\text{Ca}^{2+}$ -independent activation of the contractile proteins (for a review see ref. 3). Smooth muscle has been shown to contain both ryanodine-receptor and  $\text{IP}_3$ -receptor channels. Iino and associates<sup>26</sup> have shown

in smooth muscle that there are two separate SR  $\text{Ca}^{2+}$  stores: one releasable by both caffeine and  $\text{IP}_3$  and the other releasable by  $\text{IP}_3$  alone. Thus, the release of  $\text{Ca}^{2+}$  through one of the channels would subsequently affect the release *via* the other channel. Whether two  $\text{Ca}^{2+}$  pools also exist in the rabbit aorta and femoral artery is not clear. It is, however, possible that enflurane in part induces  $\text{Ca}^{2+}$  release through the  $\text{IP}_3$ -receptor channels. If enflurane induces  $\text{Ca}^{2+}$  release from the SR through both ryanodine- and  $\text{IP}_3$ -receptor channels, then the greater enhancement of caffeine-induced tension transients by enflurane in the aorta may result from a larger  $\text{IP}_3$ -releasable store. We have observed that in skinned aortic strips, inositol 2,4,5-trisphosphate (a synthetic  $\text{IP}_3$ ) induces larger tension transients than does caffeine (fig. 10), consistent with the larger SR content (for a review, see ref. 6). On the other hand, caffeine- and  $\text{IP}_3$ -induced tension transients in the skinned femoral arterial strips are approximately equal (fig. 10). This would suggest that the femoral artery has a smaller SR content and that enflurane causes less  $\text{Ca}^{2+}$  release from the SR in the femoral artery than the aorta. Moreover, we have observed that enflurane also enhanced NE- or  $\text{IP}_3$ -induced tension transients in skinned strips (data not shown). Whether this evidence indicates that enflurane exerts a nonspecific effect on both ryanodine- and  $\text{IP}_3$ -receptor channels remains to be examined.

Though the exact mechanisms of enflurane-induced SR  $\text{Ca}^{2+}$  release are not clear, the enflurane-induced increases in NE-activated force in isolated aortic rings

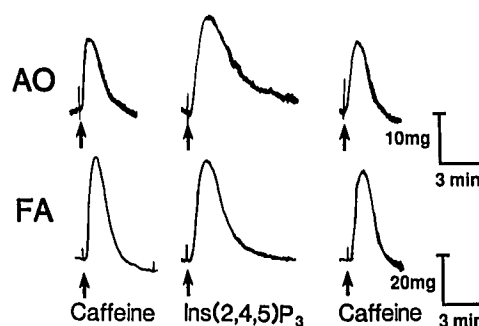


Fig. 10. Tracings showing a comparison of tension transients induced by 10 mM caffeine *versus* 10  $\mu\text{M}$  inositol 2,4,5-trisphosphate (a nonhydrolyzable synthetic inositol 1,4,5-trisphosphate) in skinned aortic (AO) and femoral arterial (FA) strips. The tension transient induced by inositol 2,4,5-trisphosphate is about threefold (mean  $\pm$  SEM [n], 315.2%  $\pm$  48.3 [8]) in AO (middle column, *top*) and about 1.5-fold (150.5%  $\pm$  11.4 [13]) in FA (middle column, *bottom*) of the caffeine-induced tension transients (first and third columns).



or after verapamil treatment in isolated femoral arterial rings are consistent, not only qualitatively but quantitatively, with the enflurane-induced  $\text{Ca}^{2+}$  release from the SR. A greater amount of  $\text{Ca}^{2+}$  release from the SR by enflurane in skinned aortic *versus* femoral arterial strips agrees with a direct increase in NE-activated force in isolated aortic rings and a smaller increase or biphasic response (initial decrease followed by increase) in isolated femoral arterial rings. This enhancement of NE-activated force in the isolated arterial rings by enflurane was further increased after treatment with the  $\text{Ca}^{2+}$  channel blocker verapamil, suggesting that enflurane acts similarly on both arterial types: blocking the voltage-operated  $\text{Ca}^{2+}$  channels in the sarcolemma and inducing  $\text{Ca}^{2+}$  release from the SR. However, the elevated baseline of the enflurane-induced tension transient suggests a direct activation of the contractile proteins by enflurane. This may also in part contribute to the enflurane-induced increase in NE-activated force development after blockade of  $\text{Ca}^{2+}$  channels in isolated arterial rings.

The influence of verapamil on the enflurane effect on isolated rings activated by NE suggests that enflurane directly blocks sarcolemmal  $\text{Ca}^{2+}$  entry in both arterial types. In voltage-clamped vascular muscle cells from the canine coronary artery, Buljubasic and associates have shown<sup>30</sup> that halothane, to a greater degree than isoflurane, reduces the  $\text{Ca}^{2+}$  inward current. Whether enflurane also has the same effect remains to be examined. The absence of a biphasic response in isolated aortic rings is consistent with a greater sensitivity of skinned strips to enflurane-induced increases in caffeine-induced tension transients, which may mask the  $\text{Ca}^{2+}$  channel-blocking effect of enflurane. However, it is also possible that there is a differential importance of  $\text{Ca}^{2+}$  influx *versus* SR  $\text{Ca}^{2+}$  for contraction in the aorta and femoral artery. The femoral artery depends more on  $\text{Ca}^{2+}$  influx than SR  $\text{Ca}^{2+}$  for initiation of muscle contraction, and thus an enflurane-induced blockade of  $\text{Ca}^{2+}$  influx agrees with the observed relaxation in isolated femoral arterial rings. On the other hand, because the aorta depends more on SR  $\text{Ca}^{2+}$  than on  $\text{Ca}^{2+}$  influx, the enflurane-induced  $\text{Ca}^{2+}$  release from the SR is then consistent with the observed enflurane-induced increase in NE-activated force in isolated aortic rings.

To summarize, in isolated arterial rings, enflurane enhances submaximum NE-activated force development in the aorta, and decreases that of force development in the femoral artery. Upon treatment with verapamil, submaximum NE-activated force develop-

ment is further enhanced in both arterial types, and at the same time the initial relaxation of the force in femoral artery is abolished. Enflurane causes  $\text{Ca}^{2+}$  release from the SR, demonstrated by the enhancement of caffeine-,  $\text{IP}_3$ -, and NE ( $\text{IP}_3$ -mediated)-induced tension transients in skinned strips from both arterial types. The enflurane-induced  $\text{Ca}^{2+}$  release from the SR is at least partially mediated by the ryanodine-receptor channel. It is concluded that enflurane has similar mechanisms of action on the aorta and the femoral artery: inducing  $\text{Ca}^{2+}$  release from the SR and blocking  $\text{Ca}^{2+}$  entry, possibly through voltage-gated  $\text{Ca}^{2+}$  channels on the sarcolemma. The enflurane-induced relaxation of the femoral artery and contraction of the aorta is due to either or a combination of (1) a lesser degree of enflurane-induced  $\text{Ca}^{2+}$  release from the SR in the femoral artery than in the aorta or (2) a differential importance of  $\text{Ca}^{2+}$  influx (femoral artery) and  $\text{Ca}^{2+}$  release from the SR (aorta) for muscle contraction in these two arterial types.

## References

1. Longnecker DE, Harris PD: Microcirculatory actions of general anesthetics. *Fed Proc* 39:1580-1583, 1980
2. Muldoon SM, Hart JL, Bowen KA, Freas W: Attenuation of endothelium-mediated vasodilation by halothane. *ANESTHESIOLOGY* 68:31-37, 1988
3. Missiaen L, De Smedt H, Droogmans G, Himpens B, Casteels R: Calcium ion homeostasis in smooth muscle. *Pharmacol Ther* 56:191-231, 1992
4. Hallam TJ, Rink TJ: Receptor-mediated Ca entry: Diversity of function and mechanism. *Trends Pharmacol Sci* 10:8-10, 1989
5. Cauvin C, Saida K, Van Breemen C: Extracellular  $\text{Ca}^{2+}$ -dependent and diltiazem inhibition of contraction in rabbit conduit arteries and mesenteric resistance vessels. *Blood Vessels* 21:23-31, 1984
6. Somlyo AP: Excitation-contraction coupling and ultrastructure of smooth muscle. *Circ Res* 57:497-507, 1985
7. Su JY, Zhang CC: Intracellular mechanisms of halothane's effect on isolated aortic strips of the rabbit. *ANESTHESIOLOGY* 71:409-417, 1989
8. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373-376, 1980
9. Snedecor GW, Cochran WG: *Statistical Methods*. Ames, Iowa University Press, 1967, pp 59-62, 93-99
10. Hellam DC, Podolsky RJ: Force measurements in skinned muscle fibers. *J Physiol (Lond)* 200:807-819, 1969
11. Endo, M: Mechanism of action of caffeine on the sarcoplasmic reticulum of skeletal muscle. *Proc Jpn Acad* 51:467-472, 1975
12. Guardabasso V, Munson PJ, Rodbard D: A versatile method for simultaneous analysis of families of curves. *FASEB J* 2:209-215, 1988
13. Su JY: Mechanisms of ryanodine-induced depression of caffeine-induced tension transients in skinned striated rabbit muscle fibers. *Plügers Arch* 411:371-377, 1988

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14. Su JY: Effects of ryanodine on skinned skeletal muscle fibers of the rabbit. *Pflügers Arch* 410:510-516, 1987
15. Su JY: Effects of ryanodine on skinned myocardial fibers of the rabbit. *Pflügers Arch* 411:132-136, 1988
16. Su JY, Kerrick WGL: Effects of halothane on  $\text{Ca}^{2+}$ -activated tension development in mechanically disrupted rabbit myocardial fibers. *Pflügers Arch* 375:111-117, 1978
17. Fink BR, Morikawa K: A simplified method for the measurement of volatile anesthetics in blood by gas chromatography. *ANESTHESIOLOGY* 32:451-455, 1970
18. Su JY, Kerrick WGL: Effects of halothane on caffeine-induced tension transients in functionally skinned myocardial fibers. *Pflügers Arch* 380:29-34, 1979
19. Su JY, Kerrick WGL: Effects of enflurane on functionally skinned myocardial fibers from rabbits. *ANESTHESIOLOGY* 52:385-389, 1980
20. Su JY, Bell JG: Intracellular mechanism of action of isoflurane and halothane on striated muscle of the rabbit. *Anesth Analg* 65:457-462, 1986
21. Pessah IN, Francini AO, Scales DJ, Waterhouse AL, Casida JE: Calcium ryanodine-receptor complex. *J Biol Chem* 261:8643-8648, 1986
22. McPherson PS, Campbell KP: The ryanodine receptor/ $\text{Ca}^{2+}$  release channel. *J Biol Chem* 268:13765-13768, 1993
23. Su JY: Influence of caffeine,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  on ryanodine depression of the tension transient in skinned myocardial fibers of the rabbit. *Pflügers Arch* 421:1-6, 1992
24. Su JY: Effects of ryanodine on SR  $\text{Ca}^{2+}$  release channels demonstrated with caffeine-induced tension transients in skinned striated and vascular smooth muscle. *Adv Exp Med Biol* 311:439-443, 1992
25. Su JY, Chang YI: Modulation of sarcoplasmic reticulum  $\text{Ca}^{2+}$ -release channels by caffeine,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  in skinned myocardial fibers of fetal and adult rats. *Pflügers Arch* 423:300-306, 1993
26. Iino M, Kobayashi T, Endo M: Use of ryanodine for functional removal of the calcium store in smooth muscle cells of the guinea pig. *Biochem Biophys Res Commun* 152:417-422, 1988
27. Stone DJ, Johns RA: Endothelium-dependent effects of halothane, enflurane, and isoflurane on isolated rat aortic vascular rings. *ANESTHESIOLOGY* 71:126-132, 1989
28. Blanck TJJ, Peterson CV, Baroody B, Tegazzin V, Lou J: Halothane, enflurane, and isoflurane stimulate calcium leakage from rabbit sarcoplasmic reticulum. *ANESTHESIOLOGY* 76:813-821, 1992
29. Miao N, Frazer MJ, Lynch C III: Volatile anesthetic actions on cardiac sarcoplasmic reticulum Ca-ATPase (abstract). *ANESTHESIOLOGY* 77:A631, 1992
30. Buljubasic N, Rusch N, Marijic J, Kampine JP, Bosnjak ZJ: Effect of halothane and isoflurane on calcium and potassium channel currents in canine coronary arterial cells. *ANESTHESIOLOGY* 76:990-998, 1992