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## Analysis of Halothane Effects on Myocardial Force-Interval Relationships at Anesthetic Concentrations Depressing Twitches but Not Tetanic Contractions

Stephen M. Vogel, Ph.D.,\* Guy L. Weinberg, M.D.,† Andreja Djokovic, B.S., David J. Miletich, Ph.D.,‡  
Ronald F. Albrecht, M.D.§

**Background:** Tetanic contractions in rat myocardium depend solely on cellular  $\text{Ca}^{2+}$  uptake, whereas twitches depend on  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum. Because halothane may cause loss of sequestered  $\text{Ca}^{2+}$ , the anesthetic was tested for its differential effects on twitch and tetanic forces. The *in vitro* effects of halothane on the twitch force-interval relationship were then evaluated, using a mathematical model that relates twitch contractile force to the  $\text{Ca}^{2+}$  content of intracellular compartments.

**Methods:** Isometric contractile force was measured in paced (0.4 Hz) rat atrial preparations. The sarcoplasmic reticulum was functionally eliminated using ryanodine ( $10^{-6}$  M), abolishing twitches. Rapid pacing (20 Hz, 10 s) caused tetanic contractions. The effects of identical halothane exposures on twitches and tetanic contractions were compared.  $\text{Ca}^{2+}$  compartment model parameters were extracted from twitch force-interval data, according to a previously employed quantitative procedure.

**Results:** Halothane (0.5–1%) depressed normal twitches, but not tetanic contractions. The anesthetic decreased the amplitude of the steady-state twitch force-frequency relationship, and accelerated the course of mechanical recovery. Halothane (0.5–1%) also accelerated the decay constant for the decline in amplitude of a series of rest-potentiated contractions. The modeling showed that a 20–30% decrease in the recirculating fraction of activator  $\text{Ca}^{2+}$  accounts for 0.5% halothane-induced negative inotropy and acceleration of the decay constant.

**Conclusions:** The differential effect of halothane on twitches and tetanic contractions implies that a functioning sarco-

plasmic reticulum is required for halothane-induced negative inotropy. The effects of halothane on the force-interval relationship suggest that halothane reduces the sequestered pool of activator  $\text{Ca}^{2+}$ .

ANESTHETIC concentrations of halothane depress cardiac output and mean arterial blood pressure.<sup>1</sup> Numerous studies indicate that, at sufficient concentrations, halothane affects virtually every step in the sequence of excitation-contraction coupling, including: (A)  $\text{Ca}^{2+}$  channel function measured either electrophysiologically or biochemically<sup>2–5</sup>; (B) the storage or release of sequestered  $\text{Ca}^{2+}$  and resulting intracellular, free  $\text{Ca}^{2+}$  transients<sup>6,7</sup>; and (C) the response of the contractile proteins to intracellular  $\text{Ca}^{2+}$ .<sup>8–10</sup> However, low halothane concentrations (0.5%) selectively depress contractility without affecting either normal or calcium-mediated action potentials.<sup>11</sup> Halothane apparently causes loss of sequestered  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum.<sup>12,13</sup>

The current study assesses whether halothane's myocardial depressant action is due largely to effects on the sarcoplasmic reticulum. Isolated rat atrium was chosen as the experimental material because of its sensitivity to  $\text{Ca}^{2+}$  and because of similarities between its behavior and that of the isolated human atrium. These similarities include the biphasic time course of mechanical recovery (restitution) seen in the normal human myocardium,<sup>14</sup> and the negative force-frequency relationship observed in the human myocardium at high extracellular  $\text{Ca}^{2+}$  concentrations<sup>15</sup> or in heart failure.<sup>16</sup> The rat atrium thus is a potentially useful model for the human case. Twitch contractions in the rat myocardium depend on release of sequestered  $\text{Ca}^{2+}$ , whereas tetanic contractions (in tissues chemically modified to deplete the pool of sequestered  $\text{Ca}^{2+}$ ) rely solely on cellular  $\text{Ca}^{2+}$  uptake. We therefore compared in isolated rat left atria, the effects of relatively low halothane concentrations on twitches and tetanic con-

\* Assistant Professor, Departments of Anesthesiology and Pharmacology.

† Assistant Professor, Department of Anesthesiology.

‡ Associate Professor, Department of Anesthesiology.

§ Professor and Head, Department of Anesthesiology.

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Address correspondence to: Dr. Vogel, Department of Anesthesiology, University of Illinois at Chicago, 1740 West Taylor Street, Chicago, Illinois 60612.



tractions. We also analyzed the force-interval relationship, and halothane's influence on it, with the aid of a mathematical model<sup>17</sup> that relates twitch contractile force to the internal  $\text{Ca}^{2+}$  compartments of the cardiomyocyte.

## Materials and Methods

### *Measurement of Contractile Force in Isolated Atria*

With approval of the University of Illinois College of Medicine Animal Care Committee, adult Sprague-Dawley rats were anesthetized using halothane, and the hearts were rapidly excised. Blood was removed by brief aortic perfusion and isolated left atria were then dissected from the perfusing hearts. The ends of the preparation were attached between a force-displacement transducer (Grass FT 03, Grass Instruments, Quincy, MA) and a fixed point by means of stainless-steel hooks. The muscles were immersed in a water-jacketed glass chamber (volume, 100 ml) containing heated ( $33^{\circ}\text{C}$ ), gassed (100%  $\text{O}_2$ ) Krebs-Henseleit solution (see later). After a 30-min equilibration period, the resting force of each muscle was adjusted to give a twitch of half the maximal amplitude. The muscles were stimulated at a frequency of 0.4 Hz by means of rectangular current pulses (duration of 0.2 ms and amplitude of 1.2 times threshold) delivered *via* a pair of platinum-plate electrodes. Contractions were recorded on a Grass Model 7 polygraph, digitized (Labmaster board; Scientific Solutions, Inc., Solon, OH), and simultaneously displayed on the video monitor of a computer (IBM personal computer AT). On-line, automated measurements of the peak amplitude of twitches and tetanic contractions (see below) were made, and when desired, the measured values were stored in a file for later analysis.

### *Tetanic Contractions with Ryanodine*

Tetanic contractions are elicited in heart muscle by rapid pacing under experimental conditions in which the sarcoplasmic reticulum is functionally removed; the only  $\text{Ca}^{2+}$  activating contraction comes from influx. The atria were treated with ryanodine ( $1.0\ \mu\text{M}$ ), a selective inhibitor of the sarcoplasmic reticulum with high affinity for the calcium-release channel. During pacing at 0.4 Hz, ryanodine rapidly abolished the normal twitch contractions. Tetanic contractions were then elicited with brief trains (10 s) of electric stimuli at a

frequency of 20 Hz (5-ms pulse duration). The external  $[\text{Ca}^{2+}]$  was raised to 10 mM to increase the tetanic contractile force to quantifiable levels. Tetanic contractions were elicited at 1/min until a stable baseline value was established. Then, halothane (0.5%–1%) was administered, and its effect on the peak tetanic force was measured.

### *Force-Frequency Relationship*

In the absence of halothane, data were obtained by increasing the stimulation frequency in steps (0.2, 0.4, 0.8, 1.6, and 3.2 Hz) and measuring the peak twitch contractile force at steady-state. The identical protocol was repeated in each preparation in the presence of halothane after an initial exposure of 20 min to the anesthetic agent.

### *Force-Interval Relationship (Mechanical Recovery) and Its Analysis with a Calcium Compartment Model*

Mechanical recovery refers to the minimum rest period after a twitch required for the restoration of contractile force. The time course of mechanical recovery was established as follows. The atria were first paced at a basic cycle length of 2.5 s. Next, a single test stimulus was interposed at a time interval ( $\Delta t$ ) of 0.15–64 s with respect to the preceding regular beat. Thirty regular stimuli (at 2.5 s) were then delivered to reestablish a baseline value, after which the procedure was repeated with a new test interval. In rat atria, if the test interval is shorter than 2.5 s, twitch amplitude is smaller and if the interval is greater than 2.5 s, twitch amplitude is larger, ultimately becoming about 100% greater than the steady-state force at 0.4 Hz. After collecting the control data, halothane (0.5 to 1%) was applied and the above protocol was repeated. A single halothane concentration was tested in each atrium. The amplitude of the test contraction was plotted *versus* log ( $\Delta t$ ) to construct the mechanical recovery curve.

Mechanical recovery in the rat myocardium exhibits distinct fast ( $\alpha$ ) and slow ( $\beta$ ) phases that are well described by summed exponentials. The rate constant and relative amplitude of each exponential component was determined from the raw data in two steps. After semilogarithmic plotting to linearize the data, a curve peeling procedure involving a linear regression analysis was performed to determine the rate constant and relative amplitude of each exponential component.

Additional experiments were performed to determine the effects of conditioning extrasystoles on the me-



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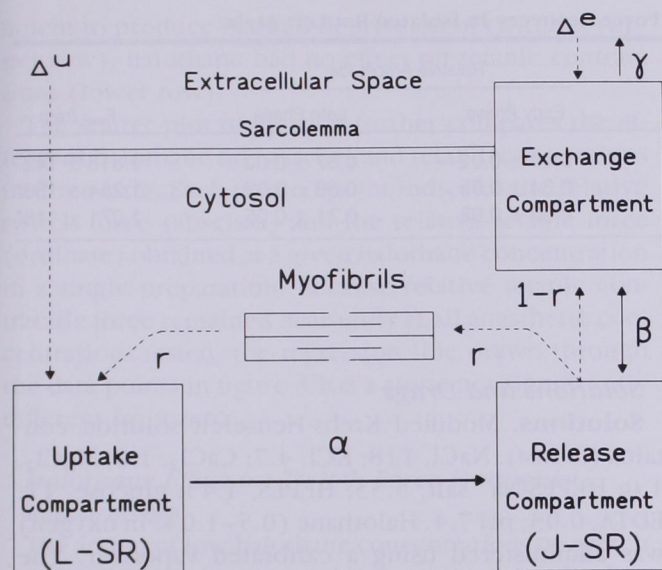


Fig. 1. Compartmental structure of the cardiomyocyte assumed for theoretical calculations. Twitch contractile force results from activation of the myofibrils by  $\text{Ca}^{2+}$ . The action potential functions, in the model, to release the entire  $\text{Ca}^{2+}$  content of the release compartment into the sarcoplasm and to provide a quantity of influxing  $\text{Ca}^{2+}$ ,  $\Delta^u$ . A fraction,  $r$ , of the released  $\text{Ca}^{2+}$  is sequestered in the uptake compartment together with  $\Delta^u$ ; the remaining fraction of the released  $\text{Ca}^{2+}$  (i.e.,  $1-r$ ) enters the exchange compartment. Stimulus-dependent  $\text{Ca}^{2+}$  uptake ( $\Delta^e$ ), which is distinct from the  $\text{Ca}^{2+}$  current ( $\Delta^u$ ) and quantitatively much smaller, increases the  $\text{Ca}^{2+}$  content of the exchange compartment. During the diastolic interval,  $\text{Ca}^{2+}$  is transferred from the uptake to the release compartment at a constant rate ( $\alpha$ ). Additional  $\text{Ca}^{2+}$  is transferred, with slower kinetics ( $\beta$ ), from the exchange to the release compartment or vice versa. The rate constant,  $\gamma$ , drains the exchange compartment ( $\gamma \ll \beta$ ). Events associated with systole (excitation,  $\text{Ca}^{2+}$  influx,  $\text{Ca}^{2+}$  release, and  $\text{Ca}^{2+}$  recirculation) are assumed instantaneous (denoted by dashed arrows) on the time scale of diastolic  $\text{Ca}^{2+}$  translocation (solid arrows =  $\alpha$ ,  $\beta$ , and  $\gamma$ -processes). The intracellular structures thought to correspond to the uptake and release compartments are, respectively, the longitudinal (L-SR) and junctional sarcoplasmic reticulum (J-SR); the exchange compartment is a functional compartment corresponding to a  $\text{Ca}^{2+}$  pool that is regulated by  $\text{Na}^+/\text{Ca}^{2+}$  exchange. Modified from figure 1 in Schouten *et al.*<sup>17</sup>

chanical restitution function. Groups of 200 extrasystoles elicited by 20-Hz stimulation for 10 s were followed by a variable pause (0.1–51.2 s) and a test beat. A 1-min rest period was then allowed, and the protocol was repeated with a new test interval.

Schouten *et al.*<sup>17</sup> derived equations that describe peak force of contraction as a function of stimulus interval and stimulus number in terms of three intracellular calcium compartments. The model includes the conventional uptake and release compartments of the sarcoplasmic reticulum, and recirculation of a fraction

( $r$ ) of activator  $\text{Ca}^{2+}$ ; the third compartment is functional, corresponding to a  $\text{Ca}^{2+}$  pool regulated by  $\text{Na}^+/\text{Ca}^{2+}$  exchange. Mechanical recovery reflects the diastolic replenishment of the release compartment, as  $\text{Ca}^{2+}$  is transferred from the uptake (early phase) and exchange compartments (late phase). The relationships among the compartments are illustrated in figure 1.

The model equations<sup>17</sup> calculate the  $\text{Ca}^{2+}$  content of the release compartment, which is assumed proportional to twitch contractile force. A  $\text{Ca}^{2+}$  content of 1.0 corresponds to maximal force ( $F_{\text{max}}$ ). A theoretical mechanical recovery curve was generated using equation 10 in Schouten *et al.*<sup>17</sup> This equation describes twitch amplitude when a time,  $\Delta t$ , has elapsed since a series of priming beats (0.4 Hz), sufficient in number to establish a steady-state of twitch contractile force. The software employed to solve equation 10 was developed in this laboratory, and checked by reproducing the modeling reported in Schouten *et al.*,<sup>17</sup> using the model parameters given in their table 1.

The six model parameters required were estimated from our experimental data (fig. 1). The  $\alpha$  and  $\beta$  rates were obtained from kinetic analysis of mechanical recovery curves (table 1). The rate constant,  $\gamma$ , was arbitrarily assigned a small and constant value because the late phase of restitution in rat atrial muscle fails to exhibit an appreciable declining phase at long test intervals ( $>100$  s). The  $r$  was approximated by the observed decay constant of potentiated beats (see later). Parameter  $\Delta^u$  acts as a scaling factor for the early phase of mechanical recovery; the  $\Delta^e$  parameter functions, in the model, to independently scale the late phase (fig. 5). In rat atria, parameter  $\Delta^u$  is extremely small (cf., however, rat ventricular trabeculae<sup>17</sup>), and the amplitude of the early phase of the recovery is effectively determined by parameter  $r$ . Maximal force,  $F_{\text{max}}$ , was measured by raising the bath  $[\text{Ca}^{2+}]$ , in two steps, to 9 and 13 mM. These  $\text{Ca}^{2+}$  concentrations were shown in preliminary experiments to yield maximal twitch amplitudes both in the absence and presence of halothane.

#### Post-rest Potentiation

The rat myocardium exhibits marked rest-potentiation, a property allowing estimation of the  $r$ . The first beat elicited after a long pause (e.g., 0.5–3 min) is potentiated compared to the preceding steady-state contractions, because the sarcoplasmic reticulum accumulates extra  $\text{Ca}^{2+}$  during the rest interval. Because of the recirculation of a constant fraction of activator



Table 1. Influence of Halothane on Various Parameters of Twitch Force Recovery in Isolated Rat Left Atria

	$\alpha$ (s <sup>-1</sup> )	$\beta$ (s <sup>-1</sup> )	Relative Amplitude		F <sub>max</sub> (mg)
			Early Phase	Late Phase	
Control (n = 10)	6.2 ± 0.3	0.096 ± 0.007	0.44 ± 0.02	0.57 ± 0.02	1,818 ± 142
Halothane (0.5%) (n = 6)	8.3 ± 1.3*	0.094 ± 0.012	0.34 ± 0.05	0.66 ± 0.02	1,234 ± 138*
Halothane (1.0%) (n = 6)	10.4 ± 1.4*	0.097 ± 0.009	0.32 ± 0.08	0.71 ± 0.05	1,071 ± 115*

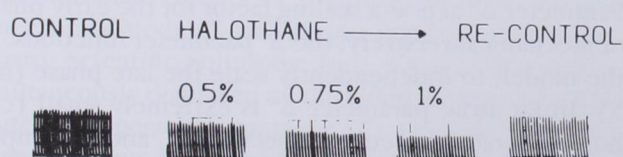
Values are mean ± SEM.

\*  $P < 0.05$  versus control.

Ca<sup>2+</sup>, subsequent beats are also potentiated, but progressively less so until a steady-state is reestablished.

In the experiments conducted, the atria were initially paced at 3 Hz and then allowed a 2-min rest period. Next, stimulation (at 3 Hz) was resumed. The recirculating fraction was estimated from the envelope of potentiated beats, which decays exponentially.<sup>18</sup> The amplitude of the  $n^{\text{th}}$  twitch ( $F_n$ ) was plotted against that of the following twitch ( $F_{n+1}$ ). The slope of the regression line fitted to the raw data gives the decay coefficient,  $D$ , i.e.,  $F_{n+1} = D \cdot F_n + C$ , where  $C$  is a constant<sup>17</sup> (cf. Riou *et al.*<sup>19</sup>). The experimental pacing frequency of 3 Hz was chosen to minimize the influence of both the  $\alpha$  and  $\beta$  processes on the decay rate, i.e., the decay coefficient is an estimate of  $r$  alone.<sup>17</sup>

## A TWITCHES



## B RYANODINE (5 µg/ml) TETANI

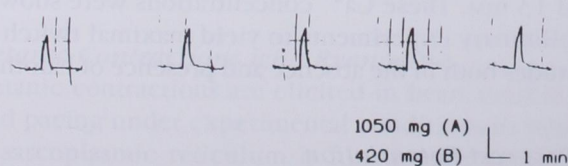


Fig. 2. Differential effect of halothane, at low concentrations, on twitch and tetanic contractile force in isolated rat left atria. Original pen writer records of twitches at 0.4 Hz (A) and ryanodine tetanic contractions at 20 Hz for 10 s (B), both in 10 mM Ca<sup>2+</sup>-Krebs, under control conditions and 15 min after halothane administration at concentrations indicated. Halothane addition was cumulative. Depressant effect of halothane on twitches was reversible on washout of the anesthetic agent (re-control).

## Solutions and Drugs

**Solutions.** Modified Krebs-Henseleit solution contains (in mM): NaCl, 118; KCl, 4.7; CaCl<sub>2</sub>, 1.0; MgCl<sub>2</sub>, 1.0; HEPES-Na<sup>+</sup> salt, 5.55; HEPES, 4.45; glucose, 11; EDTA, 0.03; pH 7.4. Halothane (0.5–1.0%, in oxygen) was administered using a calibrated vaporizer. The composition of the delivered gas mixture was continuously monitored during experiments using a DATEX Model 222 Anesthetic-Agent Monitor. The aqueous-phase concentration of halothane was measured under our experimental conditions by gas chromatography (Finnegan Model 9610). For gas phase concentrations of 0.5, 0.75, and 1% halothane in oxygen, the measured aqueous phase concentrations (in mM) are 0.23, 0.35, and 0.52, respectively. Minimum alveolar concentration of halothane, in rats, is taken as 0.95%.<sup>20</sup> (Thus, 1.0% halothane corresponds to 1.05 times the minimum alveolar concentration.)

**Drugs.** Ryanodine was purchased from Research Biochemicals International (Natick, MA), and dissolved in dimethylsulfoxide to prepare a stock solution (1 mM). For experiments, microliter volumes of the stock solution were pipetted into the bath to achieve the desired drug concentration.

## Statistical Evaluation of Experimental Data

Averaged data are reported as mean ± SEM. Statistical comparisons were made, as appropriate, using either Student's  $t$  test or analysis of variance for repeated measures with a suitable post-test for paired (Newman-Keuls test) or unpaired data (Duncan Multiple Range test). Differences between mean values were considered statistically significant at  $P < 0.05$ .

## Results

### Halothane Effects on Tetanic Contractions

Original records of twitches and tetanic contractions are given in figure 2. At concentrations (0.5–1%) suf-



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ficient to produce marked depression of twitches (upper row), halothane had no effect on tetanic contractions (lower row).

The scatter plot of figure 3 further compares the effects of halothane on twitches and tetanic contractions in three atria. Each plotted point indicates the relative twitch force (abscissa) and the relative tetanic force (ordinate) obtained at a given halothane concentration in a single preparation. Because relative tetanic contractile force remained near unity at all anesthetic concentrations tested, the regression line drawn through the data points in figure 3 has a slope not significantly different from zero.

#### Halothane Effect on Twitch Force-Frequency Relationship

The action of low halothane concentrations on twitch contractile force is presented in figure 4, which displays the typically negative force-frequency curves for atria under control conditions (open symbols) and after halothane (0.5%) treatment (filled symbols). Halothane significantly reduced the contractile amplitude at all stimulation frequencies tested (range: 0.2–3.2 Hz). As

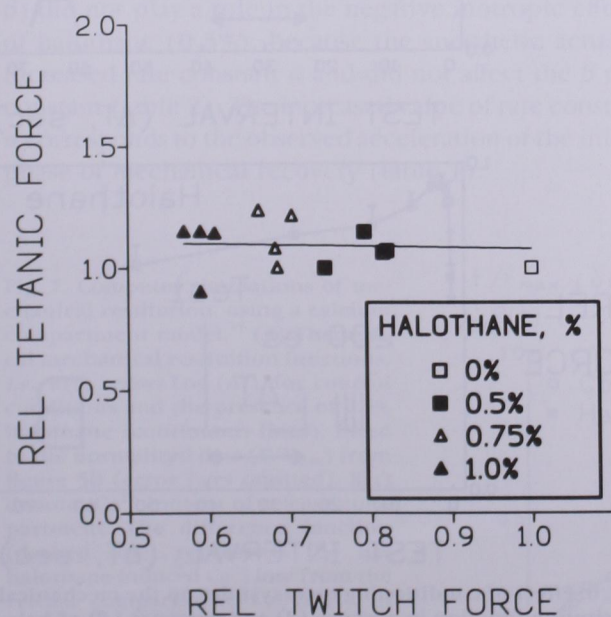


Fig. 3. Differential effect of halothane on twitches and tetanic force. Each plotted point permits comparison of relative twitch and tetanic forces, as obtained in a given atrium at a specified halothane concentration; relative force is defined as the ratio of peak force in halothane to the predrug baseline value. The solid line was obtained by regression analysis. The halothane concentrations used are indicated by individual symbols (inset).

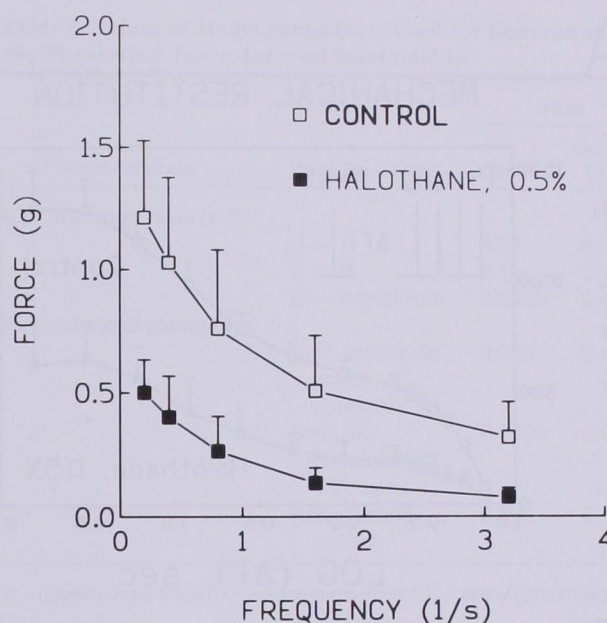


Fig. 4. Influence of 0.5% halothane on the steady-state force-frequency relationship in isolated rat left atria. Error bars = 1 SEM;  $n = 3$ .

shown in figure 4, the absolute magnitude of the effect is greater at the lower stimulation rates compared to that at the higher stimulation rates (although this is not true for the corresponding relative effects).

#### The Force-Interval Relationship (Mechanical Recovery) Analyzed with a Calcium Compartment Model

We investigated the influence of halothane on twitch recovery, which reflects the time course of replenishment of the releasable  $\text{Ca}^{2+}$  pool during diastole. The initial rapid ( $\alpha$ ) and later slow phase ( $\beta$ ) of mechanical recovery are readily identified in plots of test twitch amplitude versus  $\log(\Delta t)$  (fig. 5). In rat atria, the early phase reaches a plateau at test intervals approaching 1 s; the late phase reaches a plateau at test intervals of about 32 s. Halothane (0.5%) exposure diminished the amplitudes of both the early and late phases of the recovery process (fig. 5A). The data in figure 5A have been normalized to  $F_{\max}$ , the maximal force-generating capacity of the muscles, and replotted in figure 5B. Note that halothane significantly decreased  $F_{\max}$  (see table 1). Halothane lowered the amplitude of the normalized mechanical recovery curve (fig. 5B), *i.e.*, the effect of halothane on the amplitude of the recovery is relatively greater than its effect on  $F_{\max}$ .



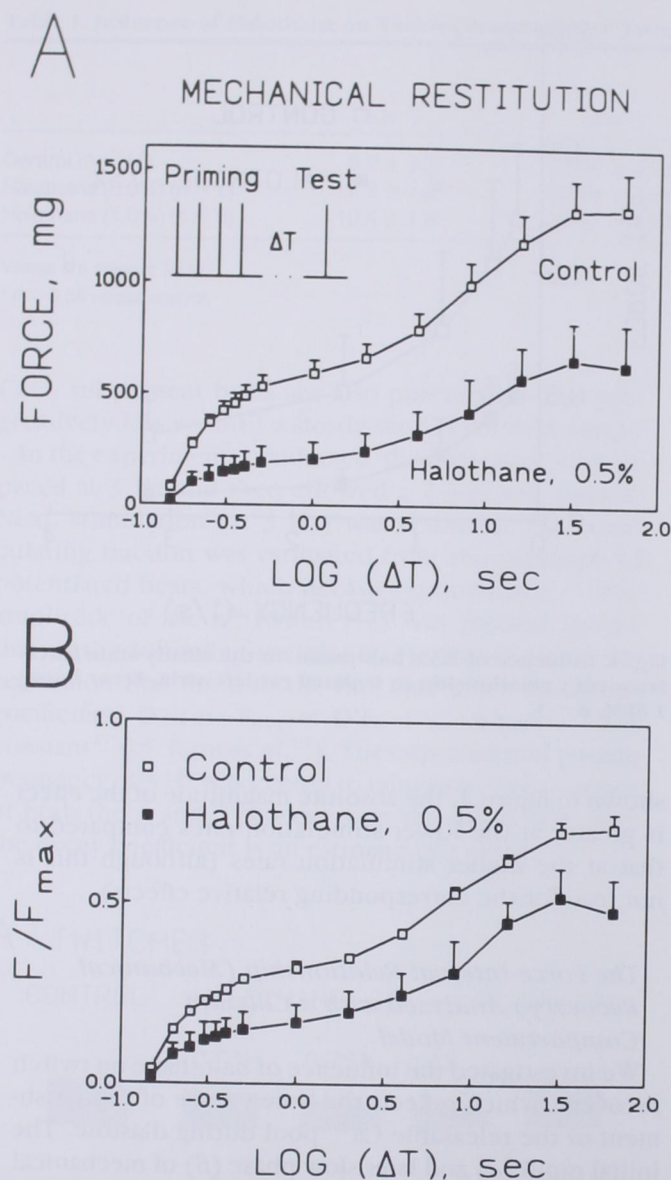


Fig. 5. Influence of halothane on mechanical recovery in isolated rat left atria. (A) Plot of twitch force (mg) versus the logarithm of the test interval ( $\Delta t$ , s) in control (open squares) and during halothane (0.5%) exposure (filled squares); stimulation protocol is illustrated (inset). (B) Replot of the data in (A) normalized to maximal force ( $F_{max}$ ), which was determined in 13 mM  $Ca^{2+}$ . Paired observations were made in four atria; in each preparation, effects of halothane were determined after a period (15–20 min) sufficient for its steady-state effects to develop.

The normalized data in figure 5B were also analyzed to determine halothane's effects on the temporal characteristics of mechanical recovery. Unexpectedly, the analysis demonstrated that the initial rate of recovery

of twitch force ( $\alpha$ ) was significantly increased by halothane (0.5%) and was increased even more at a higher anesthetic concentration (1%; table 1). In contrast, halothane (0.5 or 1%) did not significantly modify the rate of rise of the second phase of twitch recovery ( $\beta$ ; table 1). Halothane did not significantly affect the relative amplitudes of the fast and slow components (table 1), indicating that halothane uniformly decreases the height of the two phases of mechanical recovery.

We next investigated the influence of a series of extrasystoles on mechanical recovery. The conditioning extrasystoles in the protocol used are expected to impose a sudden and large  $Ca^{2+}$  load on the sarcoplasmic reticulum. The amplitude of the test beats are plotted

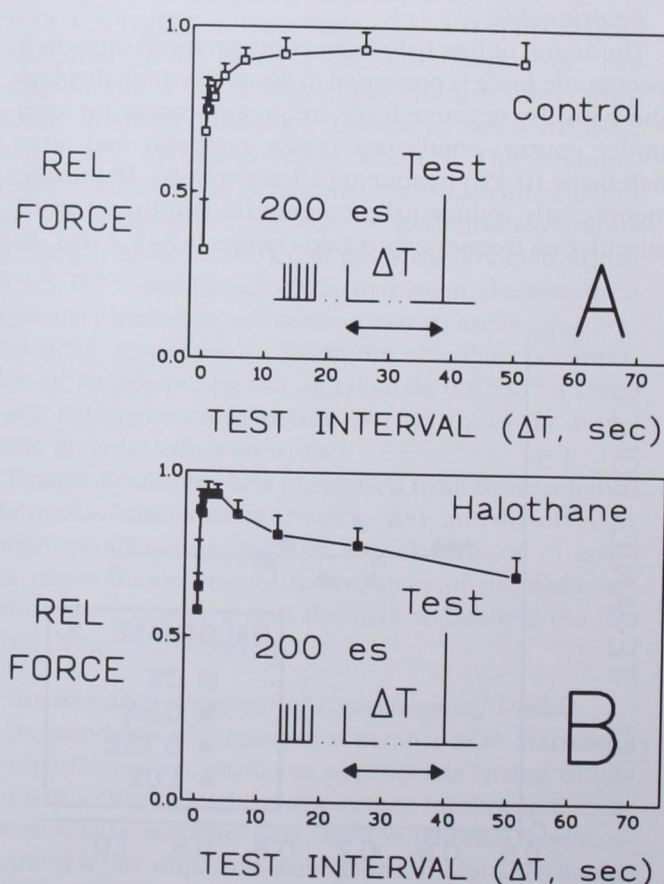


Fig. 6. Effect of conditioning extrasystoles on the mechanical restitution function in absence (A) and presence (B) of halothane (0.5%). The relative twitch contractile force has been plotted versus the test interval (linear scale). Inset depicts stimulus protocol used; es = extrasystoles. Relative values of 1.0 correspond to a developed force of  $710 \pm 98$  mg (control) and  $383 \pm 53$  (halothane). Paired observations were made 5 atria; in each preparation, effects of halothane were determined after a period (15–20 min) sufficient for its steady-state effects to develop.



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versus test interval in figure 6. (Note that the data are plotted on a linear scale to facilitate the display of the late phase.) The control mechanical recovery curve achieved a stable plateau at test intervals longer than 10 s (fig. 6A). Halothane (0.5%), by contrast, altered the shape of the mechanical recovery curve, causing an early peak at test intervals approaching 1–3 s followed by a relaxation toward lower amplitudes at longer test intervals (fig. 6B).

The calcium compartment model (fig. 1) predicted the empirical mechanical recovery time course, as shown in figure 7A. The model parameters employed are listed in table 2. The diminished amplitude of the mechanical recovery curve observed in the presence of halothane corresponds to a reduction in the  $\text{Ca}^{2+}$  content of the release compartment and was modeled by decreasing parameters  $\Delta^u$  and  $r$  (early phase) and  $\Delta^e$  (late phase). The depressed amplitude of the early phase of mechanical recovery resulted almost entirely from a smaller recirculating fraction of activator  $\text{Ca}^{2+}$  ( $r$ ; see later). Halothane's effect on  $\text{Ca}^{2+}$  inflow into the uptake compartment (*i.e.*,  $\Delta^u$ ) made only a minor contribution to overall anesthetic action. Reduced rates of  $\text{Ca}^{2+}$  transport into the release compartment ( $\alpha$  and  $\beta$ ) did not play a role in the negative inotropic effects of halothane (0.5%), because the anesthetic actually increased rate constant  $\alpha$  and did not affect the  $\beta$  rate constant (table 2). The increased value of rate constant  $\alpha$  corresponds to the observed acceleration of the initial phase of mechanical recovery (table 1).

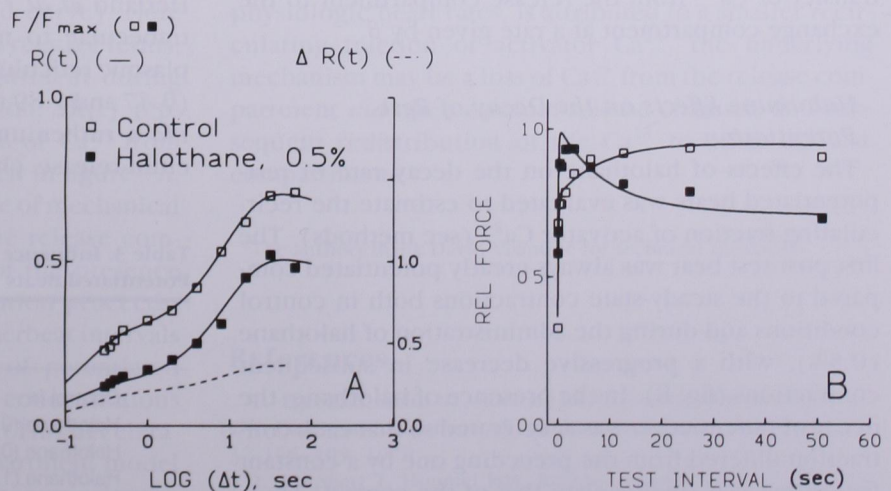
Table 2. Values of Model Parameters Used for Generating the Theoretical Force–Interval Relationship

Model Parameter	Brief Description	Value	
		Control	Halothane (0.5%)
Ca <sup>2+</sup> transport rates (s <sup>-1</sup> )			
$\alpha$	U → R	6.35	8.29
$\beta$	E ↔ R	0.1	0.1
$\gamma$	E → interstitium	0.0005	0.0005
Dimensionless parameters			
$\Delta^u$	Ca <sup>2+</sup> uptake <i>via</i> action potentials	0.003	0.0018
$\Delta^e$	Stimulus-dependent Ca <sup>2+</sup> uptake	0.0009	0.0006
r	Recirculating fraction	0.69	0.53

U = uptake compartment; R = release compartment; E = exchange compartment

The model accurately predicted the effects of a series of extrasystoles on mechanical restitution. Figure 7B shows the model representation of the phenomena depicted in figures 6A and 6B. It is noteworthy that the model predicts the relaxations observed in the presence of halothane (fig. 6B). Our experimental data are in accord with the model in which the  $\beta$ -process (fig. 1) is described by a single reversible rate constant. Hence, the slow phase of restitution was adequately fitted with  $\beta = 0.1 \text{ s}^{-1}$  for both control and halothane. In terms of

Fig. 7. Computer simulations of mechanical restitution, using a calcium compartment model.<sup>17</sup> (A) Theoretical mechanical restitution functions, *i.e.*,  $R(t)$  versus  $\text{Log}(\Delta t)$ , for control conditions and the presence of 0.5% halothane (continuous lines), fitted to the normalized data ( $F/F_{\text{max}}$ ) from figure 5B (error bars omitted).  $R(t)$  denotes  $\text{Ca}^{2+}$  content of release compartment. The difference function (dashed line) represents the net halothane-induced  $\text{Ca}^{2+}$  loss from the release compartment, *i.e.*,  $\Delta R(t)$ . In calculating the difference function, the halothane curve was multiplied by two thirds to correct for the anesthetic-induced decrease in size of the release compartment, which was operationally defined by  $F_{\text{max}}$  (table 1). (B) Theoretical restitution curves (continuous lines) fitted to the experimental data from figure 6 (error bars omitted). Model parameters  $\Delta^u$  and  $\Delta^e$  were increased to values greater than those listed in table 2, as the extrasystoles are collectively expected to greatly increase  $\text{Ca}^{2+}$  influx.





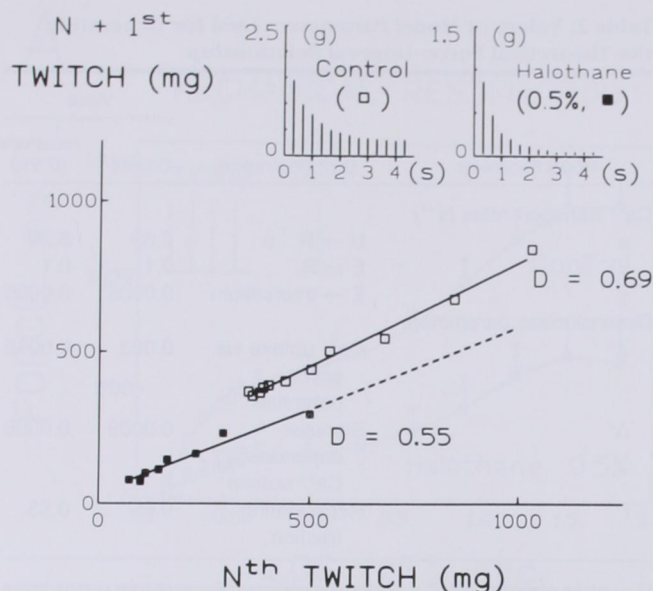


Fig. 8. Analysis of the decay rate of potentiated beats after a 2-min pause (post-rest potentiation). The linear plots give the amplitude of the  $n + 1^{\text{st}}$  twitch versus that of the  $n^{\text{th}}$  twitch ( $n \geq 2$ ), obtained in the presence (as indicated) or the absence (control) of 0.5% halothane. The insets show the respective recorded amplitudes on which the linear plots are based. The twitch amplitude is seen to decline to a steady-state level. (A decay coefficient,  $D$ , of 0.69, for example, corresponds to a 31% decrease in the variable component of the twitch.) Note that, in the presence of 0.5% halothane,  $D$  decreases from 0.69 to 0.55 (cf., table 3). The dashed segment has been added to facilitate visual comparison of the control (open symbols) and experimental slopes (filled symbols).  $D$  = decay coefficient.

the model, the relaxation in halothane results from the transfer of  $\text{Ca}^{2+}$  from the release compartment to the exchange compartment at a rate given by  $\beta$ .

#### Halothane Effects on the Decay of Rest-Potentiation

The effects of halothane on the decay rate of rest-potentiated beats was evaluated to estimate the recirculating fraction of activator  $\text{Ca}^{2+}$  (see methods). The first post-rest beat was always greatly potentiated compared to the steady-state contractions both in control conditions and during the administration of halothane (0.5%), with a progressive decrease in subsequent contractions (fig. 8). In the presence of halothane, the decay of potentiation was accelerated so that each contraction differed from the preceding one by a constant factor that was smaller than that of the control. Thus, the anesthetic agent reduced the decay coefficient,  $D$ , i.e., the factor by which the  $n^{\text{th}}$  beat is multiplied to

give the variable component of the  $n + 1^{\text{st}}$  twitch (fig. 8).

Table 3 provides a summary of decay coefficients obtained under control conditions and after halothane exposure. Halothane (0.5–1%) decreased the decay coefficient in a concentration-dependent manner. This effect, which was quite pronounced, was statistically significant at all halothane concentrations tested.

#### Discussion

The activator  $\text{Ca}^{2+}$  that causes normal twitch contractions is sequestered in terminal cisternae, the release compartment of the sarcoplasmic reticulum. During systole, most of this stored  $\text{Ca}^{2+}$  diffuses into the sarcoplasm *via* open calcium-release channels, resulting in the twitch contraction.<sup>21</sup> In our functional studies, we operationally defined the maximum  $\text{Ca}^{2+}$  content of the release compartment by  $F_{\text{max}}$ . The normalized force then represents the quantity of  $\text{Ca}^{2+}$  released relative to this maximum. Because ryanodine (in nanomolar concentrations) abolishes twitch contractile force in rat atria, we argue that twitch amplitude reflects the  $\text{Ca}^{2+}$  content of the release compartment. As halothane reduces both  $F_{\text{max}}$  and normalized force, the anesthetic must decrease the releasable pool of  $\text{Ca}^{2+}$ , thereby causing a negative inotropic action.

Evidence that halothane diminishes the  $\text{Ca}^{2+}$  content of the sarcoplasmic reticulum is that the dissolved vapor markedly reduces caffeine-induced intracellular  $\text{Ca}^{2+}$  transients<sup>22</sup> and rapid cooling contractures.<sup>23</sup> Herland *et al.*,<sup>13</sup> using force transients in skinned rat trabeculae to monitor  $\text{Ca}^{2+}$  fluxes across the sarcoplasmic reticulum, further demonstrated that halothane (0.47 and 1.89 mM) induces the release of  $\text{Ca}^{2+}$  entirely *via* a ruthenium-red sensitive pathway, i.e., the calcium-release channel. Connelly and Coronado<sup>24</sup> re-

Table 3. Influence of Halothane on the Decay Rate of Rest-Potentiated Beats

	Decay Coefficient
Control (n = 9)	0.68 ± 0.01
Halothane (0.5%) (n = 6)	0.54 ± 0.02*
Halothane (0.75%) (n = 6)	0.45 ± 0.03*
Halothane (1.0%) (n = 6)	0.31 ± 0.04*

Values are mean ± SEM.

\*  $P < 0.05$  versus control.



## HALOTHANE AND MYOCARDIAL TETANIC FORCE

cently showed that halothane (0.5–1 mM) increases the probability of the open state of single ryanodine receptor-channels, extracted from porcine left ventricles and incorporated into planar lipid bilayers.

Our modeling demonstrates that the measured reduction in  $r$ , the recirculation fraction of  $\text{Ca}^{2+}$ , accounts for the entire anesthetic-induced decrease of normalized force at physiologic heart rates ( $>1/\text{s}$ ), the fraction  $r$ , being related to the action of the  $\text{Ca}^{2+}$  ionic pump of the sarcoplasmic reticulum and  $\text{Na}^+/\text{Ca}^{2+}$  exchanger of the sarcolemma. Thus, at  $r = 0.69$ , 69% of the contractile  $\text{Ca}^{2+}$  will be resequenced, whereas 31% will exit the cell by  $\text{Na}^+/\text{Ca}^{2+}$  exchange. In the presence of 0.5% halothane, when  $r = 0.53$ , only 53% of the contractile  $\text{Ca}^{2+}$  will be taken up by the sarcoplasmic reticulum, whereas 47% will be extruded from the cells. As the halothane concentration increases (table 3), the value for the recirculating fraction  $r$  decreases, *i.e.*, the effect of the halothane becomes more pronounced. The mechanism of halothane action, therefore, involves a reduction in  $\text{Ca}^{2+}$  sequestration by the sarcoplasmic reticulum. How might such a mechanism operate in paced myocardial preparations? During the interval between beats, because halothane opens a small fraction of the calcium-release channels,<sup>24</sup>  $\text{Ca}^{2+}$  would be lost from the release compartment, and would then exit the cells via  $\text{Na}^+/\text{Ca}^{2+}$  exchange. The loss of  $\text{Ca}^{2+}$  from the release compartment results in diminished contractile  $\text{Ca}^{2+}$  and negative inotropy.<sup>7</sup> We calculated a theoretical time course for the diastolic  $\text{Ca}^{2+}$  loss, by computing the difference between the mechanical recovery functions fitted to the control and experimental (0.5% halothane) data (fig. 7A, dashed curve). Note that these theoretical curves express, in relative terms, the  $\text{Ca}^{2+}$  content of the release compartment during diastole, *i.e.*,  $R(t)$ . The difference function,  $\Delta R(t)$ , represents the net anesthetic-induced loss of  $\text{Ca}^{2+}$  from the release compartment. As can be seen in figure 7A,  $\Delta R(t)$  is at a minimum early in the course of mechanical recovery, when the  $\text{Ca}^{2+}$  content of the release compartment approaches zero regardless of the presence of halothane.  $\Delta R(t)$  increases as restitution proceeds. A loss of intracellular  $\text{Ca}^{2+}$  during the interbeat intervals would explain the accelerated decay of potentiated beats produced in the presence of the concentrations of halothane used (fig. 8 and table 3). (This acceleration is interpreted in the calcium compartment model as a reduced recirculating fraction  $r$ , or, equivalently, increased  $\text{Ca}^{2+}$  loss,  $1-r$ .) The general decrease in the amplitude of the restitution function similarly can be

understood as a relative increase in  $\text{Ca}^{2+}$  efflux (or a reduced net uptake of  $\text{Ca}^{2+}$ ) during the test intervals. Anesthetic effects on  $\text{Ca}^{2+}$  entry *per se* could also contribute to a negative inotropic effect. However, halothane (0.5–1%) did not affect tetanic contractions in ryanodine, which depend entirely on  $\text{Ca}^{2+}$  influx and subsequent  $\text{Ca}^{2+}$  action on myofibrils.

When we imposed a series of conditioning extrasystoles before test beats, the drug-treated myocardium appeared unable to sequester the increased  $\text{Ca}^{2+}$  load for more than a few seconds, presumably because the calcium-release channels of the sarcoplasmic reticulum remained open during the test intervals (fig. 7B). We argue, in this instance, that the anesthetic-induced release of sequestered  $\text{Ca}^{2+}$  into the cytosol was so great that the net direction of the  $\text{Na}^+/\text{Ca}^{2+}$  exchange reaction changed from reverse mode, typical of control conditions, to forward mode, resulting in the observed relaxation.

In extrapolating these *in vitro* results to *in vivo* conditions, some aspects should be kept in mind, among them the use of atria (rather than ventricles), the temperature used, which is lower than body temperature, and the particular oxygenation conditions employed. Also, there are well-known differences between the myocardia from rats and other mammals. Conversely, certain similarities between the human and rat myocardia render the rat model useful.

For rat atria, we conclude that the cardiac depressant action of low halothane concentrations involves a reduction in the sequestered pool of activator  $\text{Ca}^{2+}$ . Whereas the decrease observed in normalized force, at physiologic heart rates, is attributed to a smaller recirculating fraction of activator  $\text{Ca}^{2+}$ , the underlying mechanism may be a loss of  $\text{Ca}^{2+}$  from the release compartment *via* open calcium-release channels and subsequent redistribution of the  $\text{Ca}^{2+}$  to other cellular compartments.

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