

## Mechanism of the Direct, Negative Inotropic Effect of Ketamine in Isolated Ferret and Frog Ventricular Myocardium

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**Background:** Ketamine exerts both an indirect, positive inotropic effect and a direct, negative inotropic effect in isolated ferret ventricular myocardium. This negative inotropic effect becomes apparent after inactivation of the sympathetic neuroeffector junction. The aim of this study was to investigate the mechanisms of ketamine's intrinsic negative inotropic effect.

**Methods:** The authors analyzed the effects of ketamine after  $\beta$ -adrenoceptor blockade on variables of contractility and relaxation, and on the free intracellular  $\text{Ca}^{++}$  transient detected with the  $\text{Ca}^{++}$ -regulated photoprotein aequorin. Ketamine's effects were also evaluated in a preparation in which the sarcoplasmic reticulum (SR) function was impaired by ryanodine, and in frog ventricular myocardium in which the SR is poorly developed.

**Results:** Ketamine at concentrations  $\geq 3.3 \times 10^{-5}$  M decreased contractility and the amplitude of the intracellular  $\text{Ca}^{++}$  transient. After inactivation of sarcoplasmic reticulum  $\text{Ca}^{++}$  release with  $10^{-6}$  M ryanodine, a condition in which myofibrillar activation depends almost exclusively on transsarcolemmal  $\text{Ca}^{++}$  influx, ketamine caused a decrease in contractility and in the amplitude of the intracellular  $\text{Ca}^{++}$  transient, and ketamine's relative negative inotropic effect was not different from that in control muscles not exposed to ryanodine. Furthermore,  $\geq 10^{-4}$  M ketamine decreased contractility in frog ventricular myocardium, a species that is almost entirely dependent on transsarcolemmal  $\text{Ca}^{++}$  influx for its myofibrillar activation.

**Conclusions:** These findings indicate that the direct negative inotropic effect of ketamine results from a decrease in intracellular  $\text{Ca}^{++}$  availability with no changes in myofibrillar  $\text{Ca}^{++}$  sensitivity. At least part of ketamine's action is caused by inhibition of transsarcolemmal  $\text{Ca}^{++}$  influx. (Key words: Aequorin. Anesthetics, intravenous: ketamine. Heart: contractility; intracellular  $\text{Ca}^{++}$  transient.)

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KETAMINE has useful cardiostimulatory effects<sup>1</sup> that result mostly from central<sup>2</sup> and peripheral<sup>3</sup> stimulation of the sympathetic nervous system. Results of two previous studies<sup>4,5</sup> on isolated ferret papillary muscle indicated that the positive inotropic effect of ketamine results from the inhibition of neuronal and extraneuronal catecholamine uptake. In muscles from animals treated with reserpine or after  $\beta$ -adrenoceptor blockade,<sup>4</sup> ketamine did not exert a positive inotropic effect, but demonstrated a negative inotropic effect at concentrations equal to or greater than  $10^{-4}$  M. The aim of this study was to examine the mechanism(s) of the direct inotropic effects of ketamine after  $\beta$ -adrenoceptor blockade. The results indicate that ketamine decreases intracellular  $[\text{Ca}^{++}]$ , does not change myofibrillar  $\text{Ca}^{++}$  sensitivity, and decreases net transsarcolemmal  $\text{Ca}^{++}$  influx.

### Materials and Methods

This study was approved by the Animal Care and Use Committee of the Mayo Foundation. We used papillary muscles from the right ventricle of adult male ferrets (weight 1,100–1,500 g, age 16–19 weeks). The animals were anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneally), and the heart was quickly removed through a left thoracotomy. The right ventricle was opened, and suitable papillary muscles were excised and mounted in a temperature-controlled (30° C) muscle chamber that contained a physiologic salt solution of the following composition (mM):  $\text{Na}^{+}$  135;  $\text{K}^{+}$  5;  $\text{Ca}^{++}$  2.25;  $\text{Mg}^{2+}$  1;  $\text{Cl}^{-}$  103.5;  $\text{HCO}_3^{-}$  24;  $\text{HPO}_4^{2-}$  1;  $\text{SO}_4^{2-}$  1; acetate 20; and glucose 10. This solution was equilibrated with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  (pH = 7.4). Suitable preparations were selected on the basis of previously used criteria.<sup>4</sup> The muscles were

held between the lever of a force-length transducer (Innovi, Antwerp, Belgium) and a miniature Lucite® clip with a built-in stimulation electrode. Muscles were stimulated at a stimulus frequency of 0.25 Hz, with rectangular pulses of 5-ms duration and an intensity 10% above threshold. Muscles were made to contract in alternating series of four isometric and four isotonic twitches at preload only during a 2-h period of stabilization before the onset of the experiment. All experiments were carried out with the initial muscle length set at  $L_{\max}$ , *i.e.*, the muscle length at which active force development is maximal. All ferret papillary muscles were pretreated with  $10^{-7}$  M ( $\pm$ )-bupranolol HCl<sup>6</sup> before the onset of the experiment to abolish the indirect effects of ketamine.<sup>4,5</sup>

#### *Contractile Variables*

Isometric and isotonic twitches against the  $L_{\max}$  preload only were recorded during steady state at each drug concentration.<sup>4</sup> Peak developed force (DF), time to peak force (TPF), and time to 50% isometric relaxation measured from time to peak force (RTH) were measured from isometric twitches. Peak isotonic shortening (DL) was measured from isotonic twitches at the preload of  $L_{\max}$ . Maximal unloaded velocity of shortening (MUVS) was measured from "zero-load-clamped" twitches, *i.e.*, an isotonic twitch at  $L_{\max}$  in which load is rapidly (<3 ms) decreased electronically to zero load during the latent period.

#### *Detection of the Intracellular $Ca^{++}$ Transient*

In experiments in which the intracellular  $Ca^{++}$  transient was measured, muscles were prepared as follows. Electrical stimulation was stopped and multiple superficial cells were microinjected with the  $Ca^{++}$ -regulated photoprotein aequorin,<sup>7</sup> to allow for subsequent detection of the intracellular  $Ca^{++}$  transient. It was usually necessary to microinject 30–100 cells. After microinjection, muscles were allowed to rest for 1 h, and were then carefully transferred to a vertical muscle chamber that allowed for simultaneous detection of variables of contractility and of aequorin luminescence.<sup>8,9</sup> Muscles were made to contract isometrically at  $L_{\max}$  throughout experiments in which aequorin luminescence was measured. It was usually necessary to average luminescence and force signals of 16–256 twitches to obtain a satisfactory signal-to-noise ratio in aequorin luminescence signals. This was accomplished on a digital oscilloscope (Nicolet 4094B, Madison, WI). We quantified diastolic aequorin luminescence, peak

systolic aequorin luminescence, and time to peak aequorin luminescence. The time from the stimulus to the time when aequorin luminescence had decreased to 25% of its peak value (during the decline of the aequorin signal) ( $t_{1.25}$ ) was taken as a measure of the time course of the decline of the aequorin signal.

#### *Experimental Design*

Four protocols were used to examine the mechanism of ketamine's intrinsic, direct inotropic effect; each muscle served as its own control.

In group 1 muscles ( $n = 10$ ), we determined possible changes in the intracellular  $Ca^{++}$  transient during the direct, intrinsic effect of ketamine. After  $\beta$ -adrenoceptor blockade with  $10^{-7}$  M ( $\pm$ )-bupranolol HCl, a dose-response curve to ketamine was obtained in each of ten muscles (group 1). The following steps were used: control;  $10^{-6}$  M,  $10^{-5}$ ,  $10^{-4}$ , and  $3.3 \times 10^{-4}$  M ketamine; and washout. The muscles were exposed to each concentration of ketamine until a steady state of at least 5 min was achieved before contractile response was recorded. To determine whether muscle performance and aequorin luminescence decayed during the duration of the experiment, ketamine was washed out and control measurements were repeated at the end of each experiment.

Total serum concentrations of ketamine were  $60 \mu\text{M}$  5 min after intravenous administration of 2 mg/kg ketamine, and reached steady levels of 7–10  $\mu\text{M}$  in patients.<sup>10</sup> Because ketamine's plasma protein binding is 12%,<sup>11</sup> one can calculate peak free plasma ketamine concentrations of  $5.3 \times 10^{-5}$  M and sustained free plasma concentrations of 6.2 to  $8.8 \times 10^{-6}$  M. These calculations indicate that we studied ketamine concentrations ( $10^{-6}$  M to  $3.3 \times 10^{-4}$  M) that encompass the clinically relevant free (unbound) drug concentrations in plasma.

In group 2 muscles ( $n = 9$ ), we determined whether ketamine possibly alters myocardial relaxation or myofibrillar  $Ca^{++}$  sensitivity. Each of nine muscles were pretreated with  $10^{-7}$  M ( $\pm$ )-bupranolol, and subjected to "Ca<sup>++</sup>-back titration" experiments: after measurement of control variables of the isometric twitch, muscles were exposed to  $3.3 \times 10^{-5}$  M ketamine. Extracellular  $[Ca^{++}]$  was then rapidly raised by adding small aliquots of a concentrated  $CaCl_2$  solution (112.5 mM) to the bathing solution, until peak developed force was equal to that in the control twitch. This protocol allowed us to compare relaxation and time variables ( $n = 9$ ) and aequorin luminescence signals ( $n = 5$ ) in

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control and in the presence of  $3.3 \times 10^{-5}$  M ketamine at equal peak developed force. Similar "Ca<sup>++</sup>-back titration" experiments were carried out in muscles exposed to  $10^{-4}$  M (n = 5) and  $3.3 \times 10^{-4}$  M (n = 5) ketamine.

In group 3 muscles (n = 11), we attempted to determine whether transsarcolemmal Ca<sup>++</sup> exchange is affected by ketamine, by excluding the contribution of the sarcoplasmic reticulum to Ca<sup>++</sup> release by pretreatment with the plant alkaloid ryanodine. Each of 11 muscles was exposed to  $10^{-7}$  M ( $\pm$ )-bupranolol and  $10^{-6}$  M ryanodine. The effects of  $10^{-6}$  M,  $10^{-5}$  M,  $10^{-4}$  M, and  $3.3 \times 10^{-4}$  M ketamine and washout on contractile variables (n = 11) and aequorin luminescence (n = 5) were assessed.

In group 4 muscles (n = 8), we assessed the effects of ketamine on frog ventricular myocardium, a species that is primarily dependent on transsarcolemmal Ca<sup>++</sup> exchange for activation.<sup>12</sup> Ventricular strips were cut from the ventricle of pithed frogs (*Rana pipiens*), and were mounted vertically in the muscle chamber for measurements of contractility variables during ketamine dose-response experiments. The physiological salt solution was diluted to 80% of its original composition with distilled water to approximate the composition of extracellular fluid in frogs.<sup>13</sup> Frog ventricular strip experiments were carried out at 25°C. In an additional seven frog ventricular strips, we assessed the effects of  $10^{-4}$  M ketamine after exposure to  $10^{-6}$  M ryanodine.

All waveforms of aequorin luminescence, force, length, and velocity were displayed as a function of time on a four-channel digital oscilloscope (Nicolet 4094B), stored permanently on 5¼" floppy disks and recorded at slow speed on a four-channel pen recorder (Honeywell 1400, Denver, CO). All waveforms of interest recorded on the digital oscilloscope were transferred to a computer (Reason Technology 486/33 MHz, Minneapolis, MN), on which variables of contraction and relaxation, aequorin luminescence, and corresponding time values were automatically determined.

#### Statistical Analysis

The contractile responses to ketamine and peak aequorin luminescence were assessed with repeated measures analysis of variance. When appropriate, Dunnett's test was used to compare effects of individual ketamine concentrations with control. A *P* value < 0.05 was considered significant.

## Results

Table 1 shows the values of aequorin luminescence and of measurements of contractility in the ketamine dose-response experiments (group 1). Figures 1 and 2 illustrate that, after  $\beta$ -adrenoceptor blockade, ketamine did not significantly alter DF or aequorin luminescence at concentrations  $\leq 10^{-5}$  M. At concentrations  $\geq 10^{-4}$  M, ketamine caused a concentration-dependent decrease of both peak developed force and peak aequorin luminescence. Diastolic aequorin luminescence, time to peak aequorin luminescence, time to 25% decline in the peak luminescence signal ( $t_{L25}$ ), time to peak force (TPF), and time to half isometric relaxation (RTH) were not significantly changed at any ketamine concentration. In  $3.3 \times 10^{-4}$  M ketamine, the peak of aequorin luminescence occurred later ( $66.6 \pm 22.7$  ms) than in control ( $52.1 \pm 9.7$  ms), and the Ca<sup>++</sup> transient lasted longer ( $t_{L25}$   $183.7 \pm 61.8$  ms *vs.*  $139.4 \pm 24.7$  ms), yet these changes were not statistically significant. All variables of contractility and aequorin luminescence after washout of ketamine did not differ significantly from control.

To determine whether ketamine alters myofibrillar Ca<sup>++</sup> responsiveness, aequorin luminescence signals were measured in five muscles (group 2) and were compared at equal peak developed force in control (fig. 3, left) and after exposure to  $3.3 \times 10^{-5}$  M ketamine in elevated [Ca<sup>++</sup>]<sub>o</sub> (fig. 3, right). Table 2 lists the values of aequorin luminescence and of peak developed force in control; in ( $3.3 \times 10^{-5}$  M,  $10^{-4}$  M, and  $3.3 \times 10^{-4}$  M) ketamine; and in ketamine in higher [Ca<sup>++</sup>]<sub>o</sub>. Figure 4 summarizes the changes in peak aequorin luminescence during the Ca<sup>++</sup>-back titration experiments for each of the three ketamine concentrations tested. When ketamine's negative inotropic effect on DF was corrected by raising [Ca<sup>++</sup>]<sub>o</sub>, peak aequorin luminescence was not significantly different from control (*P* > 0.05, n = 5, Student's paired *t* test). In the presence of  $\geq 10^{-4}$  M ketamine in higher [Ca<sup>++</sup>]<sub>o</sub> at equal peak force as in the control, time to peak aequorin luminescence, TPF, and RTH were significantly decreased (table 2).

To assess the effects of ketamine on contractility independent of the sarcoplasmic reticulum Ca<sup>++</sup> release, aequorin luminescence (n = 5) and contractility were measured under isometric conditions in ferret papillary muscle after exposure to  $10^{-6}$  M ryanodine (fig. 5). Consistent with its effects on the SR,  $10^{-6}$  M ryanodine decreased developed force and peak aequorin lumi-

Table 1. Aequorin Luminescence and Variables of Contractility during Control Conditions in Ferret Papillary Muscles (Group 1, n = 10; mean ± SD)

	Control	Ketamine 10 <sup>-6</sup> M	Ketamine 10 <sup>-5</sup> M	Ketamine 10 <sup>-4</sup> M	Ketamine 3.3 × 10 <sup>-4</sup> M	Wash
Diastolic aequorin luminescence (nA)	0.45 ± 0.16	0.49 ± 0.16	0.45 ± 0.17	0.47 ± 0.22	0.50 ± 0.37	0.48 ± 0.30
Peak systolic aequorin luminescence (nA)	1.01 ± 0.76	1.20 ± 0.79	0.96 ± 0.71	0.49 ± 0.36*	0.16 ± 0.11*	1.00 ± 0.76
Time to peak aequorin luminescence (ms)	52.1 ± 9.7	56.8 ± 9.9	53.3 ± 10.9	60.3 ± 15.5	66.6 ± 22.7	53.9 ± 13.0
Peak developed force (mN/mm <sup>2</sup> )	20.73 ± 8.12	22.5 ± 7.7	20.6 ± 8.4	14.4 ± 5.1*	6.5 ± 4.3*	23.0 ± 8.9
Time to peak force (ms)	237.6 ± 70.6	247.6 ± 77.4	241.6 ± 76.2	225.5 ± 82.2	229.1 ± 90.1	237.9 ± 73.9
Time to half isometric relaxation (ms)	122.2 ± 30.1	131.8 ± 30.1	125.7 ± 32.2	115.6 ± 28.4	108.4 ± 25.9	119.1 ± 26.4
Time to 25% luminescence (t <sub>25</sub> )	139.4 ± 24.7	143.5 ± 23.9	141.6 ± 30.4	152.3 ± 45.3	183.7 ± 61.8	137.5 ± 30.8

\* P < 0.01 for comparison with control by repeated-measures analysis of variance and Dunnett's test.

nescence from control conditions (table 3, fig. 5, left). In muscles pretreated with ryanodine 10<sup>-6</sup> M, force and aequorin luminescence were further decreased by ketamine at concentrations ≥ 10<sup>-4</sup> M (fig. 6).

The role of the sarcoplasmic reticulum in ketamine's negative inotropic effect was further evaluated in six additional experiments summarized in figure 7. Contractility under isometric and isotonic conditions was compared in papillary muscles in which SR function was poisoned by ryanodine, and those in which SR function remained intact (historical control reported in Cook *et al.*<sup>4</sup>). Correcting for ryanodine's effect by expressing values as a percent of control reveals a virtually superimposable negative inotropic effect of ketamine in the presence or absence of SR function. The relative effects of ketamine on DF and DL with or without functional sarcoplasmic reticulum did not differ (one-way ANOVA, P > 0.2, n = 6).

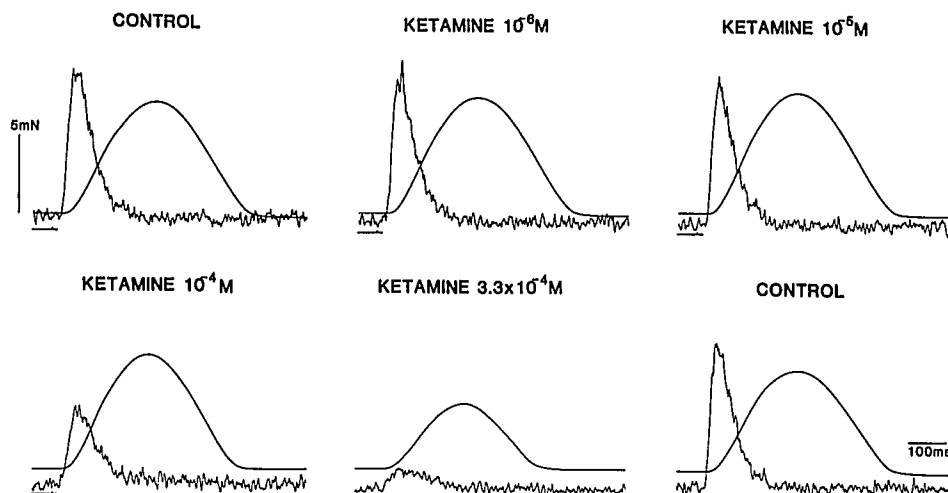
In frog ventricular muscle, ketamine had no significant effect on either peak force of isometric twitches (DF) or on peak shortening of isotonic twitches (DL) at concentrations up to 10<sup>-4</sup> M (figs. 8 and 9). Ketamine, at concentrations of 10<sup>-4</sup> M and higher caused a marked negative inotropic effect (figs. 8 and 9). Peak developed force was decreased from a control value of 14.26 ± 7.79 mN·mm<sup>-2</sup> to 11.57 ± 6.77 mN·mm<sup>-2</sup> in 10<sup>-4</sup> M ketamine, and to 7.30 ± 4.82 mN·mm<sup>-2</sup> in 3.3 × 10<sup>-4</sup> M ketamine (all values mean ± SD; n = 8). Ketamine at concentrations of 10<sup>-4</sup> M and 3.3 × 10<sup>-4</sup> M decreased DL from a control value (mean ± SD; n = 8) of 0.125 ± 0.066 L/L<sub>max</sub> to 0.101 ± 0.061 L/L<sub>max</sub> and 0.060 ± 0.042 L/L<sub>max</sub> in 10<sup>-4</sup> M and 3.3 × 10<sup>-4</sup> M ketamine, respectively. To further ascertain that the effects of ketamine on frog ventricular myocardium were independent of any potential effect on the sarcoplasmic reticulum, an additional seven frog ventricular strips were exposed to 10<sup>-6</sup> M ryanodine and then to 10<sup>-4</sup> M ketamine. Ryanodine, 10<sup>-6</sup> M, did not change DF, DL, or MUVS. Subsequent exposure to 10<sup>-4</sup> M ketamine significantly decreased these contractile variables (fig. 10).

## Discussion

The inotropic effects of ketamine on mammalian myocardium have been reported to be positive, negative, or biphasic, depending on the species studied and on the experimental conditions. Variances of this kind have been extensively discussed by others.<sup>4,5,14</sup> Previous studies have demonstrated that, at clinically rel-

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Fig. 1. Effects of ketamine on force and aequorin luminescence during isometric twitch contractions after  $\beta$ -adrenoceptor blockade. Each panel shows superimposed traces of force and of aequorin luminescence. One hundred and twenty-eight contractions were averaged.



evant concentrations, ketamine exerts an indirect positive inotropic effect on ferret ventricular myocardium.<sup>4,5</sup> This was shown to result from inhibition of neuronal catecholamine uptake mechanisms.<sup>4,5</sup> The overall effect of ketamine on contractility is the net result of this indirect positive inotropic effect and of ketamine's intrinsic negative inotropic effect on ventricular myocardium. The purpose of this study was to investigate the mechanisms of ketamine's direct negative inotropic effect.

This *in vitro* study clearly demonstrates the direct negative inotropic effects of ketamine at concentrations of  $3.3 \times 10^{-5}$  M or greater; this approximates the plasma concentrations of ketamine measured after intravenous induction doses.<sup>10</sup> The negative inotropic effect of ketamine is accompanied by a decrease in peak aequorin luminescence without any change in the kinetics of the aequorin signal. This concomitant decrease in the amplitude of contraction and of the intracellular  $\text{Ca}^{++}$  transient is most likely caused by a reduced availability of intracellular  $\text{Ca}^{++}$ . A decreased intracellular  $\text{Ca}^{++}$  availability could result from decreased  $\text{Ca}^{++}$  release by the sarcoplasmic reticulum or decreased net transsarcolemmal  $\text{Ca}^{++}$  entry, or from an enhanced  $\text{Ca}^{++}$  uptake by the SR. A significant alteration of  $\text{Ca}^{++}$  sequestration by the SR is unlikely, because the time courses of relaxation and of aequorin luminescence were not affected by ketamine.

To determine possible effects of ketamine on mechanisms other than  $\text{Ca}^{++}$  release from the SR, we studied the effects of ketamine in myocardium after SR  $\text{Ca}^{++}$  release was completely abolished by ryanodine pretreatment.<sup>15,16</sup>

The plant alkaloid ryanodine binds to specific myocardial receptors<sup>17</sup> and reduces the availability of  $\text{Ca}^{++}$  from the SR for contractile activation.<sup>18</sup> Ryanodine markedly decreases the amount of  $\text{Ca}^{++}$  released from the SR, and has no effect on the SR  $\text{Ca}^{++}$  uptake pump,<sup>19</sup> or the  $\text{Na}^{+}$ - $\text{Ca}^{++}$  exchanger. The significant reduction in all contractile variables from control measurements after  $10^{-6}$  M ryanodine is similar in magnitude to that reported previously in similar<sup>20</sup> and identical<sup>21</sup> experimental conditions, and reflects the substantial contribution of the SR to the activator  $\text{Ca}^{++}$  pool in ferret ventricular myocardium. In the presence of  $10^{-6}$  M

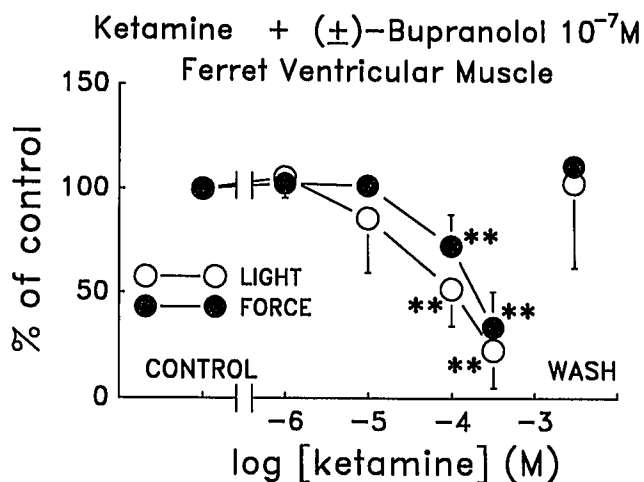
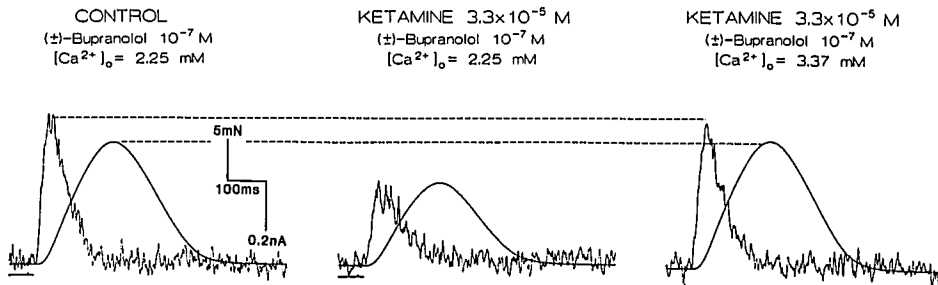


Fig. 2. Effects of ketamine on peak developed force (●) and peak aequorin luminescence (○) after  $\beta$ -adrenoceptor blockade. Values in ketamine (mean  $\pm$  SD, n = 10) are expressed as percent of control. \*\* $P < 0.01$  versus control.



**Fig. 3.**  $\text{Ca}^{2+}$ -back titration experiments (see text for details). Two hundred and fifty-six contractions were averaged. The timing of the stimulus is indicated on the short horizontal bar at the lower left corner of each panel.

ryanodine, ketamine caused a further reduction in contractile variables and of peak aequorin luminescence; this probably reflects a depressant effect of ketamine on an alternative source of activator  $\text{Ca}^{2+}$ , most likely transsarcolemmal  $\text{Ca}^{2+}$  influx.

Frog ventricular myocardium has a poorly developed SR, and is almost entirely dependent on transsarcolemmal  $\text{Ca}^{2+}$  influx to activate the myofibrillar apparatus.<sup>12</sup> Indeed,  $10^{-6}$  M ryanodine failed to change myocardial contractility in frog ventricle, an observation that indicates that the SR in frog ventricle does not regulate contractility. The ketamine-induced decrease in contractility in frog ventricle with or without ryanodine

pretreatment is, therefore, consistent with an effect of ketamine to decrease net transsarcolemmal  $\text{Ca}^{2+}$  influx.

Our findings are in agreement with those of Rusy *et al.*,<sup>22</sup> who attributed the negative inotropic effect of ketamine in isolated rabbit papillary muscle primarily to inhibition of transsarcolemmal  $\text{Ca}^{2+}$  influx with little or no effect on  $\text{Ca}^{2+}$  release by the sarcoplasmic reticulum. More recently, Baum and Tecson<sup>23</sup> observed that  $10^{-5}$  M and  $10^{-4}$  M ketamine decreased peak inward calcium current in single enzymatically dissociated guinea pig myocardial cells.

Endou *et al.*<sup>24</sup> studied the correlation between inotropic and electrophysiologic effects of ketamine in

**Table 2.** Aequorin Luminescence and Developed Force in Ferret Papillary Muscle during  $\text{Ca}^{2+}$ -Back Titration Experiments (Group 2; mean  $\pm$  SD)

	Control $[\text{Ca}^{2+}]_o$ = 2.25 mM	Ketamine $3.3 \times 10^{-5}$ M $[\text{Ca}^{2+}]_o = 2.25$ mM	Ketamine $3.3 \times 10^{-5}$ M $[\text{Ca}^{2+}]_o > 2.25$ mM	N
Aequorin luminescence (nA)	0.58 $\pm$ 0.13	0.42 $\pm$ 0.06	0.58 $\pm$ 0.09	5
Time to peak aequorin luminescence (ms)	50.6 $\pm$ 3.7	50.0 $\pm$ 4.0	49.4 $\pm$ 2.9	5
Peak developed force (mN/mm <sup>2</sup> )	30.38 $\pm$ 3.13	26.60 $\pm$ 3.88†	30.53 $\pm$ 3.27	9
Time to peak force (ms)	216.9 $\pm$ 8.3	219.6 $\pm$ 14.5†	215.0 $\pm$ 8.3	9
Time to half isometric relaxation (ms)	127.4 $\pm$ 6.6	122.4 $\pm$ 7.7	127.8 $\pm$ 7.2	9
	Control $[\text{Ca}^{2+}]_o$ = 2.25 mM	Ketamine $10^{-4}$ M $[\text{Ca}^{2+}]_o = 2.25$ mM	Ketamine $10^{-4}$ M $[\text{Ca}^{2+}]_o > 2.25$ mM	
Aequorin luminescence (nA)	4.07 $\pm$ 2.08	1.74 $\pm$ 1.32*	3.85 $\pm$ 2.73	5
Time to peak aequorin luminescence (ms)	103.4 $\pm$ 20.2	114.0 $\pm$ 17.5	52.2 $\pm$ 2.7†	5
Peak developed force (mN/mm <sup>2</sup> )	31.80 $\pm$ 20.46	23.77 $\pm$ 15.65*	34.55 $\pm$ 23.86	5
Time to peak force (ms)	402.8 $\pm$ 37.8	378.2 $\pm$ 63.7	203.4 $\pm$ 18.6†	5
Time to half isometric relaxation (ms)	230.0 $\pm$ 34.6	197.8 $\pm$ 42.3*	128.8 $\pm$ 32.1*	5
	Control $[\text{Ca}^{2+}]_o$ = 2.25 mM	Ketamine $3.3 \times 10^{-4}$ M $[\text{Ca}^{2+}]_o = 2.25$ mM	Ketamine $3.3 \times 10^{-4}$ M $[\text{Ca}^{2+}]_o > 2.25$ mM	
Aequorin luminescence (nA)	3.21 $\pm$ 1.71	0.61 $\pm$ 0.50*	3.83 $\pm$ 1.24	5
Time to peak aequorin luminescence (ms)	91.6 $\pm$ 13.2	109.8 $\pm$ 28.5	49.0 $\pm$ 3.6†	5
Peak developed force (mN/mm <sup>2</sup> )	34.46 $\pm$ 20.47	16.17 $\pm$ 12.83*	39.63 $\pm$ 27.84	5
Time to peak force (ms)	374.4 $\pm$ 25.8	335.8 $\pm$ 96.7	217.2 $\pm$ 25.3‡	5
Time to half isometric relaxation (ms)	204.0 $\pm$ 40.0	168.2 $\pm$ 42.7*	133.4 $\pm$ 26.2†	5

\*  $P < 0.05$ , †  $P < 0.01$ , ‡  $P < 0.001$ , for comparison with control by Student's paired *t* test.

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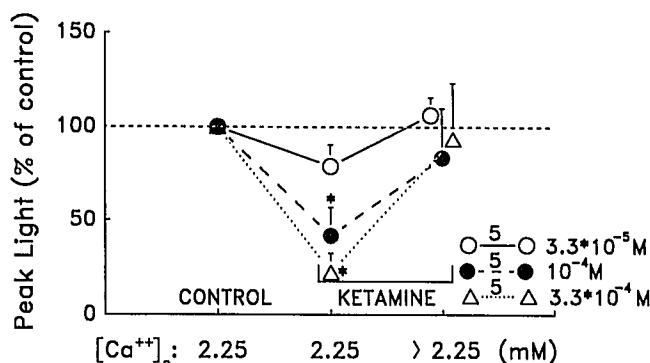


Fig. 4. Summary of  $\text{Ca}^{++}$ -back titration experiments in  $3.3 \times 10^{-5}$  M (○),  $10^{-4}$  M (●), and  $3.3 \times 10^{-4}$  M (△) ketamine ( $n = 5$  each). Peak aequorin luminescence (peak light) is plotted as percent of control (mean  $\pm$  SD;  $n = 5$  in each group) during exposure to ketamine in  $[\text{Ca}^{++}]_o = 2.25$  mM and at  $[\text{Ca}^{++}]_o > 2.25$  mM at equal peak force as in the control. \* $P < 0.05$  versus control.

atrial and ventricular myocardium of the guinea pig and rat, species in which ketamine exerts, respectively, a negative and a positive inotropic effect. The increase in force after ketamine in rat myocardium was found to result from ketamine's inhibition of the transient outward current ( $I_{to}$ ) with minimal effect on  $I_{Ca}$ , whereas, in guinea pig myocardium, the decrease in  $I_{Ca}$  after ketamine was more important than the positive inotropic effect supported by a small decrease in  $I_{to}$ . In view of these results, it is not surprising that ketamine exerts a positive inotropic effect in rat myocardium<sup>24,25</sup> that depends mostly on  $\text{Ca}^{++}$  release from the sarcoplasmic reticulum for its activation. The results of Endou *et al.*<sup>24</sup> are also consistent with ketamine's negative inotropic effect in rabbit<sup>22</sup> and ferret myocardium, species in which transsarcolemmal  $\text{Ca}^{++}$  entry plays a larger role for activation than it does in rat myocardium.

Table 3. Aequorin Luminescence and Developed Force in Ferret Papillary Muscle before and after Ryanodine  $10^{-6}$  M Group ( $n = 5$ ; mean  $\pm$  SD)

	Control	Ryanodine $10^{-6}$ M
Aequorin luminescence (nA)	$0.93 \pm 0.62$	$0.10 \pm 0.07^*$
Time to peak aequorin luminescence (ms)	$93.0 \pm 96.7$	$163.0 \pm 73.3^\dagger$
Developed force (mN/mm <sup>2</sup> )	$24.25 \pm 12.26$	$4.30 \pm 2.19^*$
Time to peak force (ms)	$252.6 \pm 93.8$	$303.6 \pm 104.7^*$
Time to half isometric relaxation (ms)	$110.4 \pm 25.1$	$99.0 \pm 9.6$

\*  $P < 0.05$ ,  $^\dagger P < 0.01$ , for comparison with control by Student's paired  $t$  test.

To determine possible effects of ketamine on myofibrillar  $\text{Ca}^{++}$  responsiveness ( $\text{Ca}^{++}$  sensitivity),  $\text{Ca}^{++}$ -back titration experiments were designed to produce isometric twitches of equal peak amplitude with and without ketamine, and to compare the  $[\text{Ca}^{++}]_i$  transients and mechanical relaxation in those conditions. The assumption implicit to this type of analysis is that the  $\text{Ca}^{++}$  occupancy of troponin C at peak force is the same in either condition, so that myofibrillar  $\text{Ca}^{++}$  sensitivity can be assessed from the relationship between  $[\text{Ca}^{++}]_i$  and  $\text{Ca}^{++}$  occupancy of troponin C. If ketamine alters reaction mechanisms "downstream" from the binding of  $\text{Ca}^{++}$  to troponin C, and modifies the relationship between  $\text{Ca}^{++}$  occupancy of troponin C and force, our approach would be invalid. Yet, so far, there is no evidence that this occurs. Moreover, it is difficult to determine, in twitch contractions of intact living muscle fibers, whether a particular intervention changes myofibrillar  $\text{Ca}^{++}$  responsiveness by comparison of force and  $\text{Ca}^{++}$  transients alone, because the relationship between force and  $[\text{Ca}^{++}]_i$  in twitch contractions does not reach steady state. When the kinetics of the  $[\text{Ca}^{++}]_i$  tran-

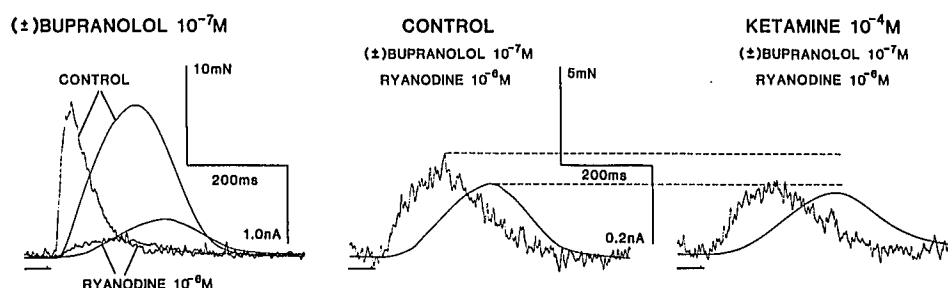


Fig. 5. Effects of ketamine on force and aequorin luminescence after  $\beta$ -adrenergic blockade and inactivation of SR  $\text{Ca}^{++}$  release by  $10^{-6}$  M ryanodine. The left panel shows superimposed traces of force and of aequorin luminescence during isometric twitches before and after  $10^{-6}$  M ryanodine. Note alteration in vertical scales in the post-ryanodine treated figures. One hundred and twenty-eight contractions were averaged in control conditions; 512 twitches were averaged in ryanodine.

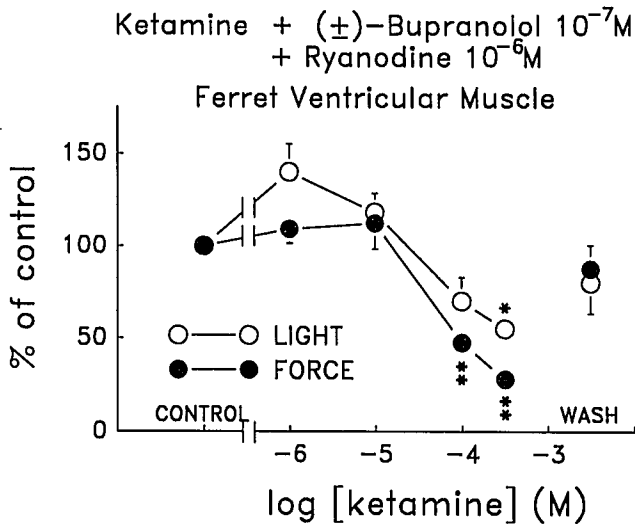


Fig. 6. Effects of ketamine on peak developed force and on peak aequorin luminescence after  $\beta$ -adrenoceptor blockade and inactivation of SR  $Ca^{++}$  release with  $10^{-6}$  M ryanodine. Values in ketamine (mean  $\pm$  SD, n = 5) are expressed as percents of control. \* $P < 0.05$ ; \*\* $P < 0.01$  versus control.

sient are dramatically altered by the intervention, the changes may be impossible to interpret in terms of changes of myofibrillar  $Ca^{++}$  responsiveness.<sup>8</sup> In our

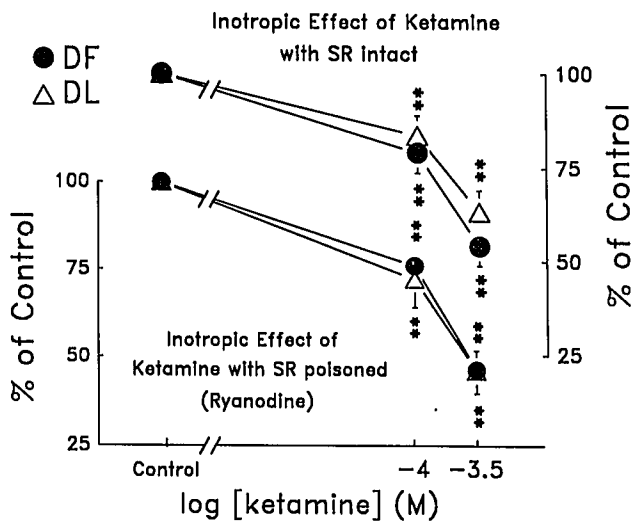


Fig. 7. Effects of  $10^{-4}$  and  $3 \times 10^{-4}$  M ketamine after  $\beta$ -adrenoceptor blockade on peak developed force (DF) and peak isotonic shortening (DL) in ferret right ventricular papillary muscles (mean  $\pm$  SD; n = 9) with an intact SR (upper graph, data from Cook *et al.*<sup>4</sup>) and after inactivation of SR  $Ca^{++}$  release (lower graph) in muscles (mean  $\pm$  SD; n = 6) exposed to  $10^{-6}$  M ryanodine before the ketamine dose-response experiment. \*\* $P < 0.01$  compared to respective control.

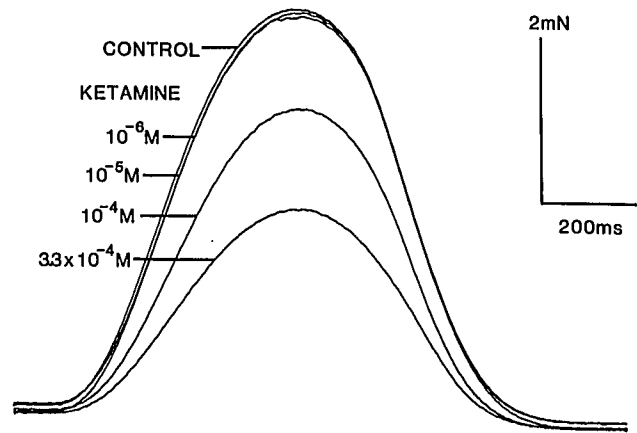


Fig. 8. Effects of ketamine of force of frog ventricular strips. Force traces of isometric twitch contractions before and during a ketamine dose-response experiment are superimposed.

experiments, because the kinetics of the aequorin luminescence signals were not changed in the  $Ca^{++}$ -back titration experiments in  $3.3 \times 10^{-5}$  M ketamine, the conclusions based on our experimental data should be valid for that ketamine concentration.

Because the amplitude and time course of the intracellular  $Ca^{++}$  transients at equal peak developed force were not different with or without ketamine, we conclude that ketamine has minimal, if any, effects on myofibrillar responsiveness to  $Ca^{++}$ . The absence of

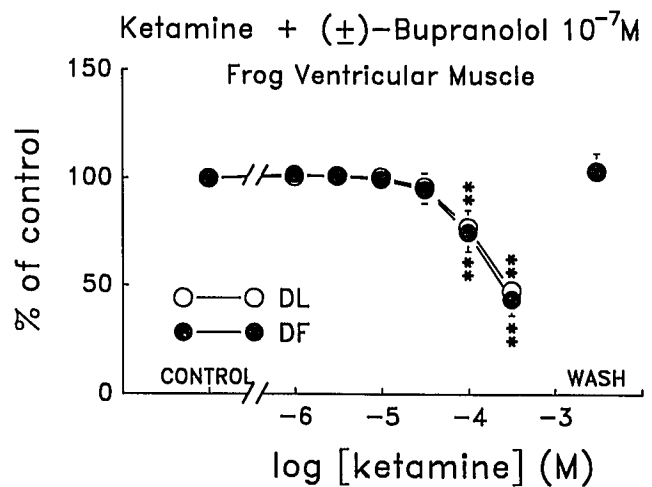


Fig. 9. Effects of ketamine on peak developed force (DF) and peak isotonic shortening (DL) of frog ventricular strips. Values in ketamine (mean  $\pm$  SD, n = 8) are expressed as percent of control. \*\* $P < 0.01$  versus control.



## KETAMINE AND MYOCARDIAL CONTRACTILITY

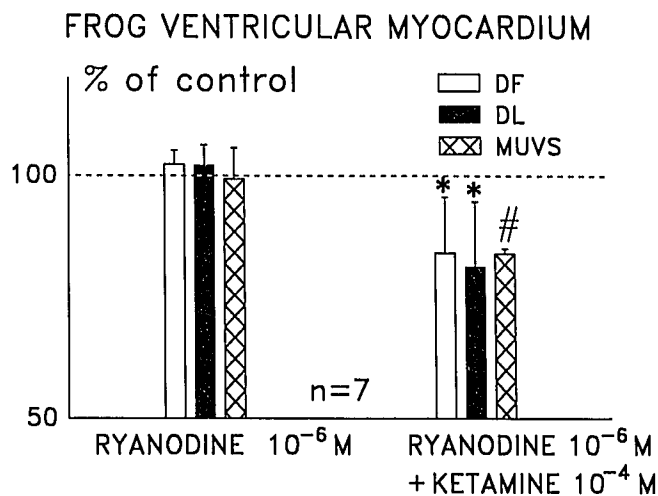


Fig. 10. Effects of  $10^{-6}$  M ryanodine (left) and of  $10^{-6}$  M ryanodine and  $10^{-4}$  M ketamine (right) on amplitude variables of contractility (DF, DL, MUVS) in frog ventricular strips. Data are mean  $\pm$  SD ( $n = 7$ ). \* $P < 0.05$  versus respective control; # $P < 0.01$  versus respective control.

specific effects of ketamine on isometric relaxation is consistent with this view. However, because  $\geq 10^{-4}$  M ketamine in higher  $[Ca^{++}]_o$  in the  $Ca^{++}$ -back titration experiments abbreviated the time course of both aequorin luminescence and isometric force (table 2), inferences about the possible absence of changes in myofibrillar  $Ca^{++}$  sensitivity in  $\geq 10^{-4}$  M ketamine are less firm.

The direct negative inotropic effect of ketamine can, therefore, be attributed to an interference with cellular mechanisms that regulate intracellular  $Ca^{++}$  availability. The evidence from the current study indicates that, at clinically relevant concentrations, ketamine exerts a mild-to-moderate direct negative inotropic effect by decreasing net transsarcolemmal  $Ca^{++}$  influx. The depressant effect may be caused by an inhibition of the sarcolemmal slow inward L-type  $Ca^{++}$  current.<sup>23,24</sup> Possible effects of ketamine on other membrane  $Ca^{++}$  exchange mechanisms ( $Na^+/Ca^{++}$  exchange,  $Ca^{++}$  ATPase export pump, etc.) cannot be excluded, and may necessitate further study.

In conclusion, ketamine exerts both a direct negative inotropic effect and an indirect positive inotropic effect, the latter resulting from inhibition of neuronal and extraneuronal norepinephrine uptake.<sup>4,5</sup> The net inotropic effect in ferret ventricular myocardium was a complex dose-response curve (positive inotropic effect at low ketamine concentrations, and a smaller positive inotropic effect at high ketamine concentrations).

We speculate that the inotropic impact of ketamine in other species, or in human myocardium, will depend on the state of sympathetic neuroeffector junction,<sup>4,5</sup> on the relative importance of intracellular  $Ca^{++}$  delivery mechanisms (SR  $Ca^{++}$  release and transsarcolemmal  $Ca^{++}$  influx<sup>4</sup>), and on specific electrophysiologic effects on  $I_{to}$  and  $I_{Ca}$ .<sup>24</sup>

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