

## Mechanism of the Direct, Negative Inotropic Effect of Etomidate in Isolated Ferret Ventricular Myocardium

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**Background:** Etomidate exerts a mild, positive inotropic effect in rat ventricular myocardium, yet has a negative inotropic effect in isolated rabbit ventricular myocardium. The aim of this study was to investigate the mechanisms of etomidate's inotropic effect and its underlying mechanism in isolated ferret ventricular myocardium (which shows similar physiologic characteristics as human ventricular myocardium) and in frog ventricular myocardium, in which  $\text{Ca}^{++}$  ions for myofibrillar activation are derived almost entirely from transsarcolemmal influx.

**Methods:** The authors analyzed the effects of etomidate 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. Etomidate's effects were also evaluated in ferret right ventricular papillary muscles in which the sarcoplasmic reticulum (SR) function was impaired by ryanodine, and in frog ventricular strips with little or no SR function.

**Results:** At concentrations  $\geq 3 \mu\text{g/ml}$ , which by far exceed the clinically useful concentration range, etomidate decreased contractility and the amplitude of the intracellular  $\text{Ca}^{++}$  transient. At equal peak force, control peak aequorin luminescence in  $[\text{Ca}^{++}]_0 = 2.25 \text{ mM}$  and peak aequorin luminescence in etomidate  $10 \mu\text{g/ml}$  and  $[\text{Ca}^{++}]_0 > 2.25 \text{ mM}$  did not differ, which indicates that etomidate does not alter myofibrillar  $\text{Ca}^{++}$  sensitivity. After inactivation of sarcoplasmic reticulum  $\text{Ca}^{++}$  release with ryanodine  $10^{-6} \text{ M}$ , a condition in which myofibrillar activation depends almost exclusively on transsarcolemmal  $\text{Ca}^{++}$  influx, etomidate caused a decrease in contractility and in the amplitude of the intracellular  $\text{Ca}^{++}$  transient, and etomidate's relative negative inotropic effect was not different from

that in control muscles not exposed to ryanodine. Etomidate  $10 \mu\text{g/ml}$  decreased contractility in frog ventricular myocardium.

**Conclusions:** These findings indicate that the direct negative inotropic effect of etomidate results from a decrease in intracellular  $\text{Ca}^{++}$  availability with no changes in myofibrillar  $\text{Ca}^{++}$  sensitivity. At least part of etomidate's action is caused by inhibition of transsarcolemmal  $\text{Ca}^{++}$  influx. Yet, these effects become apparent only at concentrations that are at least one order of magnitude larger than those encountered in clinical practice. (Key words: Aequorin. Anesthetics, intravenous: etomidate. Heart: contractility; intracellular  $\text{Ca}^{++}$  transient.)

ETOMIDATE is a short-acting intravenous anesthetic induction agent with minor cardiovascular effects.<sup>1,2</sup> It has a high therapeutic index, and is particularly useful in patients with poor cardiovascular reserve.<sup>3</sup> Although the clinical effects on myocardial performance are small in rabbit myocardium, etomidate exerts a negative inotropic effect at concentrations of 2 and 4 mg/l.<sup>4</sup> These data indicated that etomidate decreased the influx of calcium across the sarcolemma, while preserving sarcoplasmic reticulum function. In rat papillary muscle, however, etomidate has been reported to exert a mild positive inotropic effect, illustrated by an increase in the maximum velocity of shortening and no effect on isometric force development.<sup>5</sup> However, etomidate caused a slight decrease in isometric force under certain experimental conditions.<sup>5</sup> The aim of the current study was to evaluate the effect of etomidate on contractility in ferret papillary muscle and frog ventricular myocardium, and to investigate the mechanism by which etomidate exerts its effects on myocardial contractility by studying the intracellular calcium transient with the calcium-regulated photoprotein aequorin.<sup>6</sup> Potential differences of results in myocardium of different species will shed further light onto the mechanism(s) of inotropic effects of etomidate.

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## 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): Na<sup>+</sup> 135; K<sup>+</sup> 5; Ca<sup>++</sup> 2.25; Mg<sup>2+</sup> 1; Cl<sup>-</sup> 103.5; HCO<sub>3</sub><sup>-</sup> 24; HPO<sub>4</sub><sup>2-</sup> 1; SO<sub>4</sub><sup>2-</sup> 1; acetate 20; glucose 10. This solution was equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> (pH = 7.4). Suitable preparations were selected on the basis of previously used criteria.<sup>7</sup> The muscles were held between the lever of a force-length transducer (Innovi, Zaventem, 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<sub>max</sub>, *i.e.*, the muscle length at which active force development is maximal. All ferret papillary muscles were pretreated with 10<sup>-7</sup> M (±)-bupranolol HCl<sup>8</sup> before the onset of the experiment to abolish any sympathetic effects.

*Contractile Variables*

Isometric twitches at L<sub>max</sub> were recorded in control conditions and during steady state at each etomidate concentration. 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.

*Detection of the Intracellular Ca<sup>++</sup> Transient*

After the initial 2-h stabilization period, electrical stimulation was stopped and multiple superficial cells

were microinjected with the Ca<sup>++</sup>-regulated photoprotein aequorin,<sup>6</sup> to allow for subsequent detection of the intracellular Ca<sup>++</sup> transient. It was usually necessary to microinject 30–100 cells. After microinjection, muscles were not stimulated for 2 h, to allow for re-sealing of the plasma membranes of the injected cells. They were then carefully transferred to, and mounted in, a vertical muscle chamber that allowed for simultaneous detection of variables of contractility and of aequorin luminescence.<sup>9–11</sup> The aequorin-injected muscle was positioned in a narrow glass extension at the base of the organ chamber at one focal point of a bifocal ellipsoidal reflector. The photocathode of a bialkali photomultiplier‡ (EMI 9235QA) was located at the other focal point. Muscles were made to contract isometrically at L<sub>max</sub> 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 4094C, Madison, WI).

Because aequorin is consumed in the reaction with Ca<sup>++</sup>, the magnitude of successive aequorin luminescence to a given [Ca<sup>++</sup>]<sub>i</sub> decreases over time. To more validly compare aequorin luminescence signals obtained at different times during the experiment, we corrected them for aequorin consumption.<sup>12</sup> The correction method is based on the observation that the integrated aequorin luminescence is directly related to aequorin concentration in an *in vitro* solution,<sup>6,13</sup> and it is assumed that this is the case within the cytosol, as well. Aequorin luminescence was recorded every 0.5 ms throughout the experiment, and values of aequorin luminescence were integrated over the time of the entire experiment, including all twitches, and during lysis of the cells at the end of the experiment. At the end of each experiment, the physiologic salt solution was quickly removed from the muscle chamber, and 10 ml of a solution of 10 mM CaCl<sub>2</sub> and 4% Triton X-100 in distilled H<sub>2</sub>O (30° C) was flushed into the organ bath. This results in rapid cell lysis and rapid “discharge” of all remaining cytoplasmic aequorin.<sup>12</sup> Light emission from aequorin was recorded throughout this procedure and during the subsequent 15 min. Figure 1 (right) shows a typical example of the aequorin luminescence signal caused by the sudden discharge of aequorin during cell lysis at the end of the experiment. Aequorin luminescence during cell lysis is usually about 1,000 times higher than a typical aequorin signal during a twitch

‡Bialkali photomultipliers have a photocathode that is made of borosilicate, and are the best choice to match light sources emitting in the blue/green region of the visible spectrum. The photocathode is deposited as a semitransparent layer of CsSb (or BeCu in some photomultipliers) directly on the inside of the window.

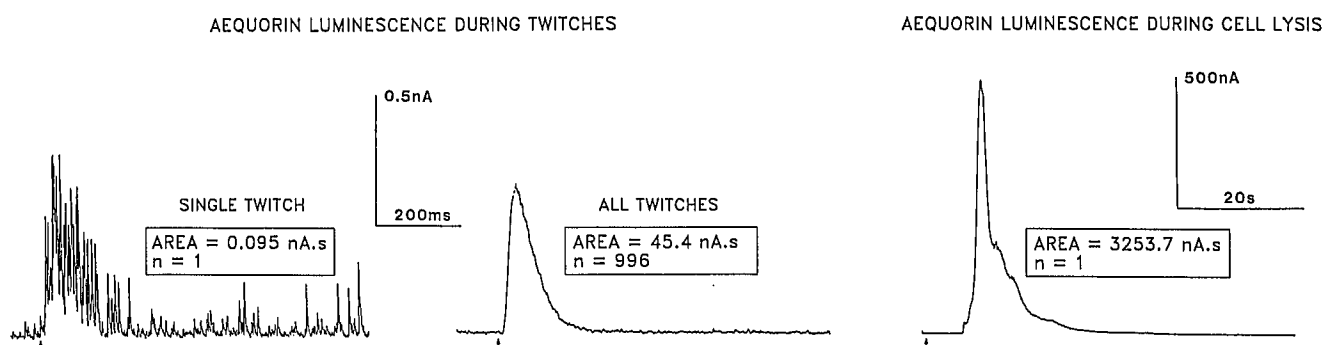


Fig. 1. Determination of aequorin consumption. (Left) Aequorin luminescence signal from a single isometric twitch of a ferret right ventricular papillary muscle. (Middle) Digitally signal-averaged aequorin luminescence signal from all twitches throughout a typical experiment; 996 twitches were averaged. The area under the aequorin luminescence curve is the sum of areas of all twitches. The arrow indicates the time of the electrical stimulus. (Right) Aequorin luminescence signal during cell lysis at the end of the experiment. A solution of 4% Triton X-100, 10 mM  $\text{CaCl}_2$  ( $30^\circ\text{C}$ ) was rapidly flushed into the muscle chamber (arrow). Note the rapid discharge of all remaining aequorin in the tissue, and that the amplitude scale is 1,000 times less sensitive than that for twitches (left and middle panels). In this example, aequorin consumption during the entire experiment was  $45.41 / (3253.71 + 45.41) = 0.0137$ , or 1.37%. In all experiments, aequorin signals at different stages of the experiment were corrected for consumption every 0.5 ms.

(fig. 1, left). Figure 1 (middle) shows the result of averaging all twitches in a typical experiment. The total amount of aequorin in the tissue ( $A_T$ ) is derived from the sum of the area under all aequorin signals during the entire experiment, including cell lysis. The baseline value from which to measure aequorin luminescence for the purpose of calculating consumption is determined from the photomultiplier anode current recorded at the end of cell lysis after all aequorin has been consumed and the muscle is still in identical optical recording conditions. At any given time during the experiment, the amount of aequorin already consumed ( $A_U$ ) is given by the sum of areas under the aequorin luminescence signal up to that time. Therefore, the amount of aequorin that remains at any time is given by  $A_T - A_U$ .

At the conclusion of the experiment, aequorin luminescence during twitch contraction was corrected point by point (every 0.5 ms) for aequorin consumption by multiplying each aequorin luminescence value every 0.5 ms with the ratio of total aequorin/aequorin remaining at that time

$$\frac{A_T}{A_T - A_U}$$

The corrected aequorin luminescence  $AL_C$  is then calculated every 0.5 ms as

$$AL_C = AL_M \cdot [A_T / (A_T - A_U)],$$

where  $AL_M$  = the measured aequorin luminescence;  $A_T$  = the area that represents total aequorin content; and  $A_U$  = the area that represents aequorin already consumed at the time of the measurement  $AL_M$ . Aequorin signals were corrected for consumption in all muscles, except for two muscles in group 3. Aequorin consumption was quite minimal during most experiments (see results section). We quantified the following variables from the aequorin luminescence signals corrected for aequorin consumption: diastolic aequorin luminescence, peak systolic aequorin luminescence, time to peak aequorin luminescence, and  $t_{L25}$  (time from the stimulus to the time when aequorin luminescence had decreased to 25% of its peak value).

#### Experimental Design

Six protocols were used to examine the mechanism of etomidate's direct inotropic effect; each muscle served as its own control.

In group 1 muscles ( $n = 8$ ), we determined possible changes in the intracellular  $\text{Ca}^{++}$  transient during exposure to etomidate. After  $\beta$ -adrenoceptor blockade with  $10^{-7}\text{M}$  ( $\pm$ )-bupranolol HCl, a dose-response curve to etomidate was obtained in each of eight muscles (group 1). The following steps were used: control, and 0.3  $\mu\text{g/ml}$ , 1  $\mu\text{g/ml}$ , 3  $\mu\text{g/ml}$ , and 10  $\mu\text{g/ml}$  etomidate. These concentrations equate to concentrations of 1.2, 4.1, 12.3, and 40.9  $\mu\text{M}$ , respectively. The muscles were exposed to each concentration of etomidate until a steady state of at least 5 min was achieved before con-

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tractile response was recorded. In group 2 muscles ( $n = 8$ ), the effects of etomidate's solvent, propylene glycol (35% v/v), were examined in concentrations that correspond to those used in group 1 muscles.

The plasma concentration of etomidate 4 min after a single intravenous 0.3 mg/kg dose is 0.3  $\mu\text{g/ml}$ ,<sup>14</sup> and may be higher sooner after intravenous injection. Yet, because about 75% of etomidate is protein-bound, the concentration range tested in this study must be considered as high, ranging from intravenous bolus dose range (0.3  $\mu\text{g/ml}$ ) to higher concentrations (1, 3, 10  $\mu\text{g/ml}$ ).

In group 3 muscles ( $n = 11$ ), we determined whether etomidate possibly alters myocardial relaxation or myofibrillar  $\text{Ca}^{++}$  sensitivity. Each of 11 muscles was 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 etomidate 10  $\mu\text{g/ml}$ . Extracellular [Ca<sup>++</sup>] was then rapidly raised by adding small aliquots of a concentrated  $\text{CaCl}_2$  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 and aequorin luminescence signals in control and in the presence of etomidate 10  $\mu\text{g/ml}$  at equal peak developed force (Student's paired  $t$  test).

In group 4 muscles ( $n = 6$ ), we attempted to determine whether transsarcolemmal  $\text{Ca}^{++}$  exchange is affected by etomidate, by excluding the contribution of the sarcoplasmic reticulum to  $\text{Ca}^{++}$  release by pretreatment with the plant alkaloid ryanodine. Each of six muscles was exposed to  $10^{-7}$  M ( $\pm$ )-bupranolol and  $10^{-6}$  M ryanodine. The effects of 0.3, 1, 3, and 10  $\mu\text{g/ml}$  etomidate on contractile variables and aequorin luminescence ( $n = 6$ ) were assessed.

In group 5 ( $n = 5$ ), we examined the effects of propyleneglycol alone after  $10^{-7}$  M ( $\pm$ )-bupranolol and  $10^{-6}$  M ryanodine, to determine whether any effects of etomidate may be attributable to its solvent.

In group 6 muscles ( $n = 8$ ), we assessed the effects of etomidate on frog ventricular myocardium, a species that is primarily dependent on transsarcolemmal  $\text{Ca}^{++}$  exchange for activation.<sup>15</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 etomidate dose-response experiments. The physiologic salt solution was diluted to 80% of its original composition with distilled water to approximate the composition

of extracellular fluid in frogs.<sup>16</sup> Frog ventricular strip experiments were carried out at 25° C.

The effects of etomidate (groups 1, 4, and 6) and of propylene glycol (groups 2 and 5) on contractility variables and on peak aequorin luminescence were assessed with repeated-measures ANOVA. When appropriate, Dunnett's test was used to compare effects of individual drug concentrations with control.  $P < 0.05$  was considered to be significant.

To compare the dose-response curve to etomidate in propylene glycol with that to propylene glycol alone (group 1 vs. group 2; group 4 vs. group 5), the following procedure was used. First, the inotropic response to drug in each muscle was expressed as percent of control. Second, the sums of percent effect over the entire drug concentration range were compared between corresponding (groups 1 and 2 and groups 4 and 5) with Student's  $t$  test.

All waveforms of aequorin luminescence, force, length, and velocity were displayed as a function of time on a four-channel digital oscilloscope (Nicolet 4094C), stored permanently on floppy disks and recorded at slow speed on a four-channel pen recorder (Honeywell 1400, Minneapolis, MN). 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.

To determine whether etomidate influences the  $\text{Ca}^{++}$ -sensitivity of aequorin, we used an aequorin *in vitro* assay apparatus<sup>6</sup> to measure aequorin luminescence in the presence and absence of etomidate 100  $\mu\text{g/ml}$  in a solution containing 150 mM KCl, 5 mM PIPES (piperazine-N,N bis-2-ethanesulfonic acid), 2 mM EGTA (ethylene glycol bis [ $\beta$ -aminoethylether]-N,N,N',N'-tetraacetic acid), and 2 mM CaEGTA, pH 7.00. This solution approximates the ionic composition of the intracellular milieu, and produces, at 22° C, a pCa of 6.4, which is in the range of myoplasmic-free  $\text{Ca}^{++}$  concentrations encountered during a twitch. The effects of 100  $\mu\text{g/ml}$  etomidate on the  $\text{Ca}^{++}$  sensitivity of aequorin luminescence was also assessed in a  $\text{Ca}^{++}$ -free solution containing only KCl and PIPES in the same concentration as listed above, to determine whether etomidate alters  $\text{Ca}^{++}$ -independent aequorin luminescence.

## Results

Among the four muscle groups, there were no statistically significant differences in muscle length at  $L_{\text{max}}$ ,

mean cross-sectional area (CSA), peak developed force (DF), and ratio of resting to total peak isometric force at  $L_{\max}$  (R/T). When all muscle characteristics are pooled,  $L_{\max}$  was  $6.1 \pm 1.2$  mm, CSA was  $0.71 \pm 0.28$  mm<sup>2</sup>, DF was  $25.51 \pm 14.62$  mN·mm<sup>-2</sup>, and R/T was  $0.17 \pm 0.07$  (all data are mean  $\pm$  SD;  $n = 33$ ). Aequorin consumption amounted to  $2.42 \pm 2.00\%$  (mean  $\pm$  SD;  $n = 8$ ) in group 1, to  $2.33 \pm 1.48\%$  (mean  $\pm$  SD;  $n = 8$ ) in group 2, to  $2.30 \pm 2.64\%$  (mean  $\pm$  SD;  $n = 9$ ) in group 3, and to  $7.15 \pm 3.47\%$  (mean  $\pm$  SD;  $n = 6$ ) in group 4. One-way ANOVA and Student's *t* tests revealed that aequorin consumption in group 4 (ryanodine + etomidate) was significantly higher than in group 1 ( $P < 0.05$ ), group 2 ( $P < 0.01$ ), and group 3 ( $P < 0.05$ ). The significantly higher aequorin consumption in group 4 may result from the longer duration of the experimental protocol in this group. Etomidate, 100  $\mu$ g/ml (in propyleneglycol), a tenfold higher concentration than that used in these experiments, did not alter the  $\text{Ca}^{++}$ -independent aequorin luminescence, nor the  $\text{Ca}^{++}$ -sensitivity of aequorin at pCa 6.4 (22° C, pH 7.0) in *in vitro* assays.

Table 1 shows the values of aequorin luminescence and of measurements of contractility in the dose-response experiments to etomidate (in propylene glycol; group 1;  $n = 8$ ) and to etomidate's solvent, propylene glycol alone (group 2;  $n = 8$ ). Table 1 and figures 2 and 3 illustrate that  $\geq 3$   $\mu$ g/ml etomidate decreased both DF and aequorin luminescence. By contrast, propylene glycol alone had no significant effects on DF or aequorin luminescence. Diastolic aequorin luminescence (except at 10  $\mu$ g/ml etomidate) and time to peak aequorin luminescence were not significantly altered by etomidate or its solvent alone. Etomidate, 10  $\mu$ g/ml, caused a slight prolongation in the duration of the decline of aequorin luminescence (table 1;  $t_{L25}$ ), whereas propylene glycol was without effect on  $t_{L25}$ . Time to peak force (TPF), and time to half isometric relaxation (RTH), were significantly shortened by etomidate and by its solvent propylene glycol alone at concentrations  $\geq 1$   $\mu$ g/ml (TPF) and  $\geq 3$   $\mu$ g/ml (RTH). Global comparison of effects on contractile variables by etomidate (in propylene glycol) and propylene glycol alone showed that: (1) there were no differences of effect on diastolic aequorin luminescence, time to peak aequorin luminescence, and time to peak force by either etomidate or its solvent; (2) etomidate's effects to decrease peak aequorin luminescence and DF were significantly different from the lack of effect of propylene glycol alone; and (3) etomidate's effect on

time to half isometric relaxation differed from that of its solvent.

To determine whether etomidate alters myofibrillar  $\text{Ca}^{++}$  responsiveness, aequorin luminescence signals were measured in 11 muscles (group 3), and were compared at equal peak developed force in control (fig. 4, left) and after exposure to 10  $\mu$ g/ml etomidate in elevated  $[\text{Ca}^{++}]_o$  (fig. 4, right). Table 2 lists the values of aequorin luminescence and of contractile variables in control, in 10  $\mu$ g/ml etomidate and in 10  $\mu$ g/ml etomidate in higher  $[\text{Ca}^{++}]_o$ . The  $[\text{Ca}^{++}]_o$  reached during back titration was  $3.35 \pm 0.28$  mM (mean  $\pm$  SD;  $n = 11$ ). As observed for group 1 muscles, 10  $\mu$ g/ml etomidate decreased aequorin luminescence, peak developed force, time to peak force, and time to half isometric relaxation. When etomidate's negative inotropic effect on DF was corrected by raising  $[\text{Ca}^{++}]_o$ , peak aequorin luminescence was not significantly different from control ( $P > 0.05$ ,  $n = 11$ , Student's paired *t* test); TPF and RTH were still significantly decreased from control values (table 2).

To assess the effects of etomidate on contractility independent of the sarcoplasmic reticulum  $\text{Ca}^{++}$  release, aequorin luminescence and contractility were measured under isometric conditions in ferret papillary muscle after exposure to  $10^{-6}$  M ryanodine (group 4; fig. 5). Consistent with its effects on the SR,  $10^{-6}$  M ryanodine decreased developed force and peak aequorin luminescence from control conditions (table 3; fig. 5, left). In muscles pretreated with  $10^{-6}$  M ryanodine, force and aequorin luminescence were further decreased by etomidate at concentrations  $\geq 3$   $\mu$ g/ml.

The role of the sarcoplasmic reticulum in etomidate's negative inotropic effect was further evaluated by comparing the relative effects of etomidate on DF and aequorin luminescence in group 1 muscles with intact SR with those in group 4 muscles with ryanodine-inactivated SR. The results are summarized in figure 6. The relative effects of etomidate on DF and aequorin luminescence with or without functional sarcoplasmic reticulum did not differ (DF,  $P > 0.5$ ; aequorin luminescence,  $P > 0.2$ ; Student's *t* test on sums of effects over entire concentration range).

The effects of propyleneglycol by itself after  $10^{-6}$  M ryanodine was assessed in an additional series of five muscles (group 5). At concentrations equivalent to those present in 0.3, 1, 3, and 10  $\mu$ g/ml etomidate, propyleneglycol increased peak developed force slightly to, respectively,  $106.7 \pm 8.5\%$ ,  $111.5 \pm 8.9\%$ ,  $113.6 \pm 10.0\%$ , and  $118.1 \pm 13.0\%$  of control

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**Table 1. Aequorin Luminescence and Variables of Contractility during Cumulative Dose-Response Experiments to Etomidate in Propylene Glycol and to Propylene Glycol Alone**

	Control	Etomidate 0.3 µg/ml	Etomidate 1 µg/ml	Etomidate 3 µg/ml	Etomidate 10 µg/ml	Etomidate + Propylene Glycol versus Propylene Glycol ( <i>t</i> test on sums)
Diastolic aequorin luminescence (nA)						
Etomidate + propylene glycol	0.41 ± 0.09	0.42 ± 0.11	0.39 ± 0.12	0.35 ± 0.11	0.33 ± 0.10*	NS
Propylene glycol only	0.37 ± 0.06	0.54 ± 0.57	0.50 ± 0.28	0.35 ± 0.05	0.33 ± 0.10	
Peak systolic aequorin luminescence (nA)						
Etomidate + propylene glycol	7.85 ± 4.35	8.12 ± 4.79	7.40 ± 4.34	5.78 ± 2.97*	3.81 ± 2.17†	<i>P</i> < 0.01
Propylene glycol only	7.14 ± 5.39	7.25 ± 5.80	6.43 ± 4.69	6.49 ± 4.92	6.31 ± 4.07	
Time to peak aequorin luminescence (ms)						
Etomidate + propylene glycol	47.8 ± 7.2	51.3 ± 7.5	48.5 ± 6.7	49.0 ± 6.6	52.8 ± 8.8	NS
Propylene glycol only	52.5 ± 8.4	52.3 ± 8.8	51.1 ± 7.1	49.3 ± 6.5	52.3 ± 7.3	
Peak developed force (mN · mm <sup>-2</sup> )						
Etomidate + propylene glycol	25.72 ± 8.64	25.28 ± 8.52	24.18 ± 8.08	21.72 ± 7.39†	14.43 ± 5.82†	<i>P</i> < 0.001
Propylene glycol only	25.95 ± 14.57	25.81 ± 13.81	26.07 ± 14.97	26.06 ± 14.67	25.86 ± 14.74	
Time to peak force (ms)						
Etomidate + propylene glycol	213.4 ± 16.6	211.4 ± 16.5	207.0 ± 16.0*	201.5 ± 15.4†	189.9 ± 15.0†	NS
Propylene glycol only	213.1 ± 16.1	210.0 ± 17.5	207.0 ± 17.2*	205.6 ± 17.7†	203.9 ± 18.9†	
Time to half isometric relaxation (ms)						
Etomidate + propylene glycol	138.1 ± 23.5	135.0 ± 23.0	132.0 ± 21.6	126.5 ± 20.2†	111.8 ± 15.5†	<i>P</i> < 0.05
Propylene glycol only	130.5 ± 24.5	128.4 ± 23.4	126.3 ± 2.4*	124.1 ± 24.0†	122.1 ± 24.1†	
Time to 25% aequorin luminescence (ms)						
Etomidate + propylene glycol	127.9 ± 9.1	128.2 ± 10.5	128.2 ± 9.2	132.4 ± 7.5	137.8 ± 10.7†	NS
Propylene glycol only	128.6 ± 12.2	130.3 ± 8.7	130.6 ± 12.3	129.3 ± 13.6	127.4 ± 15.1	

Data are means ± SD; *n* = 8 both for etomidate + propylene glycol (group 1) and for propylene glycol alone (group 2).

\* *P* < 0.05 for comparison with control by repeated-measures analysis of variance and Dunnett's test.

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

(in 10<sup>-6</sup> M ryanodine). In muscles exposed to 10<sup>-6</sup> M ryanodine, the effects of etomidate in propylene-glycol on peak developed force were significantly different from those of propyleneglycol alone (DF; *P* < 0.01; Student's *t* test on sums of effects over entire concentration range).

In frog ventricular muscle (group 6; *n* = 8), etomidate had no significant effect on either peak force of isometric twitches (DF) or on peak shortening

(DL) of isotonic preloaded twitches at concentrations up to 3 µg/ml (figs. 7 and 8). Etomidate, 10 µg/ml, caused a small negative inotropic effect (figs. 6 and 7). DF was decreased from a control value of 19.64 ± 6.59 to 18.08 ± 5.80 mN·mm<sup>-2</sup> (mean ± SD; *n* = 8) in 10 µg/ml etomidate, and DL was decreased from control value (mean ± SD; *n* = 8) of 0.197 ± 0.060 to 0.184 ± 0.060 l/Lmax in 10 µg/ml etomidate.

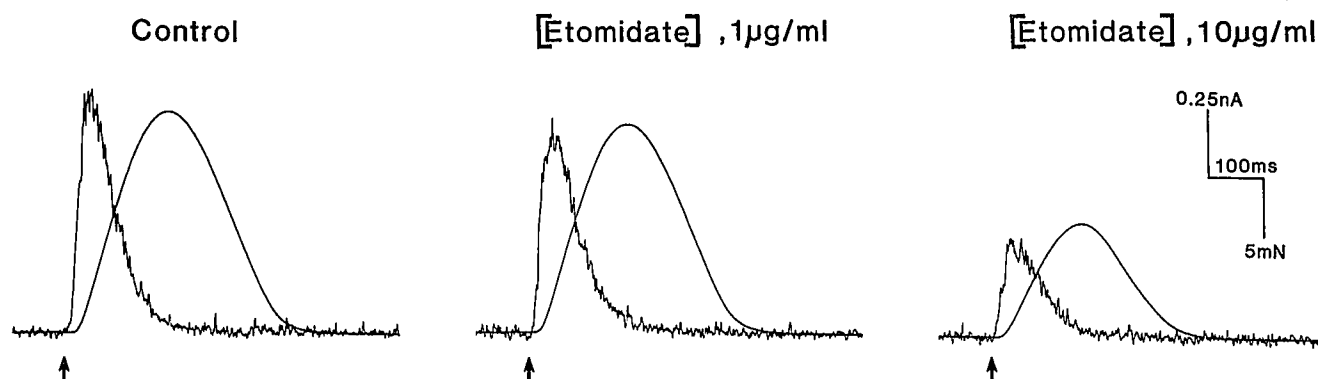


Fig. 2. Aequorin luminescence and force traces during isometric twitches of a right ventricular ferret papillary muscle in control (left) and during exposure to 1 µg/ml (middle) and 10 µg/ml (right) etomidate. Sixty-four twitches were averaged in each panel.

## Discussion

Etomidate is a short-acting intravenous anesthetic induction agent that has minor cardiovascular effects.<sup>1,2</sup> It has a high therapeutic index and is particularly useful in patients with poor cardiovascular reserves.<sup>3</sup> Although the clinical effects on myocardial performance are small, etomidate exerts a negative inotropic effect in rabbit myocardium at concentrations that exceed the clinical useful range.<sup>4</sup> These authors concluded that etomidate decreased the influx of calcium through the sarcolemma while the function of the sarcoplasmic reticulum (SR) was well preserved. By contrast, in rat papillary muscle, etomidate exerted a mild positive inotropic effect, as shown by an increased maximal velocity of shortening and no effect on isometric force

development.<sup>5</sup> In rat papillary muscle, a negative inotropic effect was observed under some experimental conditions.<sup>5</sup> Ferret ventricular myocardium is a good model to study drug actions, because it shares certain physiologic characteristics with human ventricular myocardium. The density of sympathetic innervation of the ferret right ventricle parallels that of human right ventricle.<sup>17</sup> The regulation of activator  $\text{Ca}^{++}$  by sarcolemma and sarcoplasmic reticulum is similar in the two species. This is expressed in the mechanical characteristics of the two tissues exhibiting similar force-frequency relationships and mechanical restitution curves.<sup>18,19</sup> Furthermore, information from experiments carried out in different species can help to identify the predominant site of drug action because of dif-

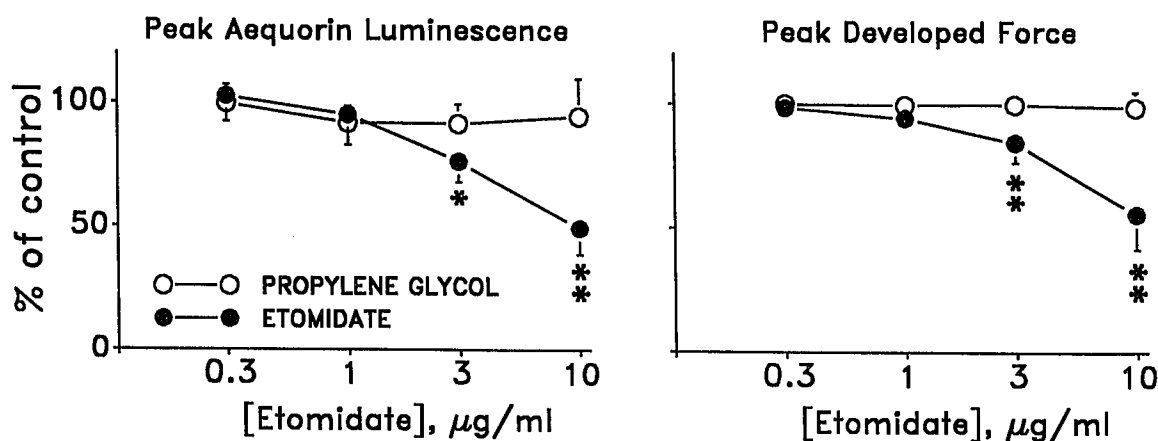


Fig. 3. Summary of dose-response experiments to etomidate in propylene glycol (n = 8; ●) and to propylene glycol alone (n = 8; ○) for peak aequorin luminescence (left) and peak developed force (right) in ferret right ventricular papillary muscle. Data are mean ± SD. \*P < 0.05; \*\*P < 0.01.

## ETOMIDATE AND MYOCARDIAL CONTRACTILITY

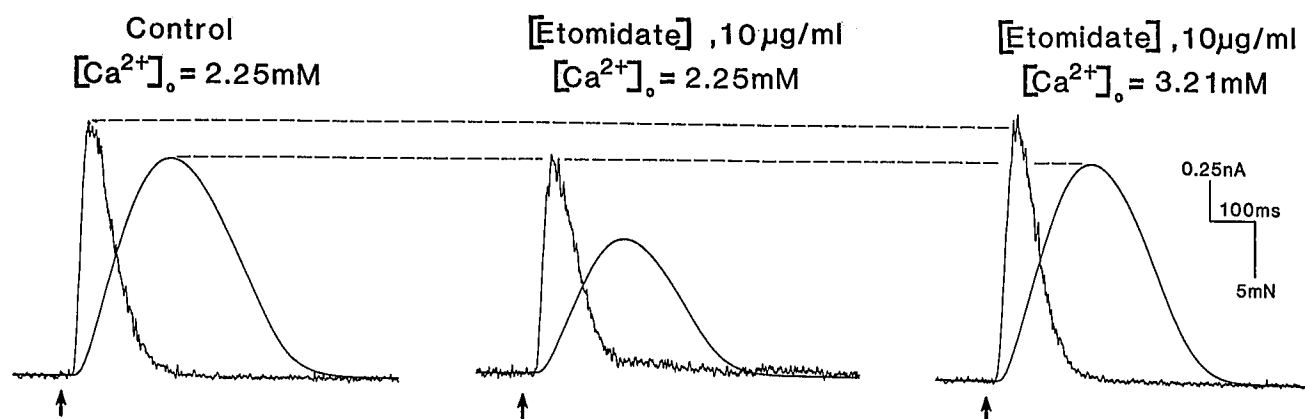


Fig. 4. Aequorin luminescence and force traces of isometric twitches during a typical  $\text{Ca}^{++}$  back-titration experiment. After an initial control (*left*), muscles were exposed to 10  $\mu\text{g}/\text{ml}$  etomidate (*middle*) and  $[\text{Ca}^{++}]_o$  was subsequently rapidly raised (*right*), so that peak force equaled that in the control. At equal peak force with (*right*) and without (*left*) etomidate, there was no difference in peak aequorin luminescence. Sixty-four twitches were averaged in each panel.

ferent contributions to excitation-contraction coupling by SR  $\text{Ca}^{++}$  release and transsarcolemmal  $\text{Ca}^{++}$  entry in rat, ferret, rabbit, and frog ventricular myocardium.

This *in vitro* study clearly demonstrates the negative inotropic effect of etomidate in ferret ventricular myocardium at concentrations of 3  $\mu\text{g}/\text{ml}$  and greater. This concentration is much higher than the free plasma etomidate concentration typically found after an intravenous bolus injection of etomidate.<sup>14</sup> Four minutes after an intravenous bolus injection of 0.3 mg/kg etomidate in humans, the plasma concentration was 0.3  $\mu\text{g}/\text{ml}$ . Yet, because about 75% of etomidate is protein bound, the concentration range studied here must be considered as higher than that encountered clinically. The negative inotropic effect of etomidate, observed in our study, is accompanied by a decrease in

peak aequorin luminescence. The concomitant decrease in the amplitude of contraction and the amplitude of the intracellular  $\text{Ca}^{++}$  transient is most likely caused by a reduced availability of intracellular  $\text{Ca}^{++}$ . This decreased intracellular  $\text{Ca}^{++}$  concentration can result from decreased  $\text{Ca}^{++}$  release by the SR or decreased net transsarcolemmal  $\text{Ca}^{++}$  entry.

To differentiate between these mechanisms, we studied the effects of etomidate in myocardium after SR  $\text{Ca}^{++}$  release was completely abolished by ryanodine pretreatment.<sup>20,21</sup> The plant alkaloid ryanodine binds to specific myocardial receptors,<sup>22</sup> and reduces the availability of  $\text{Ca}^{++}$  from the SR for contractile activation.<sup>23</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>24</sup> or the  $\text{Na}^{+}\text{-Ca}^{++}$  exchanger. The

Table 2. Aequorin Luminescence and Isometric Force Variables in Ferret Papillary Muscle during  $\text{Ca}^{2+}$  Back-titration Experiments in the Presence of Etomidate 10  $\mu\text{g}/\text{ml}$  in Propylene Glycol

	Control [ $\text{Ca}^{2+}$ ] <sub>o</sub> = 2.25 mM	Etomidate 10 $\mu\text{g}/\text{ml}$ [ $\text{Ca}^{2+}$ ] <sub>o</sub> = 2.25 mM	Etomidate 10 $\mu\text{g}/\text{ml}$ [ $\text{Ca}^{2+}$ ] <sub>o</sub> > 2.25 mM
Diastolic aequorin luminescence (nA)	0.91 ± 1.11	0.91 ± 1.06	0.81 ± 1.08
Peak systolic aequorin luminescence (nA)	8.13 ± 4.88	5.09 ± 3.81*	8.87 ± 6.16
Time to peak aequorin luminescence (ms)	52.7 ± 10.9	50.6 ± 8.8	52.6 ± 6.7
Peak developed force (mN · mm <sup>-2</sup> )	23.65 ± 12.40	13.27 ± 5.46*	23.85 ± 12.23
Time to peak force (ms)	205.3 ± 20.6	190.3 ± 18.2†	197.8 ± 20.0*
Time to half isometric relaxation (ms)	126.4 ± 29.1	109.6 ± 22.1†	120.4 ± 26.9*

Data are means ± SD; n = 11 (group 3).

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

†  $P < 0.001$  for comparison with control by Student's paired  $t$  test.



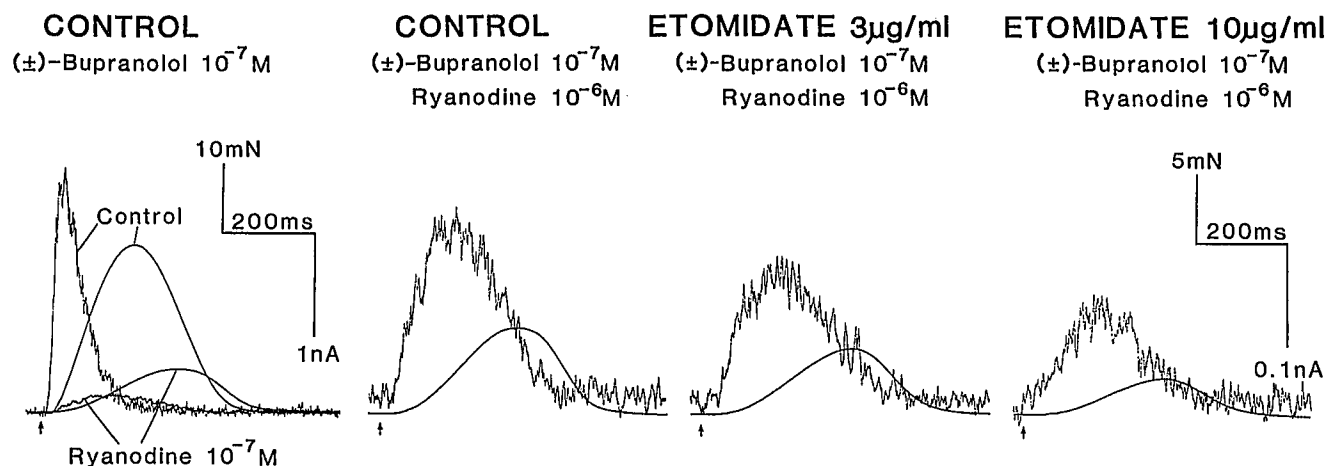


Fig. 5. Effects of etomidate on force and aequorin luminescence after inactivation of SR  $\text{Ca}^{++}$  release by  $10^{-6}$  M ryanodine. The extreme left panel shows superimposed traces of force and of aequorin luminescence during isometric twitches before and after  $10^{-6}$  M ryanodine. Note the change in vertical scales in the other panels that represent the dose-response experiment to etomidate after  $10^{-6}$  M ryanodine. One hundred twenty-eight twitches were averaged in control, and 256 twitches were averaged in  $10^{-6}$  M ryanodine.

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>25</sup> and identical<sup>26</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 ryanodine, etomidate caused a further reduction in contractile variables and peak aequorin luminescence; moreover, the *relative* negative inotropic effect of etomidate was not different from that in muscles with intact SR (fig. 6). This strongly indicates that etomidate exerts a depressant effect on

an alternative source of activator  $\text{Ca}^{++}$ , most likely transsarcolemmal  $\text{Ca}^{++}$  influx. Frog ventricular myocardium has a poorly developed SR, and is almost entirely dependent on transsarcolemmal  $\text{Ca}^{++}$  influx to activate the myofibrillar apparatus.<sup>15</sup> The etomidate-induced decrease in contractility in frog ventricle is, therefore, consistent with an effect of etomidate to decrease net transsarcolemmal  $\text{Ca}^{++}$  influx.

Our findings are in agreement with those of Komai *et al.*,<sup>4</sup> who attributed the negative inotropic effect of etomidate in isolated rabbit papillary muscle primarily to inhibition of transsarcolemmal  $\text{Ca}^{++}$  influx with little

Table 3. Aequorin Luminescence and Contractile Variables in Ferret Papillary Muscle during Dose-Response Experiments to Etomidate in the Presence of Ryanodine

		Ryanodine 10 <sup>-8</sup> M				
		Control	Etomidate 0.3 µg/ml	Etomidate 1 µg/ml	Etomidate 3 µg/ml	Etomidate 10 µg/ml
Aequorin						
luminescence (nA)	17.40 ± 13.24	1.18 ± 0.99	0.99 ± 0.79	0.87 ± 0.72	0.70 ± 0.67*	0.54 ± 0.41*
Time to peak aequorin luminescence (ms)	44.0 ± 2.8	124.17 ± 32.7	116.8 ± 18.7	138.5 ± 19.7	127.5 ± 24.78	110.0 ± 33.4
Peak developed force (mN · mm <sup>-2</sup> )	24.32 ± 11.84	6.03 ± 2.92	5.92 ± 3.24	5.01 ± 3.33	4.12 ± 2.83*	3.03 ± 1.41*
Time to peak force (ms)	196.5 ± 14.0	241.5 ± 32.9	252.3 ± 41.7	248.5 ± 51.5	242.5 ± 56.4	244.4 ± 46.5
Time to half isometric relaxation (ms)	111.3 ± 25.2	98.0 ± 14.5	98.3 ± 14.2	104.0 ± 25.4	101.2 ± 15.3	98.0 ± 12.2

Data are means ± SD; n = 6 (group 4).

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

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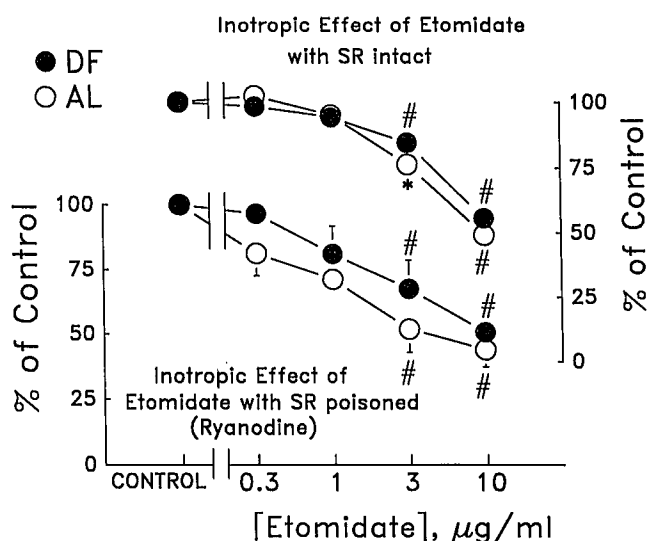


Fig. 6. Effects of etomidate on peak developed force (DF, ●) and peak aequorin luminescence (AL, ○) in ferret right ventricular papillary muscle (mean  $\pm$  SD;  $n = 8$ ) with an intact SR (upper graph; data from group 1) and after inactivation of SR  $\text{Ca}^{++}$  release (lower graph) in muscles (mean  $\pm$  SD;  $n = 6$ ) exposed to  $10^{-6}$  M ryanodine before the etomidate dose-response experiments. \* $P < 0.05$ ; # $P < 0.01$  for comparison with corresponding control.

or no effect on  $\text{Ca}^{++}$  release by the SR. The kinetics of the aequorin luminescence signal are not affected by etomidate up to and including  $3 \mu\text{g/ml}$ , but the decline was prolonged by  $10 \mu\text{g/ml}$  etomidate. This slight prolongation by  $10 \mu\text{g/ml}$  etomidate (but not by propylene glycol alone) may be consistent with a slight inhibition of the removal of  $\text{Ca}^{++}$  from the cytoplasm. At least three mechanisms may be quantitatively involved: (1) the sarcoplasmic reticulum  $\text{Ca}^{++}$  pump; (2) the  $\text{Na}^+/\text{Ca}^{++}$  exchanger; and (3) the sarcolemmal  $\text{Ca}^{++}$  pump. A decreased SR function was indicated<sup>5</sup> by a lengthening of isotonic relaxation and by a decreased postrest potentiation during etomidate exposure in rat papillary muscle, an effect that was not reported in rabbit papillary muscle.<sup>4</sup> It is known, however, that, in contrast to the rabbit, the sarcoplasmic reticulum in rat ventricular cells gains  $\text{Ca}^{++}$  during rest.<sup>27</sup> This may result from a significantly higher intracellular  $\text{Na}^+$  activity at rest in rat than in rabbit ventricle, favoring  $\text{Ca}^{++}$  entry via  $\text{Na}^+/\text{Ca}^{++}$  exchange during rest.<sup>28</sup> The decreased postrest potentiation observed in rat myocardium<sup>5</sup> after 1 min rest may, therefore, as well result from a decreased  $\text{Ca}^{++}$  entry as from a decreased SR function. Because, in rat ventricular myocardium, transsarcolemmal  $\text{Ca}^{++}$  entry plays a smaller role than in ferret

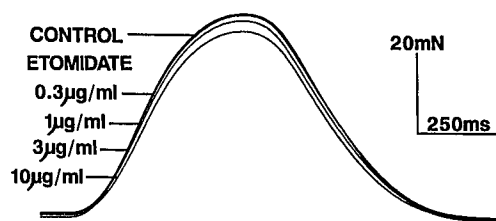


Fig. 7. Frog ventricular myocardium. Effects of etomidate on isometric force development of frog ventricular strips. Force traces of isometric twitch contractions before and during an etomidate dose-response experiment are superimposed.

or rabbit ventricular myocardium, it is not surprising that etomidate has minimal effects on contractility in the rat heart.<sup>5</sup> Although the slower decline of the aequorin luminescence in  $10 \mu\text{g/ml}$  etomidate may be consistent with a slight inhibition of  $\text{Ca}^{++}$  sequestration by the SR, or with an inhibition of other  $\text{Ca}^{++}$  extrusion mechanisms, the time course of the intracellular  $\text{Ca}^{++}$  transient is also determined and influenced by the sensitivity of the myofibrils for  $\text{Ca}^{++}$ . Therefore, it will be necessary to compare drug-induced changes in aequorin luminescence with those found during changes in extracellular  $\text{Ca}^{++}$  concentration.

To determine possible effects of etomidate on myofibrillar  $\text{Ca}^{++}$  sensitivity, we measured aequorin luminescence in "Ca<sup>++</sup> back titration" experiments in which aequorin luminescence was measured in the absence and the presence of  $10 \mu\text{g/ml}$  etomidate, at equal force obtained by adjusting the extracellular  $\text{Ca}^{++}$  concentration upwards in the presence of etomidate. The assumption implicit to this type of analysis is that the

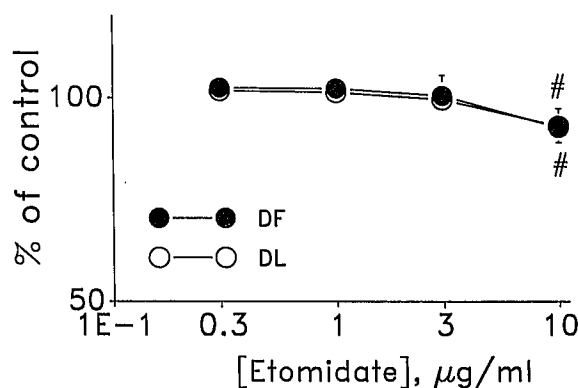


Fig. 8. Frog ventricular myocardium. Effects of ketamine on peak developed force (DF, ●) and peak isotonic shortening (DL, ○) of frog ventricular strips. Values in etomidate (mean  $\pm$  SD;  $n = 8$ ) are expressed as percent of control. # $P < 0.01$  for comparison with corresponding control.

$\text{Ca}^{++}$  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 etomidate 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. However, 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  $[\text{Ca}^{++}]_i$  transient are altered by the intervention, the changes may be impossible to interpret in terms of changes of myofibrillar  $\text{Ca}^{++}$  responsiveness.<sup>9</sup> In our experiments, because time to peak aequorin luminescence was not changed in the  $\text{Ca}^{++}$  back titration experiment, the conclusions based on our experimental data should be valid. Because there was no change in the relation between peak aequorin luminescence and peak force under etomidate, it is unlikely that the myofibrillar  $\text{Ca}^{++}$  sensitivity is changed by etomidate. We observed, however, a small decrease in RTH (time to half-isometric relaxation) during  $\text{Ca}^{++}$ -back titration. This change may be related to an earlier time to peak force, or to a decrease in myofibrillar  $\text{Ca}^{++}$  sensitivity that was not reflected in aequorin luminescence.

The negative inotropic effect of etomidate can, most likely, be attributed to an interference with cellular mechanisms that regulate intracellular  $\text{Ca}^{++}$  availability. The data from the  $\text{Ca}^{++}$  back titration experiments indicates that the slight prolongation of the decline of aequorin luminescence in 10  $\mu\text{g}/\text{ml}$  etomidate is not caused by changes in myofibrillar  $\text{Ca}^{++}$  sensitivity. It would seem more plausible to attribute these changes to an inhibition of one or more of the processes responsible for the removal of  $\text{Ca}^{++}$  from the cytosol during diastole: the sarcoplasmic reticulum  $\text{Ca}^{++}$  pump or the  $\text{Ca}^{++}$  extrusion mechanisms in the sarcolemma.

The results of this study must be interpreted in the context of the experimental conditions in which they were obtained. Results obtained here at 30° C and a stimulus interval of 4 s may differ from those that one could obtain at the more physiologic conditions of the animal, 37–38° C and 200 beats/min. Furthermore, the concentrations of etomidate at which inotropic effects are observed (3  $\mu\text{g}/\text{ml}$ ) are at least tenfold those

observed in humans 4 min after an intravenous bolus of etomidate.<sup>14</sup>

In conclusion, the evidence from the current study indicates that etomidate exerts a mild direct negative inotropic effect by decreasing transsarcolemmal  $\text{Ca}^{++}$  influx. The depressant effect may be caused by an inhibition of the sarcolemmal slow inward L-type  $\text{Ca}^{++}$  current. Possible effects of etomidate on other membranous  $\text{Ca}^{++}$  exchange mechanisms ( $\text{Na}^+/\text{Ca}^{++}$  exchange,  $\text{Ca}^{++}$  ATPase export pump, etc.) cannot be excluded, and may necessitate further study.

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