

In Vitro Effects of Etomidate on Intrinsic Myocardial Contractility in the Rat

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Etomidate is available in two different solvents: propylene glycol for induction of anesthesia and ethanol for maintenance of anesthesia. The direct effect of etomidate (1 and 5 $\mu\text{g/ml}$) and of its solvents on cardiac muscle was studied using rat left ventricular papillary muscle. Etomidate induced a slight positive inotropic effect in both solvents, as shown by an increase in maximum unloaded shortening velocity (V_{max}) but not in force. At 0.5 mM Ca^{++} 5 $\mu\text{g/ml}$ etomidate increased V_{max} ($128 \pm 18\%$, $P < 0.05$) but not force ($103 \pm 16\%$, NS). Using various afterloaded twitches, the peak power output (\dot{E}_{max}) was calculated: 1 and 5 $\mu\text{g/ml}$ etomidate increased \dot{E}_{max} ($107 \pm 8\%$, $P < 0.05$, and $108 \pm 10\%$, $P < 0.05$, respectively). This increase was related to the increase in V_{max} and not in isometric force. Etomidate did not modify the elastic components of papillary muscle, isometric relaxation, and contraction-relaxation coupling under high load. Several findings suggest that etomidate in propylene glycol impaired the sarcoplasmic reticulum (SR) function: 1) it impaired the isotonic relaxation, the contraction-relaxation coupling under low load, and the load sensitivity of relaxation; and 2) it decreased postrest potentiated contraction, which is highly dependent on the SR. Nevertheless, alteration of SR function was only significant at high $[\text{Ca}^{++}]_0$ and the beat-to-beat postrest recovery was not modified, indicating that the deleterious effects on SR function were moderate. The isotonic relaxation (max Vr) was more impaired by etomidate in propylene glycol ($78 \pm 9\%$, $P < 0.001$) and by propylene glycol alone ($69 \pm 9\%$, $P < 0.001$) than by etomidate in ethanol ($97 \pm 12\%$, NS) and by ethanol alone ($92 \pm 8\%$, $P < 0.05$). This suggests that propylene glycol was responsible for the decrease in SR function. Etomidate in propylene glycol thus has a dual action on rat myocardium: 1) a slight positive inotropic effect due to etomidate per se, and 2) a slight decrease in SR function probably related to propylene glycol. However, because etomidate in propylene glycol induced a slight decrease in isometric force under certain experimental conditions (i.e., after isometric stabilization), etomidate in propylene glycol may induce a slight negative inotropic effect in some clinical conditions as a result of its dual action on the myocardium. (Key

words: Anesthetics, intravenous: etomidate. Heart: contractility; papillary muscle; relaxation. Solvents: propylene glycol.)

ETOMIDATE is a short-acting intravenous (iv) anesthetic associated with no significant cardiovascular depression during induction of anesthesia in humans studies.^{1,2} However, an *in vitro* study reported that etomidate is a negative inotropic agent on dog papillary muscle,³ although in that study high doses of etomidate were tested. Komai *et al.*⁴ also found that etomidate had a negative inotropic effect in rabbit papillary muscle at lower concentrations. In view of the conflicting results of *in vivo* and *in vitro* studies, it still remains to be shown whether etomidate is a negative inotropic agent.

To determine the effects of anesthetic agents on myocardial contractility is difficult *in vivo* because of concomitant changes in heart rate, preload, afterload, and CNS activity.⁵ In contrast, the effects of drugs on myocardial contractility may be more accurately evaluated by *in vitro* studies. Moreover, the *in vitro* experimental model used in the present study enabled us to determine the effects of etomidate on cardiac muscle more completely than simply noting the direction and magnitude of changes produced in the strength of contraction (i.e., changes in the fundamental intrinsic mechanical properties of cardiac muscle: contraction, relaxation, contraction-relaxation coupling, energetics, and elastic components).

We conducted an extensive analysis of the intrinsic mechanical properties of rat cardiac papillary muscle *in vitro* during exposure to etomidate in propylene glycol. This preparation of etomidate is that used for the induction of anesthesia, which represents a critical period in the cardiovascular disturbances induced. However, propylene glycol itself has been shown to induce significant cardiovascular changes: 1) reflex stimulation of the vagal nerve and inhibition of efferent sympathetic activity⁶; 2) cardiac arrhythmias⁷; and 3) systemic hypotension.⁸ *In vitro* propylene glycol has been found to lengthen the refractory period⁹ and to decrease the contractile force of isolated rabbit atria.¹⁰ Thus, some of the effects observed with etomidate on rat cardiac papillary muscle may be related to propylene glycol and not to etomidate. Moreover, etomidate exists in two preparations with different solvents: a preparation for induction (etomidate in propylene glycol) and another for maintenance of anesthesia (etomidate in ethanol). We therefore studied the effects of

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Received from the Institut National de la Santé et de la Recherche Médicale, Unité 275, LOA-ENSTA-Ecole Polytechnique, Palaiseau, and the Department of Anesthesiology, C.H.U. Pitié-Salpêtrière, University of Paris VI, Paris, France. Accepted for publication September 19, 1989. Supported in part by a grant from Janssen; Drs. B. Riou and D. Chemla were supported by fellowship grants from the Fondation pour la Recherche Médicale and the Fonds d'Etude des Hôpitaux de Paris. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, New Orleans, October 14-18, 1989.

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etomidate in these two solvents and the specific effects of these solvents on the intrinsic mechanical properties of rat cardiac papillary muscle *in vitro*.

Materials and Methods

EXPERIMENTAL PROTOCOL

Care of the animals conformed to the recommendations of the Helsinki Declaration, and the study was authorized by our institution (INSERM).

After brief anesthesia with ether, hearts were quickly removed from adult male Wistar rats weighing 340–400 g. Left ventricular papillary muscles ($n = 56$) were carefully excised and suspended vertically in 60 ml Krebs-Henseleit bicarbonate buffer solution containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.1 mM KH_2PO_4 , 25 mM NaHCO_3 , 2.5 mM $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, and 4.5 mM glucose. Preparations were field-stimulated at 0.12 Hz by two platinum electrodes with rectangular wave pulses of 5 ms duration just above threshold. The bathing solution was bubbled with 95% O_2 –5% CO_2 , resulting in a pH of 7.40, and the temperature was maintained at 29° C. After a 1-h stabilization period at L_{max} (*i.e.*, the initial muscle length at the apex of the length-active isometric tension curve), papillary muscles recovered their optimal mechanical performance, which remained stable for many hours. Suitable preparations were selected as previously reported.¹¹

Control values of each mechanical parameter were recorded, and etomidate (Hypnomidate, Janssen) was then added to the bathing solution. Rat papillary muscles were divided into six groups. In group 1 ($n = 10$) 1 $\mu\text{g}/\text{ml}$ (4 $\mu\text{M}/\text{l}$) etomidate was tested at 2.5 mM $[\text{Ca}^{++}]_0$. In group 2 ($n = 10$) 5 $\mu\text{g}/\text{ml}$ (20 $\mu\text{M}/\text{l}$) etomidate was tested at 2.5 mM $[\text{Ca}^{++}]_0$. In group 3 ($n = 8$) $[\text{Ca}^{++}]_0$ was decreased from 2.5 to 0.5 mM, and etomidate, 1 $\mu\text{g}/\text{ml}$ followed by 5 $\mu\text{g}/\text{ml}$, was tested. $[\text{Ca}^{++}]_0$ was decreased for the following reasons: 1) because rat myocardial contractility is nearly maximum at 2.5 mM $[\text{Ca}^{++}]_0$,⁹ it is difficult to quantify a positive inotropic effect without decreasing $[\text{Ca}^{++}]_0$ to 0.5 mM,^{11,12} and 2) a postrest contraction study is more sensitive in the rat at a low $[\text{Ca}^{++}]_0$.^{11,13} In group 4 ($n = 10$) propylene glycol alone was tested at a concentration (0.9 mg/ml = 14 $\mu\text{M}/\text{l}$) corresponding to that obtained with 5 $\mu\text{g}/\text{ml}$ of etomidate in group 2 and at 2.5 mM $[\text{Ca}^{++}]_0$. In group 5 ($n = 9$) 5 $\mu\text{g}/\text{ml}$ etomidate in ethanol was tested at 2.5 mM $[\text{Ca}^{++}]_0$. In group 6 ($n = 9$) ethanol was tested alone at a concentration (32 ng/ml = 0.7 nM/l) corresponding to that obtained with 5 $\mu\text{g}/\text{ml}$ etomidate in group 5 and at 2.5 mM $[\text{Ca}^{++}]_0$. Concentrations of etomidate during anesthesia range from 0.2 to 2 $\mu\text{g}/\text{ml}$ ^{14,15}; nonetheless, because about 75% of etomidate is protein bound, the concentrations tested in

the present study must be considered as high (respectively, twofold and tenfold the maximum therapeutic concentration of free etomidate).

Changes in mechanical parameters were studied up to 30 min after the addition of etomidate to the bathing solution because a preliminary study showed that the effects of etomidate remain stable after 30 min.

ELECTROMAGNETIC LEVER SYSTEM AND RECORDING

The electromagnetic lever system has been previously described.¹⁶ Briefly, the load applied to the muscle was determined by means of a servocontrolled current through the coil of an electromagnet. Muscular shortening induced a displacement of the lever, which modulated the light intensity of a photoelectric transducer. All analyses were made from digital records obtained with a Hewlett Packard 1000 computer as previously described.¹¹

MECHANICAL PARAMETERS

Conventional mechanical parameters at L_{max} were calculated from four twitches. The first twitch was isotonic and loaded with the preload corresponding to L_{max} . The second twitch was abruptly clamped to zero-load just after the electrical stimulus; the maximum unloaded shortening velocity (V_{max}) was determined from this twitch. The third twitch was fully isometric at L_{max} . The fourth twitch was isotonic and was afterloaded to half-value of the isometric active force at L_{max} (fig. 1).

The mechanical parameters characterizing the contraction and relaxation phases, the coupling between contraction and relaxation, and the load sensitivity of relaxation are defined as follows (fig. 1):

Contraction phase. Maximum extent of shortening (ΔL) in the twitch with preload only; maximum unloaded shortening velocity (V_{max}) determined using the zero-load clamp technique¹⁷; maximum shortening velocity of the twitch with preload only (max Vc); maximum isometric active force normalized per cross-sectional area (AF/s); peak of the positive force derivative per mm^2 ($+dF \cdot dt^{-1} \cdot s^{-1}$). The maximum shortening velocity (V_{max}) and the maximum isometric active force (AF/s) tested the inotropic state under low and high load, respectively.

Relaxation phase. Maximum lengthening velocity of the twitch with preload only (max Vr) and the peak of the negative force derivative at L_{max} normalized per cross-sectional area ($-dF \cdot dt^{-1} \cdot s^{-1}$). These two parameters tested the lusitropic state^{18,19} under low and high loading conditions, respectively. Because changes in the contraction phase induce coordinated changes in the relaxation phase, variations of contraction and relaxation must be simultaneously considered to quantify drug-induced

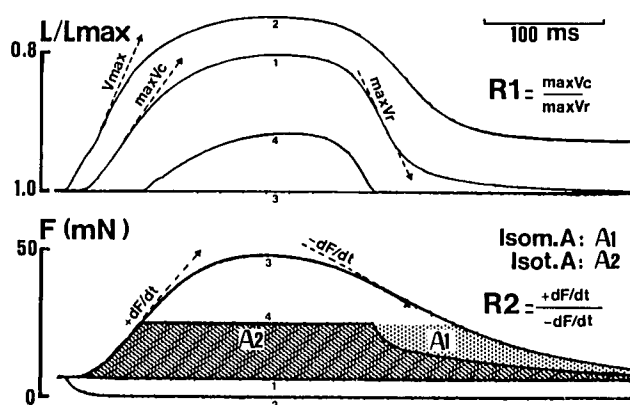


FIG. 1. Mechanical parameters of contraction and relaxation. (Upper graph), muscle shortening length (L/L_{\max}) plotted versus time. (Lower graph), force (F) plotted versus time. Twitch 1 was loaded with preload only at L_{\max} . Twitch 2 was loaded with same preload as twitch 1 and was abruptly clamped to zero-load with critical damping just after stimulation. Twitch 3 was fully isometric, and twitch 4 was loaded at 50% of the isometric active force. The index used to quantify the load sensitivity of relaxation was the ratio of two areas: the areas IsotA and IsomA were limited by force versus time curves of twitch 4 and 3, respectively, and below 50% of active force level. The isometric area (A1) includes the isotonic area (A2). This index approaches 1 when load sensitivity disappears.

changes in lusitropy. Indices of contraction-relaxation coupling were therefore developed.^{11,19,20}

Contraction-relaxation coupling. Coefficients $R1 = V_{c_{\max}}/V_{r_{\max}}$ and $R2 = +dF \cdot dt^{-1} / -dF \cdot dt^{-1}$ test the coupling between contraction and relaxation under low and high load, respectively.

Load sensitivity of relaxation. The concept of load sensitivity reflects the capacity of mammalian heart muscle to regulate the time course of relaxation according to loading conditions. In a typical load-sensitive papillary muscle, isometric relaxation of an afterloaded twitch occurs earlier than the superimposed relaxing phase of the fully isometric twitch. In a load-insensitive muscle, superimposed isometric relaxation phases almost coincide in time, irrespective of the level of afterload. Load sensitivity of mammalian cardiac relaxation is thought to reflect the presence of an effective calcium sequestering system, and in particular the sarcoplasmic reticulum (SR). The load sensitivity of relaxation is quantified by the ratio of two areas (IsotA/IsomA).²¹ This ratio (IsotA/IsomA) ranges from approximately 0.75 for a typical load-sensitive relaxation to 1 for a typical load-insensitive relaxation and provides a precise scale of measurement of load sensitivity (fig. 1).

Shortening and lengthening velocities were expressed in L_{\max}/s , force in mN/mm^{-2} , force derivative in $mN \cdot s^{-1} \cdot mm^{-2}$, and time in ms.

DETERMINATION OF HILL'S EQUATION

The force-velocity curve was derived from the peak shortening velocity (V) of seven afterloaded twitches plotted against the total force normalized per cross-sectional area (TF/s), and from that of the zero-load clamp twitch. Data were fitted according to Hill's equation²²:

$$(TF/s + a)(V + b) = (cF_{\max}/s + a)b \quad (1)$$

where a and b are the constants of the hyperbola. The peak power output (\dot{E}_{\max}), curvature of the hyperbola (G), calculated maximum shortening velocity (cV_{\max}), and calculated maximum total force normalized per cross-sectional area (cF_{\max}/s) were derived from Hill's equation as previously described.²³

DETERMINATION OF ELASTIC PARAMETERS

The resting force (RF/s) normalized per cross-sectional area was determined in five fully isometric twitches performed at various initial lengths from L_{\max} to $0.80 L_{\max}$ (L_{\max} , $-5\% L_{\max}$, $-10\% L_{\max}$, $-15\% L_{\max}$, and $-20\% L_{\max}$). Because the volume of papillary muscle during this determination was assumed to be constant, the computer corrected for the increase in cross-sectional area as L_{\max} decreased. The RF/s initial length curve was used to determine the passive elastic parameters. The RF/s was plotted against the initial muscle length (from L_{\max} to $0.8 L_{\max}$). Data were fitted according to an exponential curve as described as follows:

$$RF/s = c \cdot \exp[-d(L_{\max} - L)/L_{\max} - 1] \quad (2)$$

where c and d are constants. Coefficient d reflects the passive elastic components of papillary muscle.

POSTREST CONTRACTION

Recovery of a stable, reproducible isometric contraction after a rest interval was studied to identify the effects of etomidate on both sources of myofibrillar activating calcium: sarcolemma and SR. During rest in the rat, SR accumulates additional calcium above and beyond that accumulated with regular stimulation, and the first beat after the rest interval (B1) is more forceful than the last beat before the rest interval (B0).^{13,24} During the stimulation of the postrest recovery (B1, B2, B3 · · ·), the SR-dependent part of activator calcium decreases somewhat toward a steady state, which is reached in a few beats. Therefore, the effects of etomidate on the postrest-potentiated contraction may provide insight in a biochemically unaltered preparation on the effects of etomidate on the SR function. As previously described,⁷ the maximum isometric active force (AF/s) during postrest recovery was studied at $0.5 \text{ mM } [Ca^{++}]_o$ and after a 1-min rest

duration. The rate constant, τ , of the exponential decay of AF/s was determined as previously described.¹¹ τ was assumed to represent the time required for the SR to reset itself²⁴ and was therefore used to test the SR function.¹¹

STATISTICAL ANALYSIS

Data were expressed as mean \pm SD. Comparisons with control values were performed using the paired Student's *t* test. The Bonferroni correction was used for comparison of several means. Comparison between groups was performed by analysis of variance and Student's *t* test for unpaired data with the Bonferroni correction. Analysis of covariance was used to compare the velocity-length relationships.

To determine the Hill's equation parameters, equation 1

$$(F/s + a)(V + b) = (cF_{\max}/s + a)b \quad (1)$$

was linearized as follows:

$$F/s \cdot V = (a \cdot V) - (b \cdot F/s) + (b \cdot cF_{\max}/s) \quad (3)$$

where *a* and *b* are the constant of the hyperbola and cF_{\max}/s the calculated maximum isometric total force normalized per cross-sectional area. Multiple linear regression was performed using the least squares method.

The beat-to-beat decay of active isometric force during postrest recovery was plotted against the number of beats and fitted to an exponential curve, and regression was performed using the least squares method. Comparison of the slope of the regression curve was performed using Student's *t* test. All comparisons were two-tailed and a *P* value less than 0.05 was necessary to reject the null hypothesis.

Results

There were no statistical differences between control values for L_{\max} , cross-sectional area (*s*), ratio of resting force and total isometric force (RF/TF), contraction-relaxation coupling under low load (R1), and the load sensitivity of relaxation (IsotA/IsomA) among the six groups of papillary muscles. The mean value \pm SD (range in parentheses) of these parameters (*n* = 56) were as follows: L_{\max} , 5.6 ± 1.2 (3.5–9.0) mm; *s*, 0.89 ± 0.13 (0.64–1.20) mm²; RF/TF, 0.17 ± 0.04 (0.09–0.28); R1, 0.68 ± 0.09 (0.46–0.85); and IsotA/IsomA, 0.73 ± 0.05 (0.60–0.84).

At high $[Ca^{++}]_o$ both etomidate concentrations in propylene glycol induced a slight positive inotropic effect as shown by an increase in maximum shortening velocity (V_{\max}) with no change in maximum isometric force (AF/*s*) (table 1). Etomidate impaired isotonic relaxation (max V_r), contraction-relaxation coupling under low load (increase in R1), and slightly decreased the load sensitivity of relaxation (IsotA/IsomA) (table 1). Isometric relaxation ($-dF \cdot dt^{-1} \cdot s^{-1}$) and contraction-relaxation coupling under heavy load (R2) were not modified by etomidate (table 1).

At each level of load, peak shortening velocity (V_c) is related to the maximum extent of shortening (ΔL). There is a linear relationship between V_c and the end-shortening length ($ESL = L_{\max} - \Delta L$). However, the end shortening length seems to be one of the main determinants of the peak lengthening velocity (V_r), and there is a linear relationship between V_r and ESL.²⁵ Therefore, from pre-load to the fully isometric twitch, *i.e.*, in the physiologic range of function, we studied various afterloaded twitches and calculated ESL, V_c , and V_r for each of them. We then examined the V_c/ESL and V_r/ESL relationships

TABLE 1. Effects of Etomidate on the Intrinsic Mechanical Properties of Papillary Muscle ($[Ca^{++}]_o = 2.5$ mM)

	Group 1 (n = 10)			Group 2 (n = 10)		
	Control	Etomidate 1 μ g/ml	% Control	Control	Etomidate 5 μ g/ml	% Control
Contraction						
V_{\max} (L_{\max}/s)	3.31 ± 0.22	3.54 ± 0.38	$107 \pm 6^*$	3.03 ± 0.25	3.29 ± 0.21	$109 \pm 7^*$
max V_c (L_{\max}/s)	2.39 ± 0.23	2.61 ± 0.25	$109 \pm 7^*$	2.04 ± 0.20	2.23 ± 0.20	$109 \pm 7^*$
AF/ <i>s</i> (mN/mm ²)	5.43 ± 0.97	5.56 ± 0.86	103 ± 5	4.90 ± 1.14	4.91 ± 0.93	101 ± 9
ΔL	0.20 ± 0.02	0.20 ± 0.02	102 ± 7	0.19 ± 0.02	0.20 ± 0.02	$105 \pm 3^*$
$+dF \cdot dt^{-1} \cdot s^{-1}$ (mN \cdot s ⁻¹ \cdot mm ⁻²)	65.8 ± 13.5	74.4 ± 16.7	$113 \pm 7^\dagger$	58.7 ± 18.4	62.0 ± 13.6	109 ± 13
Relaxation						
max V_r (L_{\max}/s)	3.27 ± 0.48	2.15 ± 0.62	$75 \pm 7^\dagger$	2.79 ± 0.32	2.16 ± 0.28	$78 \pm 9^\dagger$
$-dF \cdot dt^{-1} \cdot s^{-1}$ (mN \cdot s ⁻¹ \cdot mm ⁻²)	23.5 ± 6.7	24.4 ± 4.4	105 ± 18	19.8 ± 4.2	21.0 ± 4.1	108 ± 18
Contraction-relaxation coupling						
R1 (low load)	0.70 ± 0.07	1.03 ± 0.14	$147 \pm 14^\dagger$	0.74 ± 0.07	1.05 ± 0.12	$143 \pm 16^\dagger$
R2 (high load)	2.94 ± 0.81	3.07 ± 0.56	107 ± 16	2.97 ± 0.72	3.02 ± 0.80	102 ± 8
Load sensitivity of relaxation						
IsotA/IsomA	0.73 ± 0.05	0.79 ± 0.03	$109 \pm 6^*$	0.75 ± 0.04	0.82 ± 0.04	$110 \pm 5^\dagger$

Values are mean \pm SD.

* *P* < 0.01.

$^\dagger P < 0.001$.

before and after etomidate to compare shortening and lengthening velocities at a given end shortening length. As shown in figure 2, when the papillary muscle contracted and relaxed against various levels of load, etomidate in propylene glycol slightly increased shortening velocity and markedly decreased lengthening velocity, even when simultaneous changes in end shortening length were considered.

In group 3 a decrease in contractility was observed as $[Ca^{++}]_o$ was lowered from 2.5 to 0.5 mM: V_{max} and AF/s decreased ($61 \pm 11\%$ and $53 \pm 8\%$ of the value at a $[Ca^{++}]_o$ of 2.5 mM, respectively). At a low $[Ca^{++}]_o$ both etomidate concentrations had a positive inotropic effect

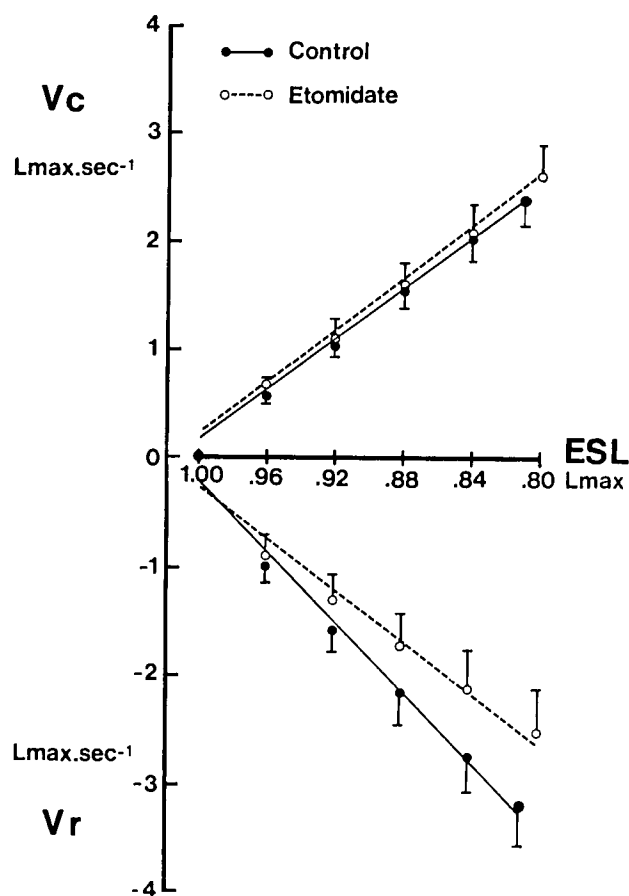


FIG. 2. Effects of 5 $\mu\text{g/ml}$ etomidate on shortening (V_c) and lengthening (V_r) velocities of various afterloaded twitches (from preload up to fully isometric twitch) in group 2 ($n = 10$). The end shortening length (ESL) increases as the load increases. The twitch with preload only corresponded to an ESL of $0.80 L_{max}$, and the isometric twitch corresponded to an ESL of $1.00 L_{max}$. The equation of the regression curve (V against ΔL) was determined for each muscle, and the velocity for a given ESL (discontinuous values from 1.00 to 0.80) was calculated from this regression curve. Thereafter the mean velocity for a given ESL was used for the determination of the regression curves indicated. Data are mean \pm SD. Analysis of covariance showed that the increase in V_c and the decrease in V_r were significant.

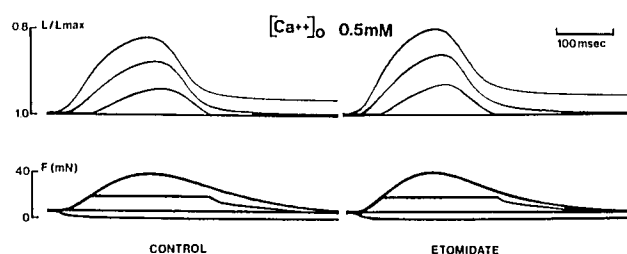


FIG. 3. Effects of 5 $\mu\text{g/ml}$ etomidate on a typical papillary muscle at a low $[Ca^{++}]_o$. (Upper graph), muscle shortening length (L/L_{max}) plotted versus time. (Lower graph), force (F) plotted versus time. This figure shows that etomidate increased the isotonic shortening velocity but not the active isometric force. Unlike at high $[Ca^{++}]_o$, etomidate did not impair isotonic relaxation at low $[Ca^{++}]_o$.

as shown by a significant increase in maximum unloaded shortening velocity (V_{max}) with no change in maximum isometric force (AF/s) (figs. 3 and 4). At a low $[Ca^{++}]_o$ 1 and 5 $\mu\text{g/ml}$ etomidate did not significantly modify contraction-relaxation coupling either under low load ($R1$, $105 \pm 8\%$ and $98 \pm 18\%$, respectively) or under high load ($R2$, $98 \pm 11\%$ and $103 \pm 16\%$, respectively) (fig. 3).

The energetics of papillary muscle were determined from the force-velocity curve. The peak power output (\dot{E}_{max}) was increased after etomidate and the curvature of the hyperbola (G) remained unchanged (table 2). This increase in \dot{E}_{max} was due to an increase in shortening velocity with no change in force. The calculated maximum shortening velocity (cV_{max}) increased after etomidate, whereas the calculated maximum total force (cF_{max}/s) remained unchanged (table 2, fig. 5).

The passive elastic components of papillary muscles were studied using the resting force (RF/s)-length curve. This curve fitted well with an exponential decay ($0.905 < R < 0.996$). The two coefficients of this exponential

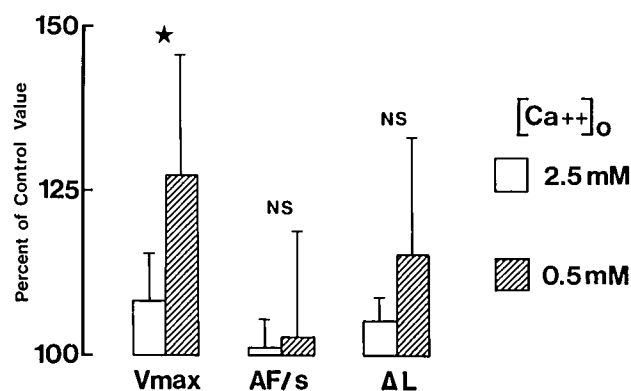


FIG. 4. Comparison of the positive inotropic effect of etomidate 5 $\mu\text{g/ml}$ at high (group 2, $n = 10$) and low (group 3, $n = 8$) $[Ca^{++}]_o$. Data are expressed as mean percent of control values \pm SD. $*P < 0.05$.

TABLE 2. Effects of Etomidate on Papillary Muscle Energetics

	cV_{\max} (L_{\max}/s)	cF_{\max}/s (mN/mm^2)	G	\dot{E}_{\max} ($mN \cdot L_{\max} \cdot s^{-1} \cdot mm^{-3}$)
Group 1 (n = 10)				
Control	3.30 ± 0.25	68.2 ± 10.9	1.44 ± 0.31	34.2 ± 0.39
Etomidate 1 $\mu g/ml$	$3.55 \pm 0.38^*$	68.7 ± 10.2	1.47 ± 0.30	$36.7 \pm 0.51^*$
Group 2 (n = 10)				
Control	3.06 ± 0.25	65.9 ± 12.1	1.39 ± 0.34	31.2 ± 6.5
Etomidate 5 $\mu g/ml$	$3.33 \pm 0.30^*$	65.6 ± 9.7	1.43 ± 0.35	$33.6 \pm 5.7^*$

Values are mean \pm SD.* $P < 0.05$ versus control values; there were no statistical differences in control values between groups (Student's *t* test for unpaired data).

curve (c and d) remained unchanged with etomidate (table 3).

Postrest recovery was studied at 0.5 mM $[Ca^{++}]_o$ and during an isometric beating period. Both etomidate concentrations decreased the maximum active force of the potentiated contraction B1 (fig. 6). In this protocol papillary muscles were stabilized in isometric conditions and a slight decrease in B0 was observed (fig. 6). The decrease in B1 was more pronounced than the decrease in B0 with 1 $\mu g/ml$ ($-14.6 \pm 4.2\%$ vs. $-7.7 \pm 4.6\%$ of control values) and with 5 $\mu g/ml$ etomidate ($-17.5 \pm 5.8\%$ vs. $-8.7 \pm 6.2\%$ of control values). The decay in the active isometric force during the postrecovery period fitted well with an exponential decay ($0.998 < R < 0.999$), and the control value of the rate constant (τ) was consistent with our previous study¹¹; 1 and 5 $\mu g/ml$ etomidate did not significantly modify τ (3.4 ± 0.2 and 3.3 ± 0.1 beats, respectively) compared with its control value (3.7 ± 0.4 beats) (fig. 7).

The role of solvents was studied in groups 2, 4, 5, and 6. Etomidate (either in propylene glycol or in ethanol) induced a slight positive inotropic effect as shown by an increase in maximum shortening velocity (V_{\max}) with no change in maximum isometric force (AF/s) (fig. 8), whereas the two solvents alone did not modify these parameters of inotropy. The positive inotropic effect of etomidate was more pronounced with ethanol than with propylene glycol.

TABLE 3. Effects of Etomidate on the Passive Elastic Components of Papillary Muscle

	c (mN/mm^2)	d (mN/mm^2)	Coefficient of Regression
Group 1 (n = 10)			
Control	0.22 ± 0.25	0.109 ± 0.010	0.98 ± 0.01
Etomidate 1 $\mu g/ml$	0.23 ± 0.21	0.101 ± 0.012	0.97 ± 0.01
Group 2 (n = 10)			
Control	0.21 ± 0.20	0.106 ± 0.008	0.96 ± 0.03
Etomidate 5 $\mu g/ml$	0.21 ± 0.20	0.107 ± 0.007	0.96 ± 0.02

Values are mean \pm SD; no differences were statistically significant. (Student's *t* test for paired data).

Etomidate in propylene glycol and propylene glycol alone impaired isotonic relaxation (max Vr) and contraction-relaxation coupling under low load (increase in R1) (fig. 9). This increase in R1 was related both to an increase in max Vc (positive inotropic effect) and a decrease in max Vr (negative lusitropic effect) with etomidate in propylene glycol, whereas it was only related to a decrease in max Vr with propylene glycol alone. Etomidate in ethanol slightly increased R1, but this increase was only due to an increase in max Vc because isotonic relaxation (max Vr) did not change.

Contraction-relaxation coupling under heavy load (R2) was modified in none of the groups (fig. 10). Nevertheless, etomidate in ethanol significantly increased $+dF \cdot dt^{-1} \cdot s^{-1}$ and $-dF \cdot dt^{-1} \cdot s^{-1}$ in comparison with etomidate in propylene glycol.

Discussion

INOTROPIC EFFECT

In humans etomidate does not modify the heart rate and only induces a slight decrease or no change in arterial

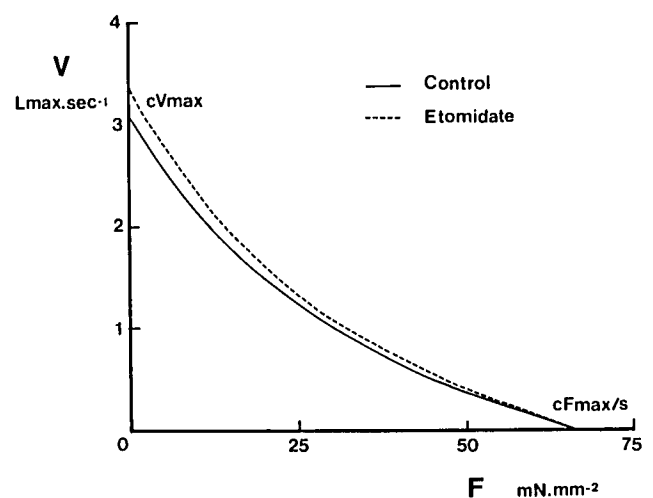


FIG. 5. Effect of 5 $\mu g/ml$ etomidate on the force (F)-velocity (V) curve (Hill's hyperbola). The data from papillary muscles of group 2 (n = 10) were used for this figure.

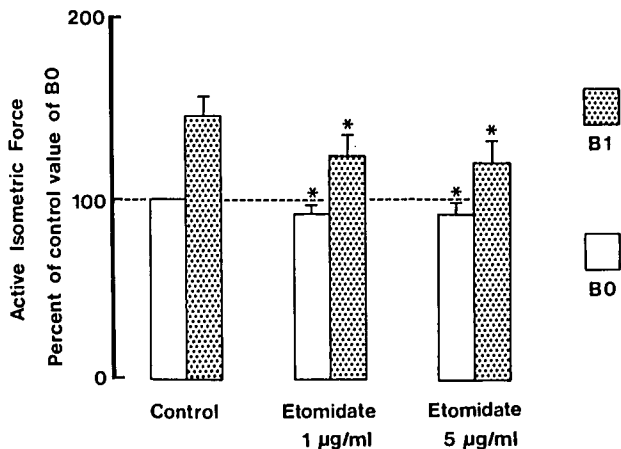


FIG. 6. Effect of etomidate on the active isometric force of the postrest potentiated contraction. B0 is the beat before rest, B1 is the first beat after rest. These data were obtained in isometrically contracting papillary muscle. The dashed line indicates 100%. Data are mean percent \pm SD. Comparison with control values: * $P < 0.05$.

blood pressure.^{1,2,26,27} These minor hemodynamic disturbances during induction of anesthesia with etomidate were reported in patients with^{2,26} or without cardiac disease.^{1,27} Kettler *et al.*²⁸ reported that etomidate did not modify the maximum rise in the rate of left ventricular pressure (dP/dt_{max}) and slightly increased the cardiac index. Brückner *et al.*¹ observed an increase in cardiac index (+4%) but a decrease in dP/dt_{max} (-9%). However, in this study the simultaneous decrease in heart rate (-4%) and arterial pressure (-14%) may explain the decrease in dP/dt_{max} , which varies with the inotropic status but also with afterload and heart rate. Using the preejection period and the left ventricular ejection time to evaluate the left ventricular contractility, Doenicke *et al.*²⁹ concluded that etomidate, unlike propanidid and methohexital, did not modify myocardial contractility. In the pig Prakash *et al.*³⁰ reported a dose-dependent decrease in cardiac output, dP/dt_{max} , and myocardial wall thickening with etomidate. Nevertheless, at the lowest dose (0.03 mg/kg) tested, equivalent to that required for surgical anesthesia, the changes in these cardiovascular parameters were minor. Moreover, the authors³⁰ concluded that these minor effects were probably related to a decrease in central sympathetic activity and a decrease in metabolic requirements, but not to a direct depressant effect on the myocardium. This study was not entirely convincing because it was performed in anesthetized and not conscious animals.⁵ However, *in vivo* evaluation of myocardial contractility is difficult because none of the parameters of contractility appear to be actually independent of preload, afterload, and heart rate.^{5,31} In contrast, myocardial contractility can be more accurately measured on isolated heart preparations.

In vitro studies^{3,4,32} have given results that apparently conflict with others or with clinical studies. The concentration of etomidate required to decrease the myocardial contractility in cat heart-lung preparation exceeded 15 μ g/ml,³² a high concentration never achieved during anesthesia. Two recent *in vitro* studies^{3,4} have reported that etomidate is a negative inotropic agent. Both Kissin *et al.*,³ who used high concentrations of etomidate (10–200 μ g/ml) on dog papillary muscle, and Komai *et al.*⁴ who tested lower concentrations (2 and 4 μ g/ml) on rabbit papillary muscle, concluded that etomidate is a negative inotropic agent.

Our study demonstrated that etomidate in rat had a slight positive inotropic effect because it increased the maximum unloaded shortening velocity (V_{max}) and did not change the maximum isometric force (fig. 4). However, when papillary muscles were allowed to contract isometrically (as in previous experimental studies^{3,4}) during the postrest potentiation study, etomidate slightly decreased the maximum isometric force (fig. 7). These apparently conflicting results may simply be related to dif-

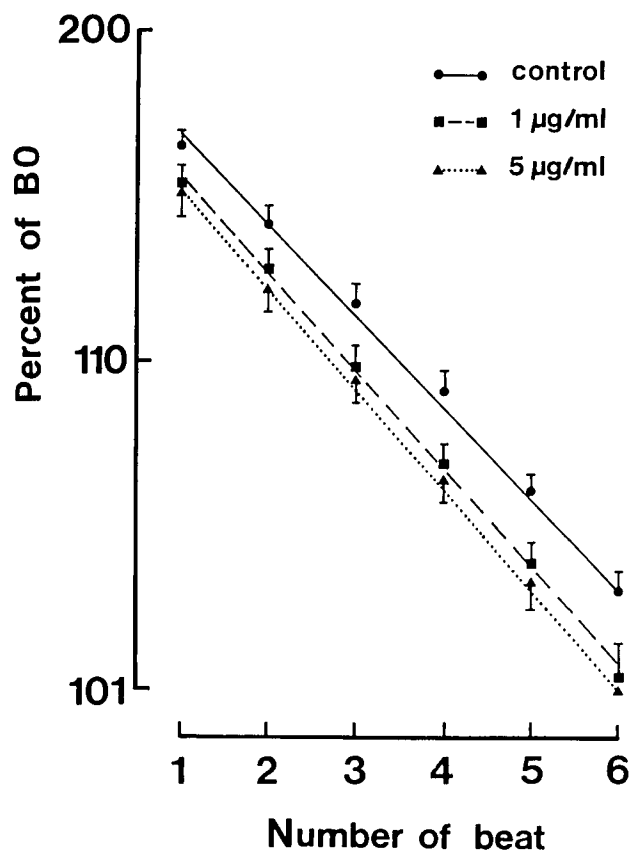


FIG. 7. Effect of etomidate on the decay of mean active isometric force during the postrest recovery period. Data are expressed as mean percent of B0 (the beat before rest) \pm SEM, and plotted on a semilogarithmic scale.

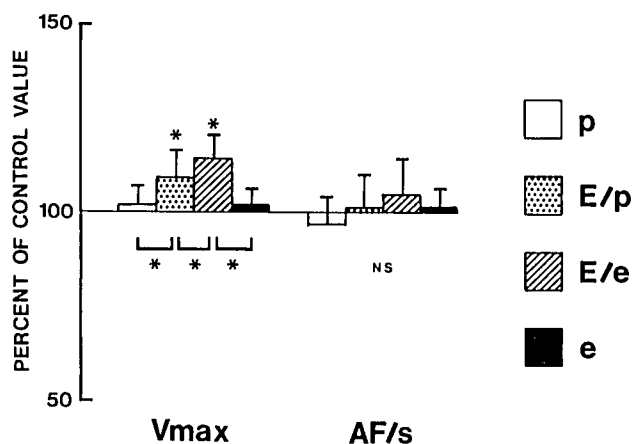


FIG. 8. Effects of etomidate (5 µg/ml) and its solvents on the maximum unloaded shortening velocity (V_{\max}) and the maximum isometric active force (AF/s). E/p = etomidate in propylene glycol; p = propylene glycol; E/e = etomidate in ethanol; e = ethanol. Values are mean \pm SD. * $P < 0.05$.

ferences in the experimental protocol used. Previous mechanical events influence the contractility of papillary muscle,³³ and it has been shown that the isometric force developed after a period of isometric beating is less than that developed after a period of isotonic beating.³⁴ The precise mechanism of this observation is not completely known, although it may partly be due to changes in SR calcium stores or myofilament sensitivity to calcium, which could depend on either isotonic or isometric stabilization.³⁴ Our findings provided additional evidence that it is important to study mechanics of isolated papillary muscles under various isotonic afterloaded conditions, and not only in isometric condition. Because we and others^{3,4} have observed that under certain experimental conditions (*i.e.*, after an isometric period of beating), etomidate may slightly decrease isometric force, it might induce a slight negative inotropic effect in some clinical conditions.

Etomidate induced a positive inotropic effect in both solvents, propylene glycol and ethanol. Because these two solvents induced no positive inotropic effect when used alone (fig. 8), the observed inotropic effect was therefore due to etomidate per se. The positive inotropic effect of etomidate was significantly more pronounced with ethanol than with propylene glycol. This was corroborated by the increase in maximum rate of rise of force ($+dF \cdot dt^{-1} \cdot s^{-1}$) which only reached statistical significance with etomidate in ethanol (fig. 10).

MECHANISM OF THE POSITIVE INOTROPIC EFFECT

The most striking result of our study was that etomidate increased the maximum shortening velocity (V_{\max}) but not the maximum active force (AF/s). Such a result was

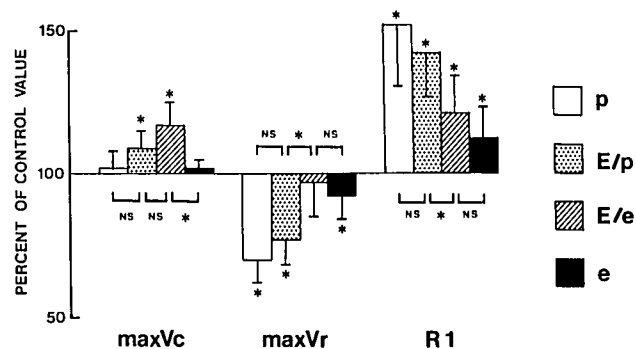


FIG. 9. Effects of etomidate (5 µg/ml) and its solvents on the maximum shortening (max Vc) and lengthening (max Vr) velocities, and on the contraction-relaxation coupling under low load ($R1 = \max Vc / \max Vr$). Values are mean percent \pm SD. * $P < 0.05$.

not surprising at a high $[Ca^{++}]_o$. Because the contractility of rat myocardium is nearly maximum,¹² it is difficult to observe a positive inotropic effect at this calcium concentration, especially during the isometric twitch.¹¹ As the $[Ca^{++}]_o$ was decreased, contractility decreased allowing the positive inotropic effect to be quantified. Even at a low $[Ca^{++}]_o$, however, etomidate increased the maximum unloaded shortening velocity without any significant change in maximum isometric force (fig. 5). Etomidate increased the peak power output (\dot{E}_{\max}), a fundamental energetic parameter derived from the Hill's hyperbola (table 2). This increase in \dot{E}_{\max} was related to an increase in V_{\max} and not in isometric force as shown in figure 5 and table 2. Most positive inotropic agents act by increasing the intracellular calcium available to the myofilaments that modulate the number and/or kinetics of cross-bridges. The absence of increase in force suggested that the number of cross-bridges remains unchanged and therefore that etomidate does not increase the quantity of calcium available to the myofilaments. Four hypotheses

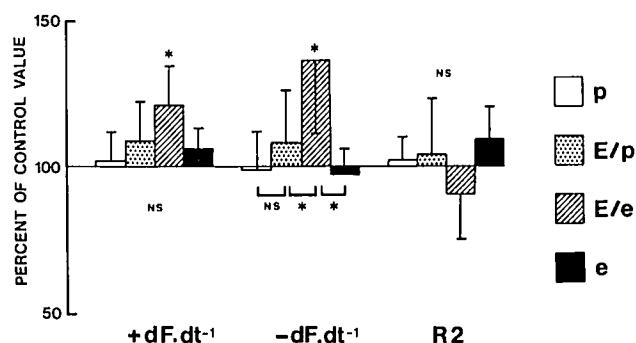


FIG. 10. Effects of etomidate (5 µg/ml) and its solvents on the maximal rate of increase ($+dF \cdot dt^{-1}$) and decrease ($-dF \cdot dt^{-1}$) of force of the isometric twitch and on contraction-relaxation coupling under heavy load. Values are mean percent \pm SD. * $P < 0.05$.

may explain this dissociation between the changes observed in V_{\max} and force: 1) a modification of elastic components; 2) a modification in initial muscle length; 3) a change in myofilament calcium sensitivity; and 4) an increase in cross-bridge kinetics.

1. The increase in velocity (and not in force) was probably not related to a modification of the elastic components of the papillary muscle because the resting force-length curve was unchanged (table 3).

2. The increase in velocity was not related to changes in initial muscle length for the following reasons: 1) the mechanical apparatus (length and force transducer) allows a precise control of the initial length; and 2) the maximum unloaded shortening velocity (V_{\max}) does not depend on initial muscle length for a wide range of initial lengths.³⁵

3. Some findings suggest that the sensitivity of myofilaments to calcium was unchanged with etomidate. The contraction-coupling under heavy load (R2) was not modified by etomidate. Under isometric conditions the amplitude of sarcomere shortening is lower than under isotonic conditions, and the sensitivity of the myofilaments to calcium is higher than under isotonic conditions and becomes the limiting step that appears to play a major role in the regulation of the time course of the isometric relaxation.³⁶ Calcium sensitivity of actin filaments is decreased when troponin I is phosphorylated by a protein kinase, which has in turn been previously activated by cAMP. Such phosphorylation results in more rapid isometric relaxation (decrease in R2) due to more rapid dissociation of calcium from troponin C.³⁷ The absence of any change in R2 under heavy load suggests that etomidate did not modify the calcium sensitivity of myofilaments. Further *in vitro* studies using skinned preparation are required to definitely rule out an effect of etomidate on myofilament calcium sensitivity. However, an increase in force would have been observed if etomidate increased the myofilament calcium sensitivity.

4. Our data suggest that the slight positive inotropic effect of etomidate in rat papillary muscle might be related to a direct effect on cross-bridge kinetics. Whatever the load applied, the shortening velocity slightly increased with etomidate (fig. 2). An increase in myosin ATPase activity might explain such an effect. There is a linear relationship between the myosin ATPase activity and the maximum unloaded shortening velocity (V_{\max}).^{23,38} This kind of mechanism has been recently suggested for adrenaline,³⁹ but the positive inotropic effect of adrenaline is largely mediated through other well-known mechanisms. However, this mechanism has not been previously recognized as the main mechanism of action for a positive inotropic agent.⁴⁰ The positive inotropic effect of etomidate was relatively slight and is probably of moderate clinical significance. However, such detailed analysis of

tension development is useful and may demonstrate other new agents, with such positive inotropic actions.⁴¹ An increase in velocity (V_{\max}) but not in force suggests a decrease in the muscle efficiency. Nevertheless, no decrease in the curvature (G) of the force-velocity relationship was observed with etomidate (table 2). G is an important energetic parameter: a decrease in G is related to a decrease in the myothermal economy.⁴² Therefore, the changes observed in V_{\max} were probably too moderate to actually modify the muscle efficiency.

SARCOPLASMIC RETICULUM FUNCTION

At a high $[Ca^{++}]_o$ relaxation was slowed (increase in max Vr and R1) (table 1). Moreover, this effect was observed whatever the afterload and even when changes in end-shortening length were considered (fig. 2). Under isotonic conditions the amplitude of sarcomere shortening is twice that observed under isometric conditions,³⁶ and isotonic relaxation occurs earlier and more rapidly than isometric relaxation, partly due to two mechanisms⁴³: 1) the easier removal of calcium from troponin C due to the decrease in myofilament calcium sensitivity, itself linked to sarcomere shortening, and 2) the rapid uptake of calcium by the SR. Under low load the SR appears to play a major role in the regulation of isotonic relaxation. Our results therefore suggest that etomidate in propylene glycol impaired the SR function. Many other findings also suggest that etomidate in propylene glycol impairs the SR function:

1. Etomidate decreased the postrest potentiated contraction, which is highly dependent on the SR.^{11,13}

2. Etomidate decreased the load sensitivity of relaxation. A decrease in load sensitivity of relaxation may result from a slowed isotonic relaxation or an abbreviated isometric relaxation, or both.⁴⁴ Because etomidate did not modify the isometric relaxation (R2, $-dF \cdot dt^{-1} \cdot s^{-1}$), the decrease in the load sensitivity of relaxation was related to the slowed isotonic relaxation, arguing in favor of a decreased SR function. This decrease in SR function was probably moderate as follows: 1) at a low $[Ca^{++}]_o$, *i.e.*, at a low SR activity, the isotonic relaxation was unchanged (fig. 3); 2) τ , the rate constant of the decay of active isometric force during the postrest recovery period, which is thought to reflect some of the SR functions,^{11,24} was unchanged (fig. 7); and 3) because a relationship between inotropy and relaxation was demonstrated in rat myocardium,²⁰ the absence of a negative inotropic effect despite an impairment of the isotonic relaxation suggested a slight deleterious effect of etomidate on the SR function.

A diminution of the isotonic relaxation (max Vr) and, consequently, a considerable alteration of the contraction-relaxation coupling under low load (increase in R1) was

observed with propylene glycol alone. Propylene glycol was therefore probably responsible *per se* for the decrease in the SR function. Propylene glycol itself is known to induce deleterious cardiovascular changes in anesthetized⁴⁵ and conscious⁸ animals. Most of the cardiovascular effects of diazepam were finally attributed to its vehicle, *i.e.*, propylene glycol.⁴⁵ When etomidate was tested in ethanol, the isotonic relaxation did not change (fig. 9). Because etomidate increased max Vc, an increase in max Vr would be expected; thus, when $[Ca^{++}]_o$ is increased to induce a positive inotropic effect, both max Vc and max Vr increase.²⁰ A slight impairment in contraction-relaxation coupling (increase in R1) was observed due to an increase in max Vc. This slight impairment in R1 was similar to that observed with ethanol alone (fig. 9). This illustrates the importance of indices, such as R1, which test contraction and relaxation coupling to assess the effect of a drug on the relaxation phase (*i.e.*, lusitropic effect) when this drug also modifies the contraction phase (*i.e.*, inotropic effect). We may therefore conclude that: 1) etomidate in propylene glycol impaired the isotonic relaxation, and this effect was due to the solvent; 2) etomidate in ethanol induced a relative impairment of isotonic relaxation, and this effect was probably related to the solvent; and 3) impairment of isotonic relaxation (and therefore impairment of the SR function) was less important with etomidate in ethanol than with etomidate in propylene glycol.

Some remarks must be included to minimize clinical relevance of our results. First, this study was an *in vitro* study and therefore only dealt with the intrinsic mechanical properties of papillary muscle. Second, this study was conducted at 29° C. Third, it was performed on rat myocardium. In rat myocardium a negative staircase is observed (an increase in stimulation frequency decreases force), the calcium-induced calcium release from the SR is more highly developed than in other species,⁴⁶ and finally, the myosin isoforms are predominantly of the V1 type, whereas in human myocardium they are predominantly of the V3 type.⁴⁷ Because we have tested high doses of etomidate, one can suggest that at lower concentrations (within the therapeutic range) there would be less or no effect. It is more important to underline the fact that this study was conducted in normal animals. Because we and others^{3,4} have observed that under certain experimental conditions etomidate may induce a decrease in isometric force (fig. 6), the possibility that etomidate might decrease myocardial contractility in diseased myocardium cannot be ruled out. Further studies are in progress in our laboratory to study the effects of etomidate on diseased myocardium *in vitro*.

Etomidate has only a slight effect on the intrinsic mechanical properties of rat cardiac papillary muscle. These

results are in good agreement with those obtained in previous clinical studies.^{1,2,26-30} The slight positive inotropic effect observed *in vitro* may have an original mechanism because our results suggest that etomidate directly modulates cross-bridge kinetics. The negative lusitropic effect of etomidate is more pronounced with propylene glycol as a solvent than with ethanol. However, the possible consequences on this slight impairment of relaxation remains speculative concerning the whole heart mechanics and requires further *in vivo* studies.

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