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Mechanism of the Positive Inotropic Effect of Ketamine in Isolated Ferret Ventricular Papillary Muscle

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Ketamine is a cardiovascular stimulant through its sympathomimetic effects; however, its direct inotropic effect has been reported as positive in rat and negative in rabbit ventricular myocardium. This study reexamines the effect of ketamine on the contractile properties of mammalian ventricular myocardium. In isolated, electrically stimulated ferret right ventricular papillary muscles, the authors assessed the inotropic effect of ketamine (10^{-6} M to 3×10^{-4} M in 0.5 log M increments) alone and in various pharmacologic conditions designed to delineate ketamine's site(s) of action. Ketamine exerted a positive inotropic effect that was maximal at 10^{-4} M. Bupranolol (10^{-7} M) abolished this positive inotropic effect, whereas phentolamine (10^{-6} M) did not. Depletion of norepinephrine stores by reserpine also eliminated ketamine's positive inotropic effect, indicating that ketamine caused indirect activation of the beta-adrenoceptor. Ketamine did not exert a positive inotropic effect in the presence of simultaneous inhibition of neuronal norepinephrine uptake with desmethylimipramine (DMI) (5×10^{-6} M) and extraneuronal uptake with corticosterone (5×10^{-5} M). It is likely that ketamine's action is to inhibit norepinephrine uptake at the neuroeffector junction rather than to augment norepinephrine release. In the presence of corticosterone, ketamine exerted a smaller positive inotropic effect than that seen with ketamine alone. Ketamine produced a small increase in force development in the presence of DMI, but this did not reach statistical significance. Inhibition of neuronal catecholamine uptake appears to be the predominant mechanism of ketamine's positive inotropic effect. (Key words: Anesthetics, intravenous: ketamine. Heart: contractility. Sympathetic nervous system, catecholamines: neuroeffector junction; norepinephrine uptake.)

KETAMINE is an intravenous anesthetic that finds particular use for its cardiostimulatory effects.[1] Experimental evidence indicates that ketamine activates the sympathetic nervous system both centrally[2] and peripherally.[3,4] Ketamine increases blood pressure, heart rate, and cardiac output; yet its use has also resulted in cardiac decompensation in critically ill patients.[5] Since ketamine was introduced into clinical practice, controversy has existed over its direct inotropic effect. To eliminate the confounding effects of sympathetic tone, vascular resistance, preload and afterload, *in vitro* studies of isolated cardiac muscle have been used to document ketamine's effect on myocardial contractility. These reports have generated conflicting results. Goldberg *et al.*[6] studied the dose-dependent effects of ketamine on isometric twitches of rat left ventricular trabeculae carneae. They found that tension development, maximal rate of tension development, and maximum velocity of shortening of unloaded muscle were depressed at ketamine concentrations greater than 2.5×10^{-4} M. In contrast, Barrigon *et al.*[7] demonstrated a dose-dependent positive inotropic response to ketamine in rat atria at concentrations greater than 10^{-5} M; this inotropic response was not altered by beta-adrenoceptor blockade or pretreatment of animals with reserpine.

More recently, Rusy *et al.*[8] studied the effects of ketamine on the slow action potential, the rested state contraction, the potentiated state contraction, and the rapid cooling contracture in rabbit papillary muscles. They concluded that ketamine exerted a negative inotropic effect and postulated that ketamine decreases transsarcolemmal Ca^{2+} influx with little or no effect on Ca^{2+} release by the sarcoplasmic reticulum. Riou *et al.*[9] studied the effects of ketamine on contraction and relaxation in rat ventricular papillary muscle. They found that at low $[Ca^{2+}]_o$, ketamine increased isometric force development and maximum unloaded velocity of shortening (MUVS).

ABBREVIATIONS

- L_{max} = resting length at which active twitch force is maximal
- DL = peak isotonic shortening
- +V = maximal shortening velocity
- −V = maximal lengthening velocity of an isotonic twitch at preload only
- MUVS = maximal unloaded velocity of shortening in a zero-load-clamped twitch
- DF = peak developed force
- +dF/dt = maximal rate of increase of force
- −dF/dt = maximal rate of decrease of force in an isometric twitch

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This positive inotropic effect was unaltered by alpha- or beta-adrenoceptor blockade. In addition, impairment of relaxation at higher ketamine concentrations led Riou *et al.* to suggest that ketamine might also have a simultaneous, competing negative inotropic effect.

The aim of this study was to resolve some of the controversy that still exists regarding the inotropic effect of ketamine. If ketamine has dual effects on myocardial contractility, these may become more evident by studying effects of ketamine over a broader range of drug concentrations. Furthermore, possible competing positive and negative inotropic effects of ketamine may be pharmacologically separable. Therefore, we quantitated the inotropic response to ketamine over six concentrations, which span the clinically relevant range (9.3×10^{-6} to 6×10^{-5} M). We then used a series of pharmacologic interventions to delineate ketamine's potential sites of action. In particular, we used alpha- and beta-adrenoceptor blockade, catecholamine depletion, and inhibition of neuronal and nonneuronal catecholamine uptake to elucidate the mechanism(s) by which ketamine exerts its positive inotropic effect. Although it will become evident that ketamine also has negative inotropic effects by way of another mechanism, that analysis will be the subject of a subsequent study.

Materials and Methods

This study was approved by the Animal Care and Use Committee of the Mayo Foundation. Forty-seven papillary muscles were taken from the right ventricle of adult male ferrets (weight, 1100–1500 g; age, 16–19 weeks). The animals were anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneally [ip]), and the heart was quickly removed through a left thoracotomy. The right ventricle was opened, and suitable papillary muscles were excised and mounted vertically in a temperature-controlled (30 °C) muscle chamber that contained a physiologic salt solution of the following composition (millimolar): Na^+ 135; K^+ 5; Ca^{2+} 2.25; Mg^{2+} 1; Cl^- 103.5; HCO_3^- 24; HPO_4^{2-} 1; SO_4^{2-} 1; acetate^{-1} 20; glucose 10. This medium was equilibrated with 95% O_2 and 5% CO_2 (pH = 7.4). Although subphysiologic, a temperature of 30° C was chosen because it allows for greater stability of our preparation over long periods of time. Suitable preparations were selected on the basis of the following criteria: length at L_{max} (*i.e.*, the length at which active twitch force was maximal) $\geq$ 3.5 mm; a mean cross-sectional area (CSA) $\leq$ 1.2 mm^2; and a ratio of resting (R) to total force (T) (R/T) $\leq$ 0.30. The muscles were held between a force-length servo transducer (Innovi, 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 allowed to contract in alternating series of four isometric and four isotonic twitches during a 2-h period of stabilization before the onset of the experiment.

Seven protocols were used to examine the mechanism of ketamine's inotropic effect; each muscle served in one protocol only. Each muscle of each series was exposed to ketamine HCl in a cumulative dose-response experiment. The following steps were used: 1) control; 2) ketamine 10^{-6} M; 3) ketamine 3×10^{-6} M; 4) ketamine 10^{-5} M; 5) ketamine 3×10^{-5} M; 6) ketamine 10^{-4} M; and 7) ketamine 3×10^{-4} M. The muscles were exposed to each concentration of ketamine until a steady-state of at least 5 min was achieved before contractile response was recorded. These concentrations were chosen because they include concentrations recently studied by other authors[8,9] and they span the range of concentrations seen during the course of an anesthetic. After an intravenous induction dose of ketamine 2 mg/kg, plasma concentrations may reach 6×10^{-5} M, whereas during steady-state infusion, plasma levels of 9.3×10^{-6} M have been documented.[10] The concentrations used in this study closely correspond to measured plasma concentration values because ketamine is approximately 90% free in plasma.[11] To determine whether muscle performance decayed during the duration of the experiment, control measurements were repeated at the end of the protocol for groups 1 and 2 (n = 17).

Isometric, isotonic, and "zero-load-clamped" twitches were recorded during steady state at each drug concentration (fig. 1). Peak developed force (DF), maximal rate of increase of force (+dF/dt), and maximal rate of force decline (−dF/dt) were measured from the isometric twitch. Peak isotonic shortening (DL), maximal velocity of shortening (+V), and maximal velocity of lengthening (−V) were measured from the isotonic twitch. MUVS was measured from the "zero-load-clamped" twitch (*i.e.*, an isotonic twitch at L_{max} in which load is rapidly [< 3 ms] decreased electronically to zero during the latent period[12]) (fig. 1). To eliminate effects of loading in preceding beats,[13] each test contraction was separated by seven isotonic twitches.

To determine ketamine's inotropic effect, a dose-response curve was obtained in eight muscles (group 1). To delineate ketamine's site(s) of action, cumulative dose-response experiments were performed in six other muscle groups in which various functions of the sympathetic neuroeffector junction were selectively altered. In group 2, pretreatment was performed with the beta-adrenoceptor antagonist (±)-bupranolol HCl 10^{-7} M (n = 9).[14] In group 3, pretreatment was performed with the alpha-adrenoceptor antagonist phentolamine HCl 10^{-6} M (n = 5).[15] To determine whether ketamine activates the beta-adrenoceptor directly or indirectly, the inotropic effect of ketamine was assessed in muscles depleted of releasable

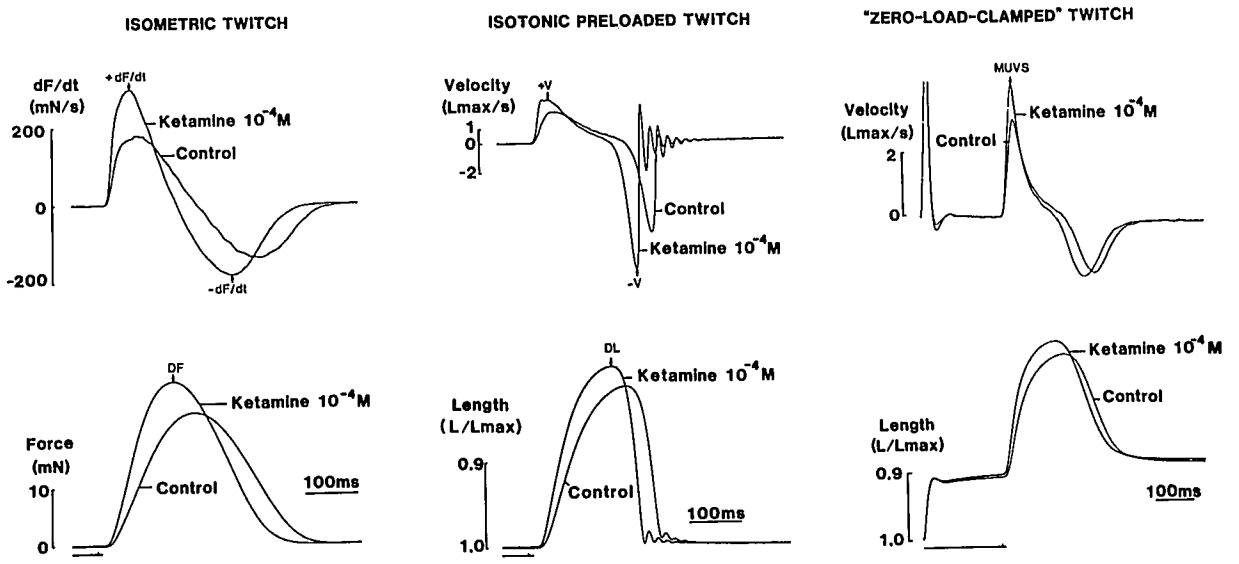

FIG. 1. Variables of contraction and relaxation determined in this study. *Bottom left:* Force traces of an isometric twitch before and during exposure to ketamine 10^{-4} M; (DF) represents peak developed force. *Top left:* Rate of increase and decrease of force of the isometric twitch represented in the lower panel; +dF/dt represents maximal rate of force development and −dF/dt the maximal rate of force decline. *Bottom center:* Shortening traces of an isotonic twitch, control and ketamine 10^{-4} M; DL represents peak shortening. *Top center:* Velocity trace of the isotonic twitch represented in the lower panel; +V represents the peak velocity of shortening and −V the peak velocity of lengthening. *Right:* Shortening (*bottom*) and velocity (*top*) traces of a zero-load-clamped twitch, control and 10^{-4} M ketamine, where load was rapidly decreased from preload to zero at the onset of the sweep. At the stimulus muscle shortening proceeds against zero load. MUVS is the maximal unloaded velocity of shortening.

norepinephrine by previous exposure of the animals to reserpine (5 mg/kg, ip, over 36 h before being killed; n = 6) (group 4). In groups 5, 6, and 7 we determined whether ketamine augmented catecholamine release from sympathetic nerve terminals, inhibited catecholamine uptake, or did both. In group 5, pretreatment was done with DMI HCl 5×10^{-6} M and corticosterone 5×10^{-5} M (n = 5); in group 6, pretreatment was done with DMI alone (5×10^{-6} M) (n = 8); and in group 7, pretreatment was done with corticosterone alone (5×10^{-5} M) (n = 6). DMI is a tricyclic compound that specifically blocks neuronal uptake of norepinephrine ($uptake_1$)[16]; a concentration of 10^{-6} M has been shown to inhibit neuronal uptake of norepinephrine by 92.5%.[17] Its potency exceeds that of cocaine,[17,18] and it lacks cocaine's local anesthetic effect. DMI has only a small effect on nonneuronal catecholamine uptake ($uptake_2$).[19] Conversely, the large steroids have been shown to be effective inhibitors of nonneuronal norepinephrine uptake.[20] Corticosterone is the most potent of this group and inhibits $uptake_2$ by as much as 94.6% at 3×10^{-5} M.[19] The rationale for these pharmacologic interventions and the sequence of experiments is illustrated schematically in figure 2.

Muscle characteristics of all seven groups are shown in table 1. The seven groups did not differ in regard to L_{max}, CSA, resting tension, or total tension by one-way analysis of variance. In group 4, to determine whether reserpine pretreatment had sufficiently depleted releasable norepinephrine stores, the contractile response to tyramine 10^{-6} M and 10^{-5} M was assessed. DF of isometric twitches was 98.94 ± 2.53% of control in tyramine 10^{-6} M (mean ± standard error of the mean [SEM]; not significantly different from control, $P = 0.30$; Student's paired t test) and 103.8 ± 3.53% of control in tyramine 10^{-5} M (mean ± SEM; not significantly different from control, $P = 0.67$;

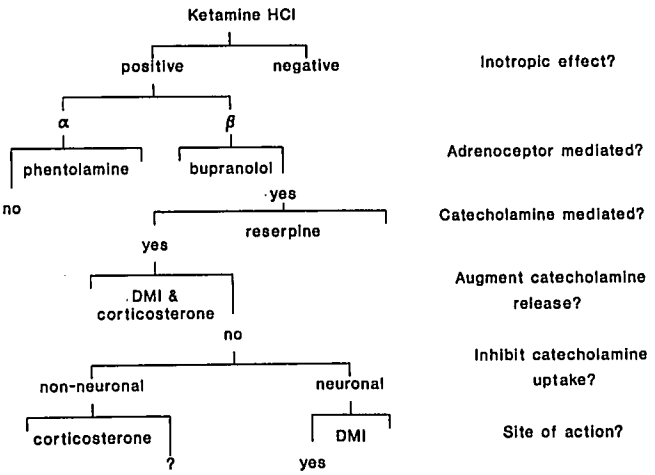

FIG. 2. Experimental steps to determine the mechanism(s) of action of the positive inotropic effect of ketamine. See text for details.

TABLE 1. Muscle Characteristics during Control Conditions at L_{max}

Group		L_{max} (mm)	CSA (mm^2)	R ($mN \cdot mm^{-2}$)	T ($mN \cdot mm^{-2}$)	R/T
1	Ketamine alone (n = 8)					
	Mean	4.73	0.73	4.72	37.32	0.18
	SD	0.76	0.16	1.94	23.76	0.06
	Range	3.60–6.00	0.49–1.05	1.30–7.60	17.47–87.00	0.12–0.24
Pretreatment						
2	Bupranolol (n = 9)					
	Mean	4.61	0.48	8.97	46.26	0.22
	SD	0.93	0.18	2.56	26.70	0.07
	Range	3.50–6.50	0.34–0.80	4.50–12.50	19.29–109.20	0.12–0.30
3	Phentolamine (n = 5)					
	Mean	4.94	0.45	13.36	79.52	0.16
	SD	0.56	0.23	7.21	29.71	0.04
	Range	4.00–5.50	0.23–1.01	4.26–25.22	33.14–123.30	0.13–0.20
4	Reserpine (n = 6)					
	Mean	4.87	0.44	7.43	36.04	0.22
	SD	0.82	0.18	2.20	17.31	0.06
	Range	4.00–6.20	0.23–0.66	3.94–9.57	19.98–65.56	0.15–0.30
5	DMI + corticosterone (n = 5)					
	Mean	5.30	0.67	8.68	38.04	0.22
	SD	0.57	0.13	2.26	14.70	0.06
	Range	4.50–6.00	0.56–0.80	6.13–11.08	22.60–53.77	0.09–0.30
6	DMI (n = 8)					
	Mean	5.30	0.60	9.88	51.53	0.22
	SD	1.13	0.30	5.57	28.73	0.09
	Range	4.00–7.90	0.32–1.08	5.51–20.63	19.59–91.67	0.09–0.30
7	Corticosterone (n = 6)					
	Mean	5.08	0.60	7.92	37.75	0.21
	SD	0.97	0.17	3.82	14.53	0.05
	Range	3.50–6.00	0.42–0.87	4.31–14.05	21.50–51.55	0.14–0.26

L_{max} = optimal muscle length; CSA = cross-sectional area; R = resting tension; T = total tension; R/T = ratio of resting to total tension at L_{max}.

Student's paired *t* test). The adequacy of the concentrations chosen for bupranolol, phentolamine, DMI, and corticosterone was determined from review of the literature.[14,15,17–19] In the six muscle groups undergoing pretreatment, contractility measurements obtained in the presence of these treatments served as control variables. This was to account for the potential inotropic effect of these pretreatments and to determine whether ketamine exerted effects in addition to those of the pharmacologic manipulations (table 2).

The contractile response to ketamine for each experiment was assessed with repeated-measures analysis of variance. When appropriate, Dunnett's test was used to compare effects of individual ketamine concentrations with control. To compare dose-response curves to ketamine among different groups, the following procedure was used. First, the inotropic response to ketamine in each muscle was expressed as the sum of individual responses at each ketamine concentration. Sums of inotropic response value were compared among groups with one-way analysis of variance, followed by Student-Neuman-Keuls tests when appropriate.

Results

At concentrations of 3×10^{-5} M and higher, ketamine exerted a positive inotropic effect in ferret ventricular muscle (fig. 3, open circles). Similarly, at concentrations of 3×10^{-5} M and higher, in zero-load-clamped twitches ketamine caused an increase of MUVS. In isotonic twitches, ketamine increased DL at a concentration of 10^{-4} M. Ketamine also increased the velocity of contraction and relaxation in isometric and isotonic twitches. The +dF/dt and −dF/dt were increased at ketamine concentrations $\geq 3 \times 10^{-5}$ M. This effect was maximal at 10^{-4} M. The +V and −V were increased at ketamine concentrations of 10^{-4} M (fig. 4). In the three types of contractions, ketamine's positive inotropic effect decreased at

TABLE 2. Inotropic Effect of Ketamine Alone and after Various Pretreatments

Ketamine Concentration	Group 1 Ketamine Alone (n = 8)	2 Bupranolol (10^{-7} M; n = 9)	3 Phentolamine (10^{-6} M; n = 5)	4 Reserpine (n = 6)	5 DMI + Corticosterone (n = 5)	6 DMI Alone (5×10^{-6} M; n = 8)	7 Corticosterone (5×10^{-5} M; n = 6)
No drug	33.96 ± 8.48	44.42 ± 8.34	72.58 ± 12.94	33.19 ± 7.03	29.45 ± 5.95	47.11 ± 10.42	33.30 ± 6.800
Pretreatment (M)		34.39 ± 8.57	77.63 ± 15.63	32.93 ± 6.79	38.77 ± 8.38	53.33 ± 10.96	29.97 ± 6.01
10^{-6}	34.69 ± 9.00	34.30 ± 8.92	79.84 ± 15.99	33.35 ± 6.69	39.27 ± 8.51	54.30 ± 11.00	30.71 ± 5.85
3×10^{-6}	36.51 ± 9.60	34.13 ± 9.01	81.97 ± 15.70	32.80 ± 6.82	39.26 ± 8.08	54.39 ± 10.86	31.04 ± 6.09
10^{-5}	38.23 ± 9.57	33.20 ± 8.71	82.97 ± 16.07	30.84 ± 6.46	38.80 ± 7.93	54.75 ± 10.50	31.99 ± 6.25
3×10^{-5}	40.59 ± 9.32**	33.77 ± 9.12	89.68 ± 19.26*	25.04 ± 5.19*	37.46 ± 7.50	55.05 ± 10.33	32.46 ± 6.46*
10^{-4}	41.07 ± 9.03**	27.11 ± 6.12**	87.37 ± 19.70	11.25 ± 2.63**	34.82 ± 7.61*	53.02 ± 10.28	31.73 ± 6.45
3×10^{-4}	38.64 ± 8.61*	17.56 ± 3.38**	82.90 ± 21.95		31.76 ± 7.71*	38.35 ± 7.91	28.08 ± 5.62

Values are mean ± SEM of DF ($mN \cdot mm^{-2}$).
* $P < 0.05$; ** $P < 0.01$ for comparison with pretreatment values, which served as control.

concentrations of 3×10^{-4} M, and this change was most obvious for DL and MUVS.

In group 2 muscles preexposed to (±)-bupranolol 10^{-7} M, ketamine's positive inotropic effect was abolished. In the three types of twitches, a negative inotropic effect was unmasked at the highest two ketamine concentrations ($P < 0.05$) (fig. 3). Bupranolol pretreatment also eliminated

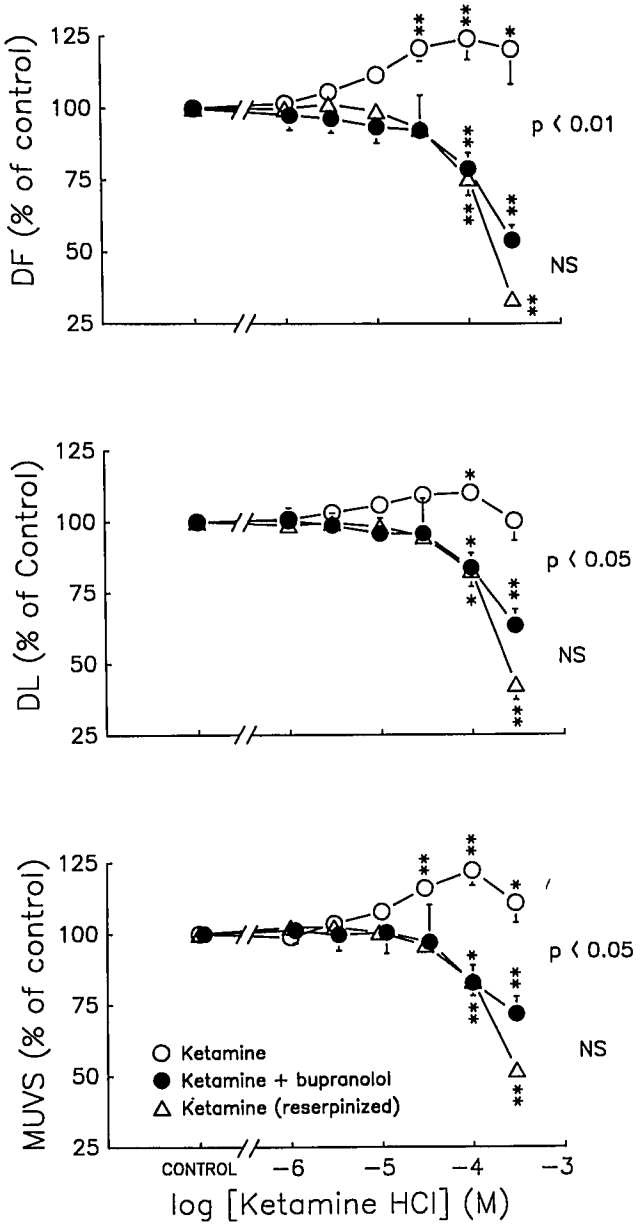

FIG. 3. The inotropic effect of ketamine alone (n = 8) (open circles) and in muscles preexposed to bupranolol 10^{-7} M (n = 9) (filled circles) or depleted of releasable norepinephrine by prior exposure to reserpine (n = 6) (triangles) (mean ± S.E.M.) on peak force (DF) (*top*), peak shortening (DL) (*center*), and maximum unloaded velocity of shortening (MUVS) (*bottom*). *$P < 0.05$; **$P < 0.01$ for comparison with control values within each group. The right side of each panel displays the levels of significance for comparison among individual groups.

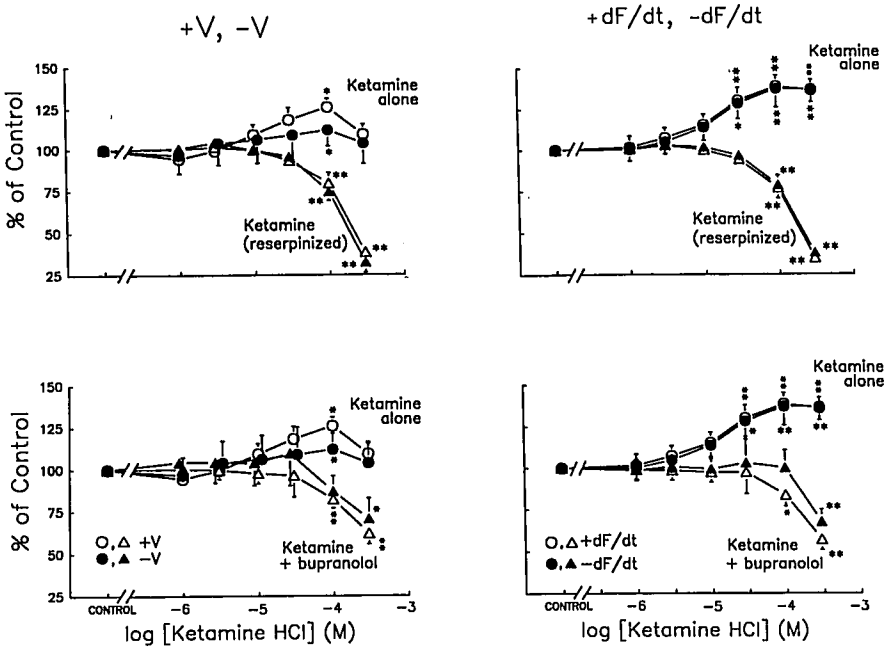

FIG. 4. Changes in the velocities of shortening and force development in response to ketamine alone (circles) and after pretreatment with bupranolol or reserpine (triangles). *Left:* Maximum velocity of shortening (open symbols) and lengthening (closed symbols). *Right:* Maximum velocity of force development and decline. $*P < 0.05$; $**P < 0.01$ for comparison with control values within each group.

the increase in velocity of contraction and relaxation caused by ketamine (fig. 4). In the presence of beta-adrenoceptor blockade, ketamine decreased the maximal rates of isometric (+dF/dt) and isotonic (+V) contraction at 10^{-4} and 3×10^{-4} M. The maximal rate of isometric (−dF/dt) and isotonic (−V) relaxation were also decreased by ketamine 3×10^{-4} M in the presence of beta-blockade (fig. 4). When muscles were preexposed to phentolamine 10^{-6} M (group 3), ketamine's positive inotropic effect was preserved (table 2). Phentolamine appeared to attenuate ketamine's positive inotropic response, yet the effects of ketamine were not significantly different among groups 1 and 3 (one-way analysis of variance). Control measurements at the end of the experiment in groups 1 and 2 did not show a decay in contractile performance (measured as DF) of the papillary muscle preparation during the course of the study (mean percentage of control ± SEM; 110.16 ± 6.93, $P > 0.05$ *vs.* control, n = 17). Because preparation stability was demonstrated in this large number of muscles, control measurements were not repeated at the end of subsequent experiments.

In group 4 (catecholamine-depleted muscles), ketamine's positive inotropic effect was abolished in the three types of contractions, and a significant negative inotropic effect was revealed at the two highest ketamine concentrations (figs. 3 and 4). The dose-response curves in muscles from reserpinized ferrets were very similar to that after beta-adrenoceptor blockade (group 2) (figs. 3 and 4). There were no statistically significant differences in ketamine's inotropic effects between group 2 (beta-blocked) and group 4 (reserpinized muscles) for all variables examined.

Pretreatment with inhibitors of neuronal norepinephrine uptake ($uptake_1$), the predominant form of norepinephrine uptake, might be expected to produce a positive inotropic effect; this was indeed observed in groups 5 and 6, in which muscles were pretreated with DMI plus corticosterone, and DMI alone, respectively (table 2). In group 7, pretreatment with corticosterone, an inhibitor of the quantitatively less important extraneuronal $uptake_2$, did not alter inotropic state as measured by DF (table 2).

In group 5, in which DMI (5×10^{-6} M) and corticosterone (5×10^{-5} M) were present and norepinephrine uptake was maximally blocked, ketamine's positive inotropic effect was abolished. As in beta-blocked or catecholamine-depleted muscles, the dose-response curve to ketamine was flat over the concentration range 10^{-6} to 3×10^{-5} M, and a negative inotropic effect was evident at ketamine concentrations $\geq 10^{-4}$ ($P < 0.05$) (fig. 5). This impairment of contractility was seen in the three twitch types. The dose-response curve to ketamine in group 5 was statistically significantly different ($P < 0.05$) from that in group 1 (ketamine alone) for the variables DF and DL. The statistical methods used did not demonstrate a significant difference between groups 1 and 5 for MUVS.

In groups 6 and 7, the inotropic response to ketamine was assessed in the presence of DMI (group 6) and corticosterone (group 7) individually. Pretreatment with DMI resulted in a significant 17% increase in force development ($P < 0.05$). Prior inhibition of $uptake_1$ with DMI eliminated ketamine's positive inotropic effect, whereas inhibition of $uptake_2$ with corticosterone (group 7) did not. In the presence of corticosterone, ketamine increased DL

and MUVS at concentrations of 3×10^{-5} M and 10^{-4} M ($P < 0.05$), whereas the increase in DF reached statistical significance only at concentrations of 3×10^{-5} M (fig. 5). Ketamine caused a small increase in contractility in the presence of either uptake blocker individually (fig. 5), yet the changes were not statistically significant. Figure 5 illustrates that the dose-response curves of groups 6 (DMI) and 7 (corticosterone) lie between those of ketamine alone (group 1) and ketamine in the presence of complete norepinephrine uptake blockade (group 5).

In the presence of DMI, the stimulation threshold increased at the highest two ketamine concentrations in some muscles. In three of five experiments in group 5 and four of eight experiments in group 6, the stimulus voltage had to be increased to complete the experiment.

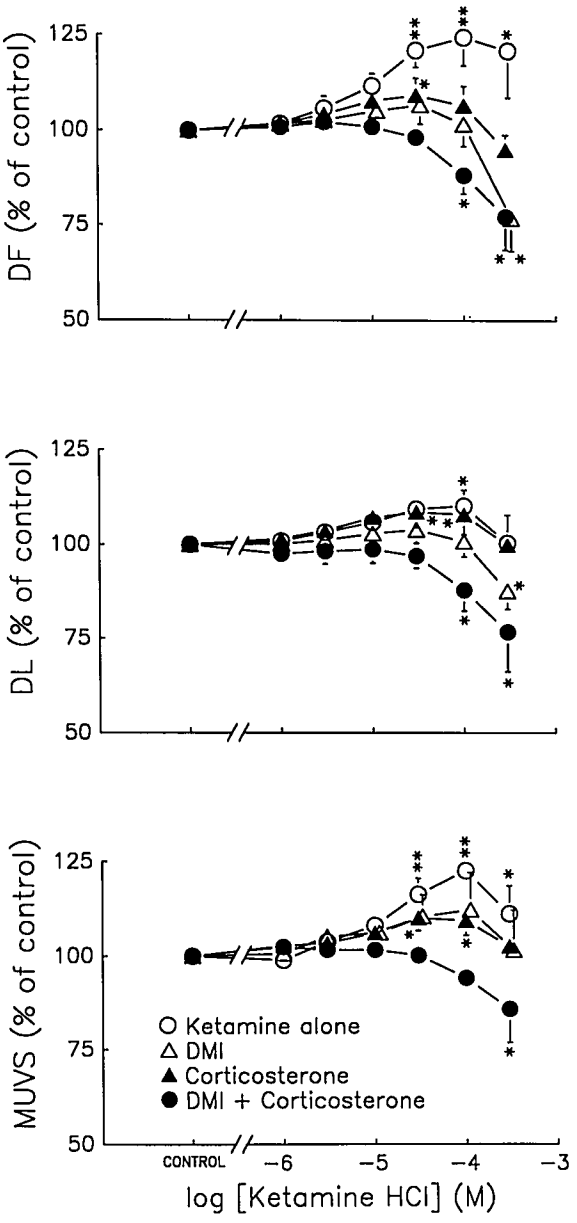

FIG. 5. Effect of inhibitors of catecholamine uptake on the inotropic effect of ketamine for peak developed force (DF) (*top*), for peak shortening (DL) (*center*), and for maximum unloaded velocity of shortening (MUVS) (*bottom*). *$P < 0.05$; **$P < 0.01$ for comparison with control values within each group.

Discussion

The results of this study demonstrate that a clinically relevant concentration of ketamine exerts an indirect positive inotropic effect on ventricular myocardium. This study also shows that ketamine acts as a myocardial depressant through a different mechanism; this direct depressant effect becomes evident when adrenergic transmission is impaired. At the concentrations tested, when the sympathetic neuroeffector junction was intact, ketamine's indirect positive inotropic action exceeded its direct depressant effect.

After demonstrating ketamine's positive inotropic effect, we sought to identify its mechanism. Because ketamine increases central nervous system sympathetic outflow[2] and inhibits catecholamine uptake in smooth and skeletal muscle,[3,4] we examined the effects of beta- and alpha-adrenoceptor blockade and of catecholamine depletion on the inotropic response to ketamine. We determined that in our model ketamine's positive inotropic effect is mediated through activation of the beta-adrenoceptor and that ketamine's effect is indirect (*i.e.*, ketamine does not act as a beta-adrenoceptor agonist but, rather, modifies the effect of and/or increases the supply of endogenous norepinephrine).

Next, we sought to determine whether ketamine augments catecholamine release from sympathetic nerve terminals or inhibits catecholamine uptake. In the presence of both DMI and corticosterone, both neuronal and extraneuronal norepinephrine uptake are maximally inhibited. If ketamine augments norepinephrine release, a positive inotropic effect would be expected when uptake is blocked, but under these conditions ketamine exerted no positive inotropic effect. Therefore, augmentation of norepinephrine release does not appear to be ketamine's mechanism of action. As is the case in smooth muscle, these findings are consistent with the hypothesis that ketamine increases myocardial contractility by inhibiting norepinephrine uptake at the neuroeffector junction.

Studies done in the presence of either DMI or corticosterone individually suggest that ketamine inhibits both neuronal and nonneuronal catecholamine uptake. In groups 6 and 7, ketamine increased contractility, but the absolute effect was small in each group and only reached statistical significance in the experiments in the presence of corticosterone. This suggests that ketamine primarily inhibits neuronal catecholamine uptake in ferret ventricular muscle; however, the papillary muscle preparation may not be sufficiently sensitive to assess the relative importance of the inhibition of the two forms of cate-

cholamine uptake. If ketamine increases contractility primarily by inhibiting neuronal uptake of norepinephrine, one would expect a similar inotropic response to pretreatment with DMI. Furthermore, the inotropic effect of ketamine should not exceed that produced by complete uptake inhibition with DMI and corticosterone. As can be seen in table 2, experimental results are consistent with these hypotheses. In groups 5 and 6, pretreatment with DMI plus corticosterone, or DMI alone resulted in a positive inotropic effect (*i.e.*, average increases in DF of 31% and 17%, respectively). The 24% increase in DF produced by ketamine alone was quantitatively similar to that seen in groups 5 and 6 and did not exceed the positive inotropic effect seen in the presence of both DMI and corticosterone. These observations provide additional indirect evidence that ketamine acts as a norepinephrine uptake inhibitor.

Our findings are similar to those of Riou *et al.*[9] in that we demonstrated a positive inotropic response to ketamine and described a competing negative inotropic effect at high concentrations. Riou *et al.* attributed ketamine's positive inotropic effect at concentrations of 10^{-5} M to an increase in transsarcolemmal Ca^{2+} influx, which is consistent with a catecholamine-mediated mechanism. However, in their study in rat ventricular myocardium, beta-adrenoceptor antagonism did not abolish ketamine's inotropic effect in $[Ca^{2+}]_o$ 0.5 mM. They attributed the decrease in contractility of ketamine 10^{-4} M to an impairment of sarcoplasmic reticulum function. Phentolamine did not alter the positive inotropic effect of ketamine in either rat or ferret ventricular myocardium, which suggests that ketamine has no direct effects on myocardial alpha-adrenoceptors.

In contrast to our findings, Rusy *et al.*[8] described a negative inotropic effect to ketamine in rabbit ventricular muscle and attributed it to a decrease in transsarcolemmal Ca^{2+} entry with little or no effect on Ca^{2+} release by the sarcoplasmic reticulum.

The findings of Rusy *et al.*[8] and Riou *et al.*[9] are not necessarily inconsistent with our own for the following reasons. Both studied only two ketamine concentrations (8.4×10^{-5} M and 1.68×10^{-4} M, and 10^{-5} M and 10^{-4} M, respectively). It is possible that a dose-response curve to ketamine similar to ours, shifted to the right or to the left, might become evident in rat and rabbit myocardium if additional ketamine concentrations were studied. We have shown that ketamine has two competing mechanisms of action, one that augments and one that depresses contractility. Differences between results in rat,[9] rabbit,[8] and ferret myocardium may be explained by variations in relative importance of these two mechanisms in different species.

Although one might attempt to explain the species differences on the basis of the density of adrenergic innervation or the ratio of adrenoceptor subtypes alone, this proves unsatisfactory. Of the three species, the rabbit has the greatest density of sympathetic innervation.[21–23] One could therefore expect ketamine's indirect sympathomimetic effect to be predominant in rabbit myocardium and overcome ketamine's direct negative inotropic effect; this is not seen. Rat and rabbit ventricular myocardium have approximately the same density of alpha- and beta-adrenoceptors,[24] so that adrenoceptor differences alone do not adequately explain the species differences. Density of sympathetic innervation and adrenoceptor subtypes probably play only a secondary role in determining the inotropic response to ketamine.

More important, perhaps, are differences among species regarding the primary source of activator Ca^{2+}. The rabbit is most dependent on transsarcolemmal Ca^{2+} movement for myofibrillar activation. The rat is primarily dependent on the sarcoplasmic reticulum as the source of myoplasmic Ca^{2+}.[25] If ketamine's direct depressant effect results mainly from impairment of transsarcolemmal Ca^{2+} movement, this effect might be quite evident in the rabbit and of much less consequence in the rat. Relative sensitivity to each of ketamine's two competing mechanisms may therefore explain the conflicting results that have made this literature intriguing. The negative inotropic effect of ketamine in rabbit was indeed attributed to a decrease in transsarcolemmal Ca^{2+} influx.[8] If ketamine inhibits norepinephrine uptake in rabbit ventricular myocardium, the intrinsic direct effect of ketamine in this species would be even larger than that observed by Rusy *et al.*[8]

A methodologic observation also must be made. Our goal was to determine the direct myocardial effect of ketamine. It is well known that sympathetic nerve terminals remain functional in an isolated muscle preparation and may alter contractile response.[26] We used a stimulation voltage 10% above threshold to minimize sympathetic activation. It therefore follows that, with supramaximal voltage field stimulation rather than punctate 10% above threshold stimulation, sympathetic activation would be more profound and ketamine's indirect effect may have been even more pronounced.

In the intact heart it is difficult to determine the importance of the background sympathetic tone in maintaining contractility, but in ferret ventricular muscle we have been able to quantitate norepinephrine release under basal conditions and document its increase with stimulation (unpublished data). Therefore, as an inhibitor of norepinephrine uptake, ketamine has the potential to increase basal sympathetic tone in the myocardium in addition to the augmentation it produces under conditions of sympathetic activation.

Although we must be cautious in making inferences to human physiology, particularly in light of the species differences described, our finding that ketamine's positive inotropic effect is dependent on an intact sympathetic

neuroeffector junction may have clinical implications. The myocardium of patients in chronic congestive heart failure has been shown to undergo depletion of norepinephrine.[27] Similar findings have been reported in post–cardiac transplant patients in whom sympathetic nerve terminals undergo degradation.[28] Finally, one might expect ketamine to have adverse consequences in those acutely or subacutely beta-blocked in whom "up regulation" of the beta-adrenoceptor has not had time to occur. In these groups, ketamine's direct myocardial depressant effect might predominate over its indirect cardiostimulatory effect. Variable intactness of the sympathetic neuroeffector junction may explain in part the diversity in clinical response seen with the use of ketamine.

In summary, ketamine, at a clinically relevant concentration, increased contractility in isolated mammalian ventricular muscle. In our model, ketamine activated the beta-adrenoceptor indirectly through the inhibition of neurotransmitter uptake at the sympathetic neuroeffector junction. Our data suggest that inhibition of neuronal uptake of catecholamine was the dominant mechanism of ketamine's positive inotropic effect. This conclusion is based on the indirect evidence that ketamine's sympathomimetic effect is eliminated by prior blockade of uptake$_1$ with DMI. This awaits confirmation by biochemical techniques that can quantitate ketamine's effects on norepinephrine handling in the synaptic cleft. Finally, additional studies are required to delineate the mechanism of ketamine's intrinsic depressant effect in isolated myocardium.

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