Br. J. Pharmac. (1982), **76**, 085–093

THE MECHANISM OF THE POSITIVE INOTROPIC ACTION OF KETAMINE ON ISOLATED ATRIA OF THE RAT

SANTOS BARRIGON, BEATRIZ DE MIGUEL, JUAN TAMARGO & TERESA TEJERINA

Department of Pharmacology, School of Medicine, Universidad Complutense, Madrid-3, Spain

1 The effect of ketamine (10^{-7} M–5×10^{-4} M) on electrical and mechanical properties of isolated atria of the rat was investigated.

2 On spontaneously beating right atria, ketamine produced a dose-dependent positive inotropic effect which was accompanied by a progressive reduction in atrial rate.

3 In electrically driven left atria, ketamine produced a dose-dependent positive inotropic effect which was accompanied by a parallel increase in df/dt_{max} and by a prolongation in the time to peak tension and in the time for total contraction. The positive inotropic effect occurred concomitantly with an increase in the height and duration of the plateau phase of the action potential of atrial fibres.

4 The positive inotropic effect of ketamine varied with the concentration of Ca and Na in the bathing media and the rate of stimulation.

5 Ketamine decreased post-extrasystolic potentiation and the amplitude-interval relationship.

6 The positive inotropic effect of ketamine was inhibited by verapamil (10^{-6} M) and by caffeine (4×10^{-3} M).

7 Ketamine, 5×10^{-5} M and 10^{-4} M, increased ^{45}Ca uptake in electrically driven left atria. At 10^{-4} M and 5×10^{-4} M, ketamine also increased ^{45}Ca efflux.

8 These results suggest that ketamine produces its positive inotropic effect by two possible mechanisms. One of these is presumed to be an effect on the cell membrane which leads to an increased Ca influx into atrial fibres; the other is probably related to the inhibition of Ca sequestration by the sarcoplasmic reticulum.

Introduction

Ketamine is a dissociative anaesthetic agent which has been shown to produce marked increases in arterial blood pressure, heart rate and cardiac output in man and in several animal species (Domino, Chodoff & Corssen, 1965; McCarthy, Chen, Kaump & Ensor, 1965). The mechanism(s) whereby ketamine produces these changes is still uncertain, although some suggestions have been made. For example, the cardioexcitatory effects of ketamine have been ascribed to: release of catecholamines from peripheral tissue stores (Virtue, Alanis, Mori, Lafargue, Vogel & Metcalf, 1967), a cocaine-like effect (Nedergaard, 1973), stimulation of central cardiovascular mechanisms resulting in increased sympathetic efferent discharge (McCarthy *et al.*, 1965; Traber & Wilson, 1969), diminution of frequency response of the carotid sinus baroreceptors (Dowdy & Kaya, 1968), inhibition of the vagal component of the baroreceptor reflex (McGrath, MacKenzie & Millar, 1975) and release of renal renin (Tanaka & Pettinger, 1974). Ketamine has also been shown to produce dose-dependent negative chronotropic and inotropic effects in rabbit isolated perfused heart (Dowdy & Kaya, 1968), in left ventricular trabeculae carneae of the rat (Goldberg, Keane & Phear, 1970), in dog ventricle (Schwartz & Horwitz, 1975) and in guinea-pig atria (Adams, Parker & Mathew, 1977). The consensus, therefore, is that ketamine increases cardiac function by modifying autonomic nervous system discharge (Goldberg *et al.*, 1970; Schwartz & Horwirtz, 1975). However, Adams *et al.* (1977) described a positive inotropic effect of ketamine in guinea-pig atria concomitantly exposed to catecholamines or a dibutyryl derivative of cyclic AMP and found that this effect was independent of heart rate or direct or reflexogenic autonomic nervous system changes. For this reason it was thought of interest to confirm whether, under different experimental conditions, ketamine could exert a positive inotropic effect in rat isolated atria. The results described in this paper demonstrate that ketamine produced a dose-dependent positive inotropic effect in isolated rat atria. The role of Ca influx on the ketamine-induced positive inotropic effect has been also evaluated.

0007-1188/82/050085–09 $01.00

Methods

Sprague-Dawley rats (200–250 g) were killed by a blow on the head and the heart was rapidly removed. Right and left atria were dissected and placed in 10 ml organ baths containing Tyrode solution of the following composition (mM): NaCl 136, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9 and glucose 5.5. The solution was maintained at 34°C and oxygenated with 95% O_2 and 5% CO_2. Under these conditions, right atria started beating spontaneously. Left atrial preparations were stimulated regularly at a basal rate of 3 Hz through bipolar platinum electrodes by square wave pulses (1 ms duration, twice threshold strength) delivered to the preparations from a Grass stimulator Model S4. The frequency and amplitude of contractions were recorded isometrically by means of a Grass FTO3 force-displacement transducer on a Grass polygraph. Resting tension was adjusted to 1.0 g and a 30 min equilibration period was allowed before control measurements were made. Peak contractile force, time to peak tension, maximal rate of contractile force (df/dt_{max}) and the time for total contraction were obtained from isometric force tracings and averaged from three successive contractions measured with the chart speed set at 100 mm/s. Post-extrasystolic potentiation was obtained by delivering a second stimulus just after the refractory period (De Mello, 1976). The influence of ketamine on the restitution kinetics of Ca availability from intracellular stores was determined according to the methods described by Bayer, Hennekes, Kaufmann & Mannhold (1975). After the development of steady contractions in atria stimulated at a basal rate of 3 Hz, a single test contraction was evoked following various rest intervals (0.5–8 s). The force of this contraction was plotted as a percentage of the rest interval to obtain the amplitude-interval relationship.

After control values for each parameter were recorded, ketamine was added to the bath in a cumulative manner at 10 min intervals, since time-response studies indicated that the effects of the drug has stabilized in less than 10 min. In order to compare the results, the control values of the measured parameters in each experiment were taken as 100%. Values obtained in the presence of ketamine were calculated as a percentage of control values for each preparation.

For experiments on the interaction of ketamine with the responses to isoprenaline the following procedure was followed. Control cumulative dose-response curves for chronotropic and inotropic responses to isoprenaline were performed in right atria. Once stable curves were obtained, the same procedure was repeated 10 min after the addition of ketamine to the bathing media. Dose-response curves were expressed as a percentage of maximal response vs. dose of the drug.

Transmembrane potentials were recorded through glass microelectrodes filled with 3 M KCl, displayed via high-impedance, capacity-neutralizing amplifiers (WPI) and photographed on film (Rodriguez & Tamargo, 1980). The maximum rate of depolarization (V_{max}) was obtained by electronic differentiation.

^{45}Ca uptake and ^{45}Ca efflux

To determine ^{45}Ca uptake, left atria driven at a basal rate of 3 Hz were incubated in Tyrode solution for 30 min. In each experiment half of the atrium served as control and the other half as experimental preparation. The atria were then incubated in ^{45}Ca-labelled Tyrode solution (specific activity 2 μCi/ml; Radiochemical Centre, Amersham). After 2 h, ketamine was added to the experimental preparations and the uptake was measured. At the desired time intervals atria were removed, blotted on filter paper, dipped into Tyrode solution, reblotted and weighed. The atria were then placed in scintillation vials and 0.5 ml of Soluene-350 (Packard) added and the atria digested overnight at 50°C. Radioactivity was assayed in a liquid scintillation counter (Intertechnique Model SI-3000) as previously described by Barrigon, Tamargo & Garcia de Jalón (1978).

To determine ^{45}Ca efflux, left atria were incubated in Tyrode solution for 30 min. The preparations were then kept in labelled Tyrode solution for 2 h and then immersed for an accurately measured period of time in successive vials containing non-radioactive solution. In some vials, ketamine (10^{-4} M and 5×10^{-4} M) was added to the non-radioactive solution. The preparations were stimulated at 3 Hz throughout the experiment.

Drugs

The following drugs were used: ketamine hydrochloride (Specia), reserpine phosphate (Ciba-Geigy), practolol (ICI), isoprenaline bitartrate (Winthrop), caffeine (Sigma) and verapamil hydrochloride (Knoll). Drugs were dissolved in deionized distilled water. Ascorbic acid (10^{-4} M) was added to each solution of isoprenaline, made up freshly every day. Rats treated with reserpine were injected (10 mg/kg, i.p.), 24 h before the experiments were carried out. Concentrations of all drugs refer to the salt.

Throughout the paper results are expressed as mean ± s.e.mean. Statistical significance was determined by Student's t test and differences were considered significant when $P < 0.05$.

Results

Dose-responses to ketamine in right and left atrial preparations

The effects of ketamine in concentrations between 10^{-7} M and 5×10^{-4} M were studied on rate and amplitude of spontaneous contractions in 16 right atria. Results are shown in Figure 1. Control values for both parameters were 200 ± 14 beats/min and 598 ± 43 mg, respectively. Ketamine, $< 10^{-6}$ M, did not modify the rate and amplitude of contractions. At higher concentrations ketamine produced a dose-dependent decrease in atrial rate which was accompanied by a significant increase in peak contractile force. The onset of the effect was almost immediate and the maximal effect on both parameters was usually observed within 5–10 min after the addition of the drug to the organ bath. At the highest concentration tested (5×10^{-4} M) ketamine decreased atrial rate by $40 \pm 5\%$ ($P < 0.001$) and increased peak contractile force by $95 \pm 12\%$ ($P < 0.001$). All these effects were rapidly reversed by washing the atria with normal Tyrode solution.

The effects of cumulative concentrations of ketamine on the different parameters of isometric contractions were also studied in left atria driven at a basal rate of 3 Hz (Figure 2). Control values (mean ± s.e.mean) for each parameter in 14 atria were as follows: peak contractile force = 646 ± 45 mg, time to peak tension = 50 ± 2 ms, time for total contraction = 146 ± 4 ms and df/dt_{max} = 13 ± 2 mg/ms. Ketamine ($> 5 \times 10^{-5}$ M) also produced a dose-dependent increase in peak contractile force. This positive inotropic effect was accompanied by a parallel increase in df/dt_{max}, and by a prolongation in the time to peak tension and in the time for total contraction (Figure 2). The increase in peak contractile force induced in left atria was less than that observed in spontaneous right atria. Thus, at 5×10^{-4} M, ketamine increased contractile force in left atria by only $40 \pm 7\%$ with respect to control values ($P < 0.001$). If right atria were driven electrically, the effect of ketamine on contraction was similar to that seen in left atria. Contracture (increase in resting tension) was not observed in either right or left atria with any of the doses of ketamine studied.

The effect of ketamine (5×10^{-4} M) was also studied in left atria driven at 4 and 0.5 Hz. Ketamine increased peak contractile force by $57 \pm 9\%$ and $27 \pm 3\%$ in atria driven at 4 and 0.5 Hz, respectively. Thus the lower rate of stimulation increased the amplitude of contractions so that the action of ketamine was less apparent.

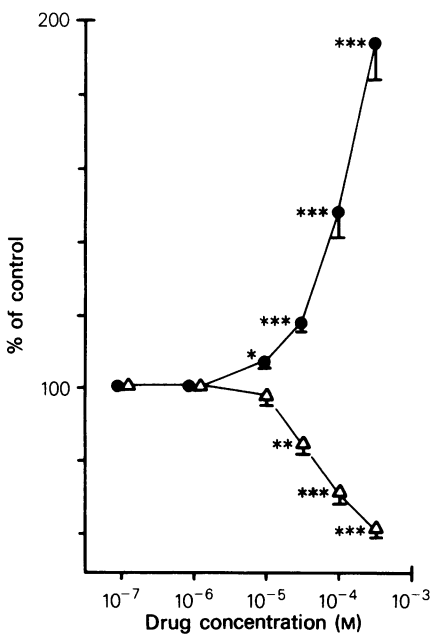

Figure 1 Effect of ketamine on peak contractile force (●) and rate (△) of spontaneous contractions in isolated right atria. Ordinate scale: % of control values. Abscissa scale: drug concentration (M). Each point represents the mean of 16 experiments; vertical lines show s.e.mean. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

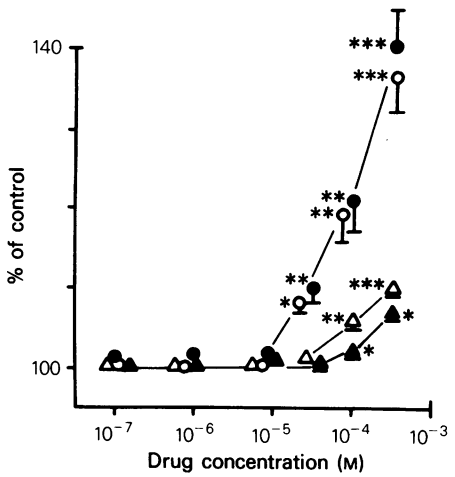

Figure 2 Effect of ketamine on peak contractile force (●), maximal rate of contractile force (df/dt_{max}, ○), time to peak tension (▲) and time for total contraction (△) in electrically driven left atria. Ordinate scale: % of control values. Abscissa scale: drug concentration (M). Each point represents the mean of 14 experiments; vertical lines show the s.e.mean. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Effect on transmembrane potentials of atrial fibres

The effects of ketamine (10^{-5} M–5×10^{-4} M), on transmembrane potentials of atrial fibres are shown in Table 1. Ketamine did not significantly modify the resting membrane potential, action potential amplitude and V_{max}. However, it increased the amplitude and duration of the plateau phase of the action potential and decreased the slope of phase 3. Both effects explain the dose-dependent lengthening in action potential duration, measured both at 50 and 90% of repolarization. Measurements of twitch tension and action potential made simultaneously showed that the increase in amplitude and duration of the plateau and of peak tension produced by ketamine occurred simultaneously.

Effect of ketamine on amplitude-interval relationship and on post-extrasystolic potentiation

As shown in Figure 3, a progressive prolongation in resting interval increased the amplitude of the recorded single contraction. To study the post-extrasystolic potentiation phenomenon, left atria were driven with single stimuli at a basal rate of 3 Hz and then 8 successive pairs of double stimuli were applied (Figure 4). The second stimulus was then removed and the post-extrasystolic potentiation measured. Comparison of these events with those elicited in the same preparation during the period of positive inotropic effect accompanying incubation with 10^{-4} M and 5×10^{-4} M ketamine shows that both amplitude-interval relationship and post-extrasystolic potentiation were reduced in a dose-dependent manner during this period.

Effects of variations of the extracellular concentration of calcium and sodium on the positive inotropic effect of ketamine

It is known that amplitude of cardiac contractions increase when the Ca concentration in the organ bath is increased or the Na is reduced (Wildbrandt & Koller, 1948). In both situations Ca influx into atrial cells is increased (Niedergerke, 1963). To investigate the effect of extracellular Ca concentration on the positive inotropic effect of ketamine, atria were incubated in Tyrode solution containing different Ca concentrations (1.8, 3.6 and 5.4 mM), at least 20 min before the addition of ketamine. As is shown in Figure 5, the effect of ketamine was dependent upon

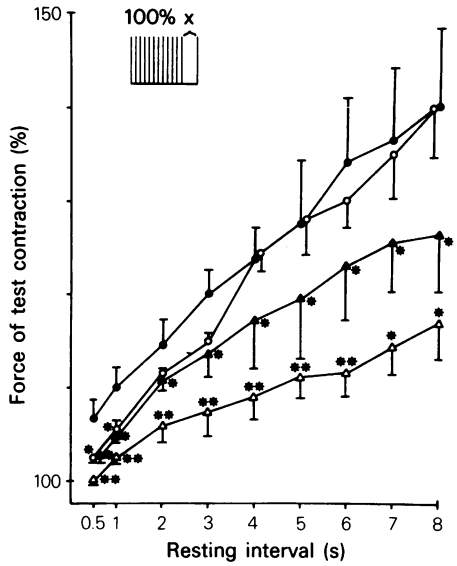

Figure 3 Amplitude-interval relationship of test single contractions elicited after a period of rest following frequent stimulation. The steady-state amplitude attained during conditioning stimulation was taken as 100%, as shown in the inset of the figure. Ordinate scale: force of test contraction (% of control values). Abscissa scale: resting interval (s). Each point represents the mean of 10 experiments; vertical bars show the s.e.mean. (●) Without ketamine; (○) ketamine, 5×10^{-5} M; (▲) ketamine, 10^{-4} M; (△) ketamine, 5×10^{-4} M. $^{*}P<0.05$; $^{**}P<0.01$.

Table 1 Electrophysiological effects of ketamine on rat atrial fibres

Drug concentration (M)		*Amplitude* (mV)	*Resting potential* (mV)	V_{max} (V/s)	APD_{50} (ms)	APD_{90} (ms)
						46.7 ± 4.3
10^{-5}	(10)	96.2 ± 1.8	78.6 ± 1.1	56.4 ± 6.2	24.3 ± 1.9*	66.1 ± 6.3*
5×10^{-5}	(10)	95.8 ± 1.7	78.3 ± 1.6	56.0 ± 6.8	26.1 ± 2.4**	71.8 ± 6.1**
10^{-4}	(12)	96.1 ± 2.7	78.1 ± 1.9	55.8 ± 6.5	28.5 ± 2.9**	75.5 ± 5.9**
5×10^{-4}	(12)	94.2 ± 2.2	77.7 ± 1.4	56.5 ± 6.7	39.0 ± 2.7***	110.5 ± 3.8***

Values are mean ± s.e.mean. Number of observations (n) are given in parentheses. Readings started after 10 min of perfusion with ketamine. APD_{50} and APD_{90} refer to action potential duration to 50% and 90% repolarization, respectively.
$^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

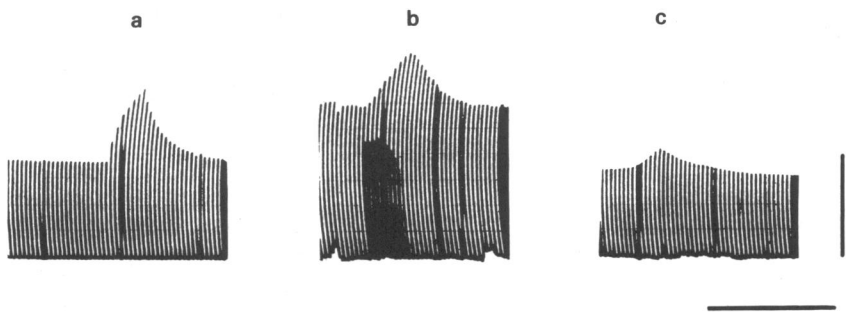

Figure 4 Effect of ketamine on the post-extrasystolic potentiation phenomenon in electrically driven left atria. (a) Post-extrasystolic potentiation recorded before ketamine; (b) and (c) were recorded 10 min after the addition of ketamine, 10^{-4} M and 5×10^{-4} M, to the organ bath. Vertical calibration: 0.5 g (a) and (b) and 1.0 g (c). Horizontal calibration: 10 s.

the extracellular Ca concentration, the positive inotropic effect being less apparent when the Ca concentration in the bathing medium increased.

In another group of experiments, the effect of ketamine was studied in 8 right atria equilibrated for 30 min in Tyrode solution with Na concentration reduced to 70%. The results obtained showed that in 70% Na solution, when Ca influx is increased, the positive inotropic effect of ketamine, 5×10^{-4} M, was significantly reduced ($49 \pm 8\%$ as compared to $89 \pm 7\%$; $P < 0.001$).

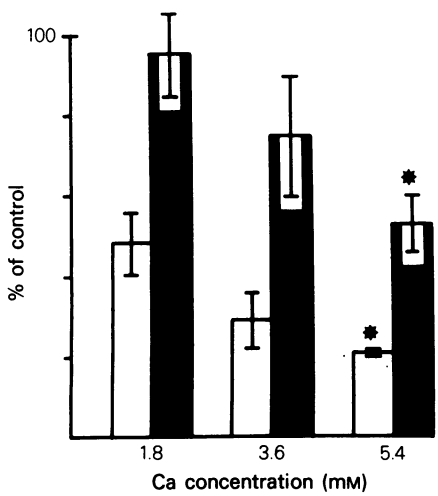

Figure 5 Effect of ketamine, 10^{-4} M (open columns) and 5×10^{-4} M (solid columns) on the contractile force of spontaneously beating right atria in the presence of normal (1.8 mM) or high (3.6 and 5.4 mM) Ca medium, expressed as percentage of control contractile force. Values are mean of 8 experiments; vertical lines show the s.e.mean. Significantly different from normal Ca medium (*$P < 0.05$).

Ketamine interaction with reserpine, practolol and isoprenaline

These experiments were performed to determine whether the positive inotropic effect of ketamine was mediated by the release of catecholamines from cardiac sympathetic stores. The positive inotropic effect of ketamine was studied in 6 spontaneously beating right atria obtained from reserpine-treated animals and in another 6 atria pretreated with practolol (8×10^{-6} M). In these experiments, the positive inotropic effect of ketamine (5×10^{-4} M) was not significantly modified in either atria from reserpine-treated rats ($88 \pm 12\%$ as compared to $95 \pm 12\%$, $P > 0.05$) or practolol pretreated atria ($99 \pm 9\%$ as compared to $94 \pm 12\%$, $P > 0.05$).

Dose-response curves for chronotropic and inotropic responses to isoprenaline on 7 right atria were obtained in control Tyrode solution and in the presence of ketamine (5×10^{-5} M–5×10^{-4} M). As is shown in Figure 6, ketamine at these concentrations produced a dose-dependent positive inotropic effect and caused a parallel shift of the dose-response curve upward. The normalized data represented in the upper part of the figure, however, indicate that ketamine did not significantly affect the shape of the positive inotropic responses induced by isoprenaline.

Effect of verapamil and caffeine on the positive inotropic effect of ketamine

The positive inotropic effect of ketamine (10^{-4} M and 5×10^{-4} M) was studied in electrically driven left atria in the absence and presence of verapamil (10^{-6} M). Verapamil itself decreased peak contractile force by $34 \pm 5\%$ ($n = 10$) and almost abolished the positive inotropic effect of ketamine, 10^{-4} M ($7 \pm 6\%$ as compared to $22 \pm 5\%$, $P < 0.001$). In the presence of verapamil, ketamine 5×10^{-4} M did not increase but

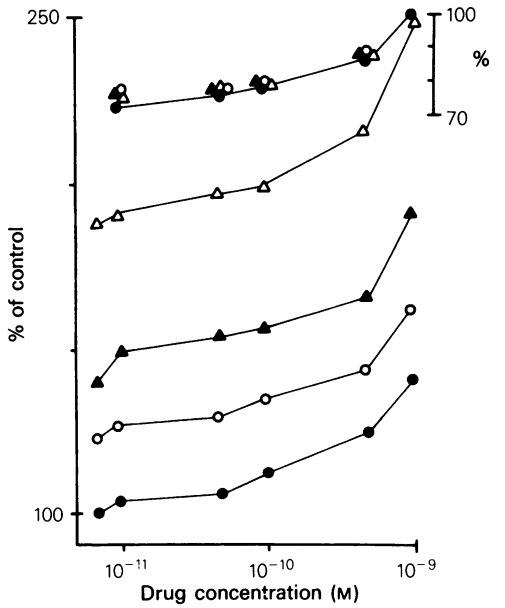

Figure 6 Effect of ketamine on the positive inotropic responses to isoprenaline. Ordinate scale: increase in contractile force (% of control values). Abscissa scale: drug concentration (M). Each point is the mean of 7 experiments. The upper part of the figure shows the normalized averaged data. (●) Without ketamine; (○) ketamine 5×10^{-5} M; (▲) ketamine, 10^{-4} M; (△) ketamine, 5×10^{-4} M.

decreased peak contractile force by $55 \pm 14\%$. These results suggest that the positive inotropic effect of ketamine might be due, at least in part, to an increase in transmembrane Ca influx, since it was abolished by verapamil, a Ca-antagonist.

The possible contribution of the sarcoplasmic reticulum to the positive inotropic effect of ketamine was investigated by exposing the atria to caffeine, a drug which releases Ca from and inhibits Ca uptake into the sarcoplasmic reticulum (Blinks, Olson, Jewell & Braveny, 1972). In 6 experiments, ketamine (5×10^{-4} M) increased peak contractile force by $36 \pm 5\%$ at the time caffeine (4×10^{-3} M) was given. Under these conditions, caffeine still increased contractile force by $42 \pm 6\%$ ($92 \pm 15\%$ over control values). In another approach, atria were exposed first to caffeine and then to caffeine plus ketamine. Caffeine increased contractile force by $90 \pm 13\%$, but ketamine decreased contractile force in the presence of caffeine by $13 \pm 3\%$ ($P < 0.05$).

Effect of ketamine on ^{45}Ca uptake and ^{45}Ca efflux

The effect of ketamine on ^{45}Ca uptake was studied in paired left atria driven at a basal rate of 3 Hz after 2 h of incubation in labelled Tyrode solution. At this time the uptake curve had already reached its plateau level and it was easy to distinguish the effect of the drug (Figure 7). Ketamine, 5×10^{-5} M increased ^{45}Ca uptake at all times, whereas at 10^{-4} M the increase in ^{45}Ca uptake was observed only after 5 and 10 min of incubation in labelled Tyrode solution. At 5×10^{-4} M, however, ketamine did not modify ^{45}Ca uptake at any time. Ketamine also increased ^{45}Ca efflux in electrically driven left atria. Results obtained in the presence of ketamine, 10^{-4} M and 5×10^{-4} M, are shown in Figure 8. Although the possibility exists that ketamine at concentrations below 5×10^{-4} M produces a net increase of intracellular Ca concentration, it seems more likely that at 5×10^{-4} M it causes an increased turnover of Ca in atrial fibres.

Discussion

The results of the present study show that in rat isolated atria ketamine decreased atrial rate in a dose-dependent manner similar to that previously observed in isolated preparations of other species (Dowdy & Kaya, 1968; Traber, Wilson & Priano, 1968; Goldberg *et al.*, 1970; Adams *et al.*, 1977). Paradoxically, and in contrast to previous reports, ketamine in either spontaneously beating right atria or in electrically driven left atria produced a dose-

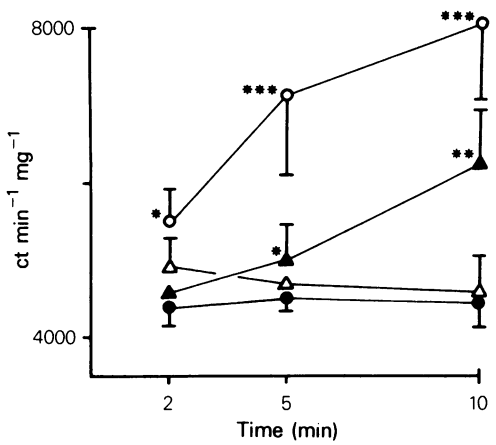

Figure 7 Effect of ketamine on ^{45}Ca uptake in electrically driven left atria. Ketamine was added after 2 h of incubation in labelled Tyrode solution. Ordinate scale: ^{45}Ca uptake in ct min^{-1}mg^{-1} of atria. Abscissa scale: time (min) in radioactive solution. Each point represents the mean of at least 5 experiments; vertical lines show the s.e.mean. (●) Without ketamine; (○) ketamine, 5×10^{-5} M; (▲) ketamine, 10^{-4} M; (△) ketamine, 5×10^{-4} M. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

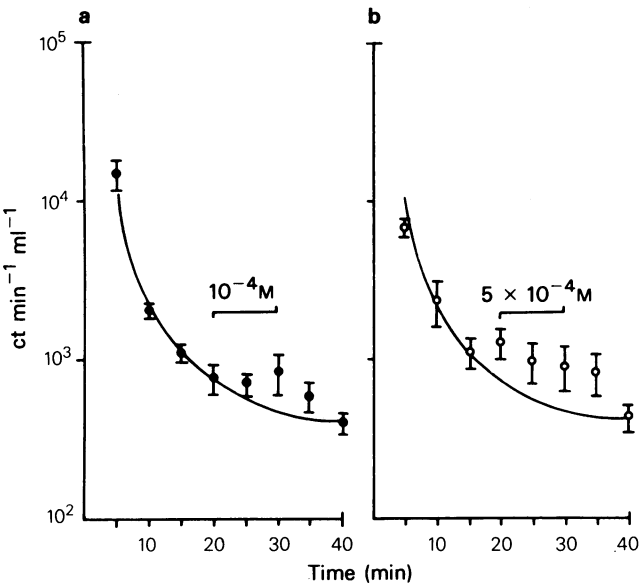

Figure 8 Effect of ketamine, 10^{-4} M (●, a) and 5×10^{-4} M (○, b), on ^{45}Ca efflux in electrically driven left atria. Ordinate scale: ^{45}Ca in ct min^{-1}ml^{-1} on a semi-logarithmic scale. Abscissa scale: time (min). Each point represents the mean of 6 experiments: vertical bars show the s.e.mean.

dependent increase in peak contractile force. In the left atria this effect was accompanied by a parallel increase in df/dt_{max} and by a prolongation in the time to peak tension and in the time for total contraction. Since in the rat myocardium a decrease in atrial rate is accompanied by an increase in contractile force (Koch-Weser & Blinks, 1963), the positive inotropic effect of ketamine could, at least partly, be attributed to its negative chronotropic effect. This was confirmed by the greater positive inotropic effect of the drug in spontaneously beating right atria than in left atria driven at constant rate. The plasma levels (6×10^{-5} M to 2×10^{-4} M; Cohen & Trevor, 1973) of anaesthetic doses of ketamine (20–40 mg/kg) after its intravenous injection into rats are precisely the concentrations of ketamine that produced the positive inotropic effect in the rat isolated atria. The discrepancy between the results of this study and those reported by other authors could be attributed to species differences (Dowdy & Kaya, 1968; Traber *et al.*, 1968; Adams *et al.*, 1977).

The results of this study are clearly consistent with the hypothesis that the positive inotropic effect of ketamine may represent an increase in transmembrane Ca influx into atrial fibres. The evidence for this is 5 fold: (1) Ketamine produced a dose-dependent increase in peak contractile force and df/dt_{max} in both right and left atria. The rapid onset of the positive inotropic effect of ketamine and the excellent recovery after washing might suggest that its action could be located on the outer surface of the sarcolemma. (2) The positive inotropic effect was abolished by verapamil, a Ca-antagonist that inhibits Ca influx (Fleckenstein, 1977) and prevents the replenishment of sarcolemmal Ca binding sites which may regulate the influx of Ca during excitation (Langer, Frank & Brady, 1976). (3) The increment in Ca influx produced by ketamine seems to be essential for the positive inotropic effect of the drug since its effect was highly dependent on the Ca and Na concentration in the bathing media. The inotropic effect of ketamine was significantly reduced when the atria were incubated in low Na or in high Ca solutions, i.e. when the Ca influx was already largely increased (Niedergerke, 1963). (4) Ketamine, at concentrations below 5×10^{-4} M, increased significantly the ^{45}Ca uptake in electrically driven atria, which seems to confirm that the increase in Ca influx is involved in the positive inotropic effect of the drug. However, at 5×10^{-4} M ketamine did not increase ^{45}Ca uptake at any time interval. Since at this concentration ketamine also increased ^{45}Ca efflux, it seems likely that the failure to demonstrate an increase in ^{45}Ca uptake could indicate that ketamine at this concentration increased the turnover of Ca in atrial fibres. (5) It is known that an increase of the action potential duration can prolong Ca influx (Morad & Trautwein, 1968; Wood, Heppner & Weidmann, 1969; Braveny & Sumbera, 1970). Ketamine also caused a dose-dependent increase in height and duration of the

plateau and prolonged the action potential duration; both effects occurred concomitantly with the increase in peak contractile force. In the rat myocardium the slow inward current flowing during the plateau phase of the action potential is carried by Ca ions (Coraboeuf & Vassort, 1968). Therefore, changes in the plateau and in peak contractile force suggest that the drug is increasing transmembrane Ca influx during the plateau of the action potential into atrial fibres. Because ketamine increased Ca influx, the amount of Ca stored intracellularly is secondarily increased and the subsequent depolarizations will release more Ca from the sarcoplasmic reticulum (SR); an augmented amount of Ca to the myofilaments per unit of time would increase the intensity of the active state and the df/dt_{max}.

At high concentrations ($>10^{-4}$ M), ketamine not only increased the intensity but also the duration of the active state. A possible explanation for both effects is that ketamine slows the rate of Ca sequestration. Although there are, unfortunately, no data about the effects of ketamine on uptake of Ca into intracellular storage sites, such as the SR, some results might suggest that it acts through an inhibition of Ca sequestration system. Thus, ketamine prolonged the time to peak tension and pharmacological interventions (strontium, caffeine) which produce such prolongation have been related to decreased uptake of intracellular Ca by the SR (Reiter, 1964; Blinks *et al.*, 1972). Ketamine also inhibited the post-extrasystolic potentiation. The mechanism of this potentiation has been explained by the increase in Ca concentration of internal stores caused by the increased influx of Ca by the second action potential (Edmonds, Greenspan & Bailey, 1972). Assuming that this theory is correct, it can be expected that ketamine would decrease post-extrasystolic potentiation if the drug decreases Ca sequestration by the SR. Finally, ketamine depressed the amplitude-interval relationship. The contraction elicited by the test stimuli after resumption of stimulation is determined by the Ca stored in the SR at the beginning of the rest period as well as by the rate of Ca efflux (Bass, 1976). Therefore, the reduction of the amplitude-interval relationship produced by ketamine is not associated only with a reduced store of Ca but also with an increased Ca efflux during the resting period. However, ketamine did not increase resting tension. This finding makes it unlikely that ketamine decreases Ca uptake by the SR. The absence of contracture could be explained because ketamine increased ^{45}Ca efflux, thus preventing the increase of intracellular Ca above the threshold level for the development of tension. On the other hand, the absence of contracture may also be presumed to indicate that the Ca-sequestering mechanism of rat myocardium is able to keep the sarcoplasmic Ca concentration below the threshold for tension development, even in the face of considerable inhibition by ketamine. A similar mechanism has been proposed to explain the low susceptibility of the kitten heart to develop contracture in the presence of caffeine (Blinks *et al.*, 1972). Further studies are required to determine the effect of ketamine on intracellular stored Ca.

The increase of contractile force produced by ketamine has been attributed to its ability to release endogenous catecholamines (Virtue *et al.*, 1967) or to a cocaine-like effect (Nedergaard, 1973). However, catecholamines produce effects just opposite to those produced by ketamine (Entman, Levery & Epstein, 1969). The results of this study also demonstrate that in both practolol-treated atria or in atria obtained from reserpine-treated animals, ketamine still produced its positive inotropic effect. Moreover, ketamine did not alter the positive inotropic and chronotropic responses to isoprenaline. Therefore, these results suggest that the positive inotropic effect of ketamine is not mediated through stimulation of cardiac β_1-adrenoceptors, the release of endogenous catecholamines or the stimulation of catecholamine-responsive adenylate-cyclase.

References

ADAMS, H.R., PARKER, J.L. & MATHEW, B.P. (1977). The influence of ketamine on inotropic and chronotropic responsiveness of heart muscle. *J. Pharmac. exp. Ther.*, **201,** 171–183.

BARRIGON, S., TAMARGO, J. & GARCIA DE JALON, P. (1978). Interaction of cocaine and bunaphtide on the ^{3}H-norepinephrine uptake and retention on isolated left atrium of guinea-pig. *Experientia*, **34,** 770–771.

BASS, O. (1976). The decay of the potentiated state in sheep and calf ventricular myocardial fibers. *Circulation Res.*, **39,** 396–399.

BAYER, R., HENNEKES, R., KAUFMANN, R. & MANNHOLD, R. (1975). Inotropic and electrophysiological actions of verapamil and D600 in mammalian myocardium. *Naunyn-Schmiedebergs Arch. Pharmac.*, **290,** 49–68.

BLINKS, J.R., OLSON, C.B., JEWELL, B.R. & BRAVENY, P. (1972). Influence of caffeine and other methylxanthines on mechanical properties of isolated mammalian heart muscle. *Circulation Res.*, **30,** 367–392.

BRAVENY, P. & SUMBERA, J. (1970). Electromechanical correlations in the mammalian heart muscle. *Pflügers Arch.*, **319,** 36–48.

COHEN, M.L. & TREVOR, A.J. (1973). On the cerebral

accumulation of ketamine and the relationship between metabolites of the drug and its pharmacological effects. *J. Pharmac. exp. Ther.*, **189**, 351–358.

CORABOEUF, E. & VASSORT, G. (1968). Effects of some inhibitors of ionic permeabilities on ventricular action potential and contraction of rat and guinea-pig hearts. *J. Electrocardiol.*, **1**, 19–30.

DE MELLO, W.C. (1976). On the mechanism of histamine action in cardiac muscle. *Eur. J. Pharmac.*, **35**, 315–324.

DOMINO, E.F., CHODOFF, P. & CORSSEN, G. (1965). Pharmacological effects of Cl-581, a new dissociative anesthetic in man. *Clin. Pharmac. Ther.*, **6**, 279–290.

DOWDY, E.G. & KAYA, K. (1968). Studies on the mechanism of cardiovascular responses to Cl-581. *Anesthesiol.*, **29**, 931–943.

EDMONDS, R.E., GREENSPAN, K. & BAILEY, J.C. (1972). Role of premature action potential in contractile potentiation; a study of paired potentiation. *Cardiovascular Res.*, **6**, 368–374.

ENTMAN, M.L., LEVEY, G.S. & EPSTEIN, S.E. (1969). Mechanism of action of epinephrine and glucagon on the heart: evidence for a cyclic 3′,5′-AMP mediated increase in cardiac sarcotubular stores. *Circulation Res.*, **25**, 429–438.

FLECKENSTEIN, A. (1977). Specific pharmacology of calcium in myocardium cardiac pacemakers and vascular smooth muscle. *A. Rev. Pharmac.*, **17**, 149–166.

GOLDBERG, A.H., KEANE, P.W. & PHEAR, W.P. (1970). Effects of ketamine on contractile performance and excitability of isolated heart muscle. *J. Pharmac. exp. Ther.*, **175**, 388–394.

KOCH-WESER, J. & BLINKS, J.R. (1963). The influence of the interval between beats on myocardial contractility. *Pharmac. Rev.*, **15**, 601–652.

LANGER, G.A., FRANK, J.S. & BRADY, A.J. (1976). The myocardium. *Int. J. Physiol.*, **9**, 191–237.

McCARTHY, D.A., CHEN, G., KAUMP, D.H. & ENSOR, C. (1965). General anesthetic and other properties of 2-(O-chlorophenyl)-2-methylamino cyclohexane HCl (CI-581). *J. New Drugs.*, **5**, 21–33.

McGRATH, J.C., MacKENZIE, J.E. & MILLAR, R.A. (1975). Effects of ketamine on central sympathetic discharge and the baroreceptor reflex during mechanical ventilation. *Br. J. Anaesth.*, **47**, 1141–1147.

MORAD, M. & TRAUTWEIN, W. (1968). Effect of the duration of the action potential on contraction in mammalian heart muscle. *Pflügers. Arch.*, **299**, 66–82.

NEDERGAARD, O.A. (1973). Cocaine-like effect of ketamine on vascular adrenergic neurones. *Eur. J. Pharmac.*, **23**, 153–161.

NIEDERGERKE, R. (1963). Movements of Ca in beating ventricles of the frog's heart. *J. Physiol.*, **167**, 551–580.

REITER, M. (1964). Electrolites and myocardial contractility. In Pharmacology of Cardiac Function. *Proc. Second Intern. Pharmac. Meeting* (Prague). ed. Krayer, O., Vol. 5, pp. 25–42. Oxford: Pergamon Press.

RODRIGUEZ, S. & TAMARGO, J. (1980). Electrophysiological effects of imipramine on bovine ventricular muscle and Purkinje fibres. *Br. J. Pharmac.*, **70**, 15–23.

SCHWARTZ, D.A. & HORWITZ, L.D. (1975). Effects of ketamine on left ventricular performance. *J. Pharmac. exp. Ther.*, **194**, 410–414.

TANAKA, K. & PETTINGER, W.A. (1974). Renin release and ketamine-induced cardiovascular stimulation in the rat. *J. Pharmac. exp. Ther.*, **188**, 229–233.

TRABER, D.L., WILSON, R.D. & PRIANO, L.L. (1968). Differentiation of the cardiovascular effects of CI-581. *Anesth. Analg.*, **47**, 769–777.

TRABER, D.L. & WILSON, R.D. (1969). Involvement of the sympathetic nervous system in the pressor response to ketamine. *Anesth. Analg.*, **48**, 248–252.

VIRTUE, R.W., ALANIS, J.M., MORI, M., LAFARGUE, R.J., VOGEL, J.H. & METCALF, D.R. (1967). An anesthetic agents: 2-orthochlorophenyl, 2-methylamino cyclohexanone HCl (CI-581). *Anesthesiol.*, **28**, 823–833.

WILDBRANDT, W. & KOLLER, H. (1948). Die Calcium-Wirkung am Forscherzen als Funktion des Ionengleichgewichts Zwischen Zellmembran und Umgebung. *Helv. physiol. pharmac. Acta*, **6**, 208–221.

WOOD, E.H., HEPPNER, R.L. & WEIDMANN, S. (1969). Inotropic effects on electric currents: I. Positive and negative effects of constant electric currents or current pulses applied during cardiac action potentials. II. Hypothesis: calcium movements, excitation-contraction coupling and inotropic effects. *Circulation Res.*, **24**, 409–445.

(Received August 6, 1981. Revised January 6, 1982.)