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Electrophysiologic Mechanisms Responsible for Inotropic Responses to Ketamine in Guinea Pig and Rat Myocardium

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Inotropic and electrophysiologic effects of ketamine were investigated in cardiac preparations isolated from guinea pigs and rats. Ketamine produced a concentration-dependent negative inotropic effect in electrically driven guinea pig papillary muscles, an effect that was accompanied by a decrease in action potential duration at the 0-mV level (APD_0). In contrast, ketamine produced a concentration-dependent positive inotropic effect in rat left atria in the presence of 10^{-6} M propranolol. The increase in force of contraction was accompanied by an increase in APD_0. Experiments using patch clamp techniques revealed that ketamine reduced the transsarcolemmal Ca^{2+} current (I_{Ca}) as well as the inward rectifier K^+ current and delayed outward K^+ current in guinea pig single ventricular cells. These results indicate that the shortening of APD_0 observed in guinea pig papillary muscles might result from the suppression of I_{Ca}. In rat single ventricular cells ketamine reduced the Ca^{2+}-insensitive transient outward current (I_{to}) and did not enhance I_{Ca}, suggesting that the ketamine-induced prolongation of APD_0 observed in rat left atria is due to a decrease in I_{to} rather than an increase in I_{Ca}. Treatment of rat left atria with the specific Ca^{2+}-insensitive I_{to} inhibitor 4-aminopyridine (2 mM) produced a positive inotropic effect and prolongation of APD_0, and these effects were equivalent to those caused by the highest concentration of ketamine. In the presence of 4-aminopyridine, ketamine failed to induce a positive inotropic effect and instead caused a negative inotropic one. In conclusion, the negative and positive inotropic effects of ketamine may result from the suppression of I_{Ca} and I_{to}, respectively. The inhibitory action on these membrane currents may at least in part explain the species and tissue differences in inotropic responses to ketamine. Key words: Heart: action potential; inotropic effect. Anesthetics, intravenous: ketamine. Species: guinea pig; rat. Ions: calcium; potassium. Pharmacology: 4-Aminopyridine.

KETAMINE possesses a direct negative inotropic effect on a variety of mammalian heart preparations, such as isolated rabbit heart,[1] guinea pig atrium,[2] and canine atrium[3] and ventricle.[4] Ketamine inhibits the maximum rate of rise of slow action potential without affecting a rapid cooling contracture in rabbit papillary muscles,[5] suggesting that inhibition of transsarcolemmal Ca^{2+} influx through Ca^{2+} channels is in part responsible for the negative inotropic effect of ketamine. In this regard, ketamine appears to have an action similar to other intravenous and inhalation anesthetics.[6–9] In contrast, Barrigon *et al.* have reported that ketamine causes a direct positive inotropic effect on rat atria, an effect that is accompanied by an increase in action potential duration (APD) and is abolished completely by verapamil, a Ca^{2+} channel blocker.[10] From these findings they postulated that ketamine may increase transsarcolemmal Ca^{2+} influx in rat atria. However, it remains unanswered as to why ketamine exerts disparate inotropic actions on myocardium of different species.

In this study, we examined the inotropic effects of ketamine on cardiac muscles isolated from guinea pigs and rats. We found that ketamine produces a negative inotropic effect in guinea pig papillary muscle and, as reported by Barrigon *et al.*,[10] a positive inotropic effect in rat left atrium. Because changes in APD were opposite in these cardiac tissues—a shortening of APD in guinea pig papillary muscles and a prolongation in rat atria—we examined effects of ketamine on membrane current system in isolated ventricular cells of guinea pigs and rats using patch clamp techniques.

Materials and Methods

Measurement of Force of Contraction

This study was approved by the Hokkaido University School of Medicine Animal Care and Use Committee. Guinea pigs of either sex weighing 250–400 g were killed by a blow on the head, and male Wister rats weighing 200–400 g were anesthetized with diethyl ether. Then, the hearts were excised rapidly and transferred to a dissection bath filled with oxygenated Krebs-Henseleit solution (*p*H 7.4) at room temperature. The solution was of the following composition (millimolar concentrations): Na^+ 143.9, K^+ 6.0, Ca^{2+} 1.8, Mg^{2+} 1.2, Cl^- 127.4, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, HCO_3^- 24.9, and glucose 10.0. The right ventricular papillary muscle of guinea pig, left ventricular papillary muscle of rat, and left atrium of each species were dissected carefully.

The papillary muscle, less than 1 mm in diameter, was mounted vertically in a 10-ml water-jacketed bath containing Krebs-Henseleit solution bubbled with 95% O_2 and 5% CO_2 at 30° C. The lower end of the papillary muscle was fixed on a hook, and the other end was connected by a silk thread to a force transducer (Nihon Koh-

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den, TB-612T). Isometric tension developed in the preparation was recorded on a pen recorder (Nihon Kohden, RJG-3026) through a preamplifier (Nihon Kohden, RP-5). The resting tension applied to the preparation was adjusted to 0.5 g. The papillary muscle was paced electrically by rectangular pulses 0.5 Hz in frequency, 5 ms in duration and two times the threshold voltage, delivered by a pair of spiral platinum electrodes connected to an electric stimulator (Sanei Sokki, 3F46) through an isolation unit (Sanei Sokki, 5361).

The left atrium was mounted vertically in a water-jacketed bath containing 50 ml of Krebs-Henseleit solution bubbled with 95% O_2 and 5% CO_2 at a temperature of 30°C. The lower end of the preparation was pinned on a pair of hook electrodes for stimulation, and the other was connected by a silk thread to a force transducer (Nihon Kohden, TB-612T). The muscle was stimulated electrically and the isometric developed tension was recorded as described for the papillary muscle except that the resting tension applied to the preparation was adjusted to 1.0 g and the rectangular pulse was 1 ms in duration.

The atria and the papillary muscles were equilibrated for 75 min and 120 min before starting the experiments, respectively. The concentration–response curves for the inotropic effect of ketamine were determined in a cumulative manner by increasing its concentration in steps of 0.5 log unit. In a series of experiments to examine the influence of 4-aminopyridine (4-AP) on the inotropic effect of ketamine, ketamine was applied cumulatively about 30 min after the administration of 2 mM 4-AP, when an increase in the force of contraction by 4-AP reached a steady level. When prazosin was used, it was added to the bath 30 min before the administration of ketamine. All of the experiments were carried out in the presence of propranolol (10^{-6} M) to eliminate the possibility that endogenous norepinephrine released by ketamine might modify the direct inotropic effect of ketamine.[3]

Measurement of Action Potential

The left atrial strips and papillary muscles of guinea pig and rat were placed in a 5-ml tissue bath and superfused at a rate of 10 ml/min with Krebs-Henseleit solution. The solution was gassed with 95% O_2 and 5% CO_2, and the temperature was kept at 30° C. One end of the preparation was hooked to an extension of the lever arm of a force transducer (Nihon Kohden, TB-651T), and the other end was pinned to the bottom of the tissue chamber. The transducer was mounted on a micromanipulator, and resting tension applied to the preparation was adjusted to 200 mg.

The preparations were stimulated at a rate of 0.5 Hz through platinum field electrodes. Stimuli were rectangular pulses of 1-ms duration at twice the threshold voltage, delivered from an electronic stimulator (Nihon Kohden, SEN-3101) through an isolation unit (Nihon Kohden, SS-101J). Transmembrane potentials were recorded using glass microelectrodes filled with 3 M KCl (resistance of 10–20 MΩ). The microelectrode was coupled *via* an Ag/AgCl junction to a high-impedance capacitance neutralizing amplifier (Nihon Kohden, MEZ-8201). An agar bridge containing 3 M KCl was used as a reference electrode. An electronic differentiator whose output was linear from 50 to 1,000 V/s was used for measuring the maximum rate of rise of action potentials. These amplified signals were displayed on an oscilloscope (Nihon Kohden, VC-10), photographed on 35-mm film, and recorded on a chart recorder (Watanabe Sokki, WR-3101). The preparations were equilibrated for at least 120 min before the commencement of the experiments. The experiments studying the effects of ketamine were carried out in the presence of propranolol (10^{-6} M). Only the results from the experiments in which a stable impalement was maintained are presented here.

Measurement of Membrane Currents

Single ventricular cells of guinea pig and rat hearts were obtained, as described previously.[11] In an anesthetized animal whose lungs were being mechanically ventilated, the heart was quickly excised and cannulated by a Langendorff apparatus. The heart was perfused with a normal HEPES–Tyrode solution (36° C) until the rate of beating became stable (about 5 min). Then, the perfusate was changed to a nominally Ca^{2+}-free Tyrode solution for 5 min, resulting in the cessation of the heart's beating. The quiescent heart was perfused with the solution containing 0.04% (weight/volume) collagenase (type 1, Sigma, St. Louis, MO) for 5–10 min in guinea pig hearts and for 20 min in rat hearts.

After the digestion by collagenase, a Kraftbrühe solution[12] was used to wash the collagenase solution off, and the ventricular tissue was cut into small pieces. The cell suspension in the Kraftbrühe solution was filtered through a 100-μm pore stainless steel mesh and stored in a refrigerator (4° C) for later use. The normal HEPES–Tyrode solution was of the following composition (millimolar concentrations): Na^+ 143.33, K^+ 5.4, Ca^{2+} 1.8, Mg^{2+} 0.5, Cl^- 153, $H_2PO_4^-$ 0.33, glucose 5.5, and HEPES–NaOH buffer (*p*H 7.4) 5.0. The Kraftbrühe solution was of the following composition (millimolar concentrations): K^+ 130, Mg^{2+} 3, Cl^- 46, $H_2PO_4^-$ 20, L-glutamic acid 50, taurine 20, glucose 10, EGTA 0.5, and HEPES–KOH buffer (*p*H 7.4) 10. The internal solution, in the recording pipette, was of the following composition (millimolar concentrations): K^+ 150, Mg^{2+} 1.0, Cl^- 22, aspartate 110, ATP 5.0, phosphocreatine 5.0, EGTA 0.5–10, and HEPES–KOH buffer (*p*H 7.4) 5.0.

Single ventricular cells were placed in the recording chamber (1-ml volume) attached to an inverted microscope (Olympus, IMT-2), and superfused with a HEPES–Tyrode solution at a rate of 4–6 ml/min. The temperature of the external solution was kept at 36° C. The whole-cell membrane currents were recorded by the patch clamp method,[13] using glass patch electrodes with a tip diameter of 4–5 μm and a resistance of 1.5–2.5 MΩ. The tip of the electrode, filled with the internal solution, was attached to cell surface membrane using a micromanipulator (Leitz), and negative pressure (20–30 cmH_2O) was applied to the electrode. After the giga-sealing between the tip and the membrane was established, the membrane patch was disrupted by applying more negative pressure to make the whole-cell voltage-clamp mode. The electrode was connected to an input of a current–voltage converter with a feedback resister of 100 MΩ.

The signals were displayed on a storage oscilloscope (Tektronix, 5113 OP03) and were simultaneously fed to a data recording system consisting of a video cassette recorder (National, NV-F1) and a PCM converter system (Sony, PCM-501ES) as a backup. The current and voltage signals were filtered at 2 KHz, digitized by an analog–digital converter (Canoopus Electronics, ADX-98) at 2 KHz and stored in the 20-Mb hard disk of a personal computer (NEC, PC-98XA) for later analysis. A liquid junction potential between the internal solution and the bath solution of −7 mV was corrected.

Drugs

The following compounds were used: ketamine hydrochloride (kindly given by Warner Lambert, Tokyo, Japan), DL-propranolol hydrochloride (Sigma), prazosin hydrochloride (Taito-Pfizer, Tokyo, Japan), 4-aminopyridine (Wako Pure Chemical Industries, Osaka, Japan), and pinacidil (Shionogi, Osaka, Japan). All drugs except pinacidil were dissolved in distilled water. Pinacidil was dissolved 0.1 N HCl.

Statistics

All values are presented in terms of means ± SE. Analysis by Student's t test was performed for paired or unpaired observations. Comparisons of variables obtained during the dose–response curves with baseline were made by one-way analysis of variance. When an overall statistical significance was found, modified t tests were used to compare an intervention with the control value. $P < 0.05$ was considered significant.

Results

Inotropic Effects of Ketamine in Guinea Pig Papillary Muscle and Rat Left Atrium

Figure 1 (top) depicts representative inotropic responses of electrically driven guinea pig papillary muscle and rat left atrium to ketamine at a concentration of 3

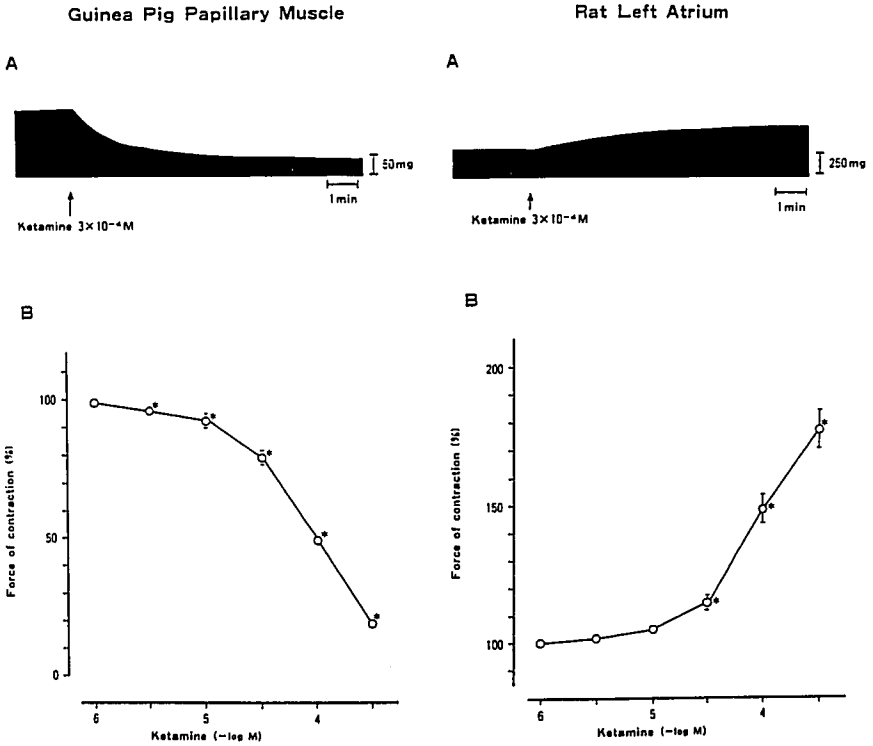

FIG. 1. Effects of ketamine on the force of contraction in guinea pig papillary muscle (*left*) and rat left atrium (*right*). *A*: Typical effect of ketamine at a concentration of 3×10^{-4} M. *B*: Concentration-dependent inotropic effects of ketamine. Values after the administration of ketamine were expressed in percentage of predrug control values taken as 100%. Points are means ± SE of six preparations. *$P < 0.05$ *versus* the respective control values.

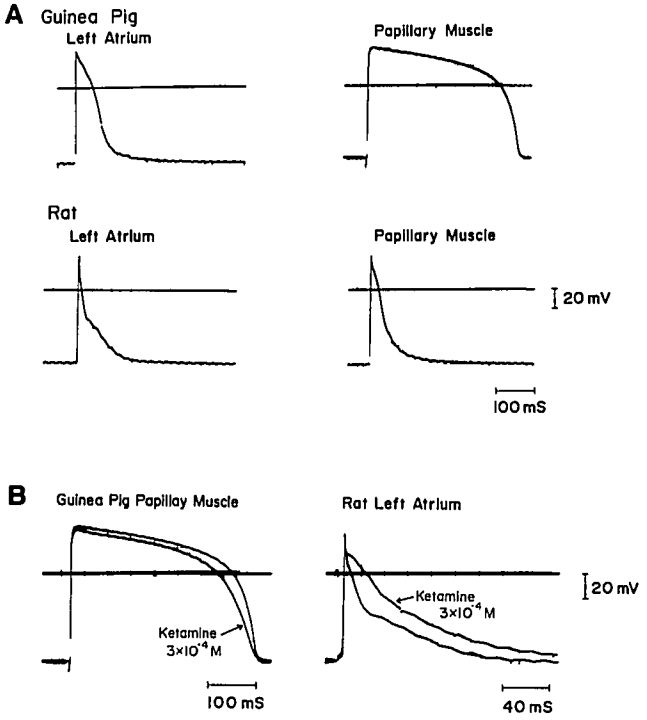

FIG. 2. *A*: Representative action potential configurations of left atrium and papillary muscle of guinea pig and rat, electrically driven at 0.5 Hz. *B*: Effects of ketamine at a concentration of 3×10^{-4} M on action potentials of guinea pig papillary muscle and rat left atrium. The horizontal line indicates a 0-mV level.

$\times 10^{-4}$ M. Ketamine produced a negative inotropic effect in a concentration-dependent manner in guinea pig papillary muscles (fig. 1, left). Ketamine at 3×10^{-4} M decreased the force of contraction to 19.0 ± 1.0% of the basal level (n = 6). In marked contrast, ketamine increased the force of contraction of rat left atria in a concentration-dependent manner (fig. 1, right). The maximum level of the force of contraction obtained at 3×10^{-4} M was 178 ± 7% of the basal value (n = 6). In concentrations greater than 3×10^{-4} M, ketamine decreased the force of contraction (data not shown). The positive inotropic effect of ketamine observed in rat left atria was not affected by an α_1-adrenoceptor antagonist, prazosin (10^{-7} M). The onset of both inotropic responses to ketamine was rapid, and the force of contraction returned to its basal level after the drug was washed out.

Electrophysiologic Effects of Ketamine

In order to clarify the mechanisms of the negative and positive inotropic effects of ketamine, we examined the effects of ketamine on action potentials of guinea pig papillary muscle and rat left atrium. It is noteworthy that action potential configurations of both tissues are quite different from each other, as shown in figure 2A. The action potential configuration of guinea pig papillary muscle is characterized by lack of an early repolarization phase and a very long plateau phase at positive potentials. On the other hand, the APD at the level of 0 mV (APD_0) in rat left atrium is very short.

The plateau phase of the action potential is maintained by a fine balance between inward and outward currents.[14] Inward currents produce depolarization, whereas outward currents contribute to repolarization of the cells. It is well known that Ca^{2+} current flowing through voltage-dependent Ca^{2+} channels (I_{Ca}) is the main inward current flow during the plateau phase.[14] Recently, however, the Ca^{2+}-insensitive transient outward current (I_{to}) has been reported to be responsible for the early repolarization phase of action potential and to participate dominantly in the determination of the plateau duration in both atrial and ventricular cells in various species, including rat ventricular myocardium.[15–18] Judging by the above information, the rapid repolarization phase of rat left atrium seems to be due to in part, if not all, to the flow of Ca^{2+}-insensitive I_{to}, although the existence of this current in rat atrial cells has not been demonstrated. However, this speculation was supported by the finding that the specific Ca^{2+}-insensitive I_{to} inhibitor 4-AP markedly prolonged the APD in rat left atria, as it did in rat papillary muscles. The APD_0 levels in rat left atria and papillary muscle were prolonged by 10.0 ± 2.1 ms (n = 4) and 14.0 ± 1.3 ms (n = 4), respectively by 4-AP at 2 mM.

In both guinea pig papillary muscles and rat left atria, ketamine at a concentration of 3×10^{-4} M produced a depolarization of the resting membrane and decreased the amplitude and the maximum rate of rise of action potentials (table 1). However, as shown in figure 2B, ketamine shortened APD in guinea pig papillary muscle, whereas it prolonged APD in rat left atrium: APD_0 was decreased by about 44 ms in the former and increased by about 8 ms in the latter by ketamine at 3×10^{-4} M. Because changes in APD about the 0-mV level profoundly affect the magnitude of force of contraction,[19,20] it seems likely that the opposite directional changes in APD in guinea pig papillary muscles and rat left atria may be responsible for the negative and positive inotropic effects of ketamine in the respective tissues. Indeed, in rat atria, the time course of the increase in the force of contraction induced by ketamine is parallel with that of the prolongation of APD_0 (fig. 3).

In order to clarify the underlying mechanisms responsible for the ketamine-induced changes in action potential configuration, we examined the effect of ketamine on the transmembrane ionic currents using patch clamp techniques. Figure 4A shows the effects of ketamine on the currents elicited by a 300-ms test pulse from a holding potential of −37 mV to various potentials in a guinea pig ventricular cell, and the current–voltage relationship in

TABLE 1. Effects of 3×10^{-4} M Ketamine on Action Potential Parameters

	RMP (mv)	APA (mv)	APD_0 (ms)	APD_{-40} (ms)	APD_{-70} (ms)	$\dot{V}_{max}$ (V/s)
Rat LA						
Control	−79.3 ± 1.4	110.3 ± 1.7	6.6 ± 1.0	43.0 ± 2.4	119.5 ± 3.3	186 ± 12
Ketamine	−76.5 ± 1.0*	94.8 ± 0.4*	14.9 ± 1.3*	68.3 ± 0.9*	176.5 ± 3.0*	114 ± 11*
Rat PM						
Control	−80.5 ± 1.3	117.3 ± 2.6	19.3 ± 4.0	35.0 ± 4.4	82.0 ± 13.2	197 ± 4
Ketamine	−78.0 ± 1.4*	108.0 ± 2.0*	29.8 ± 5.6*	59.8 ± 7.6*	172.0 ± 21.0*	141 ± 4*
Guinea pig LA						
Control	−80.3 ± 0.6	116.5 ± 2.5	36.9 ± 5.0	64.0 ± 5.9	107.8 ± 1.5	139 ± 11
Ketamine	−79.0 ± 0.7*	108.3 ± 2.6*	49.0 ± 6.4*	91.8 ± 10.2*	171.8 ± 11.1*	107 ± 14*
Guinea pig PM						
Control	−82.3 ± 1.1	127.0 ± 1.5	340.0 ± 2.7	384.4 ± 4.1	396.3 ± 6.4	154 ± 26
Ketamine	−80.5 ± 1.1*	121.5 ± 1.4*	296.3 ± 3.7*	363.8 ± 4.0*	390.0 ± 4.0	117 ± 23*

Values are means ± SE of four experiments in each tissue.
RMP = resting membrane potential; APA = action potential amplitude; APD_0, APD_{-40}, and APD_{-70} = action potential duration at 0, −40, and −70 mV, respectively; $\dot{V}_{max}$ = maximum rate of rise; LA = left atria; PM = papillary muscles.
* Significantly different compared to respective control values.

this cell is depicted in figure 4B. As illustrated clearly, the peak inward current elicited by test pulses ranging from −17 to +32 mV, namely I_{Ca}, was decreased by ketamine in a concentration-dependent manner. The current amplitude elicited by a test pulse to +2 mV, which was defined by subtraction of the late current at the end of the step pulse from the initial peak inward current, was decreased to 69.1 ± 3.6% of control amplitude by ketamine at 10^{-4} M in seven cells. The current–voltage relationship also revealed that ketamine inhibited the inward rectifier K^+ current (I_{Krect}) and the delayed outward K^+ current (I_K). On hyperpolarization to −47 mV, I_{Krect} almost reached its maximum value in the outward direction, and ketamine at 10^{-4} M decreased this current at −47 mV to 46.7 ± 3.4% of the control amplitude (n = 5). The amplitude of I_K at +42 mV, defined by subtraction of the initial current from the late current at the end of the clamp pulse, was also decreased to 48.5 ± 4.0% of control (n = 5). These findings indicate that the ketamine-induced shortening of APD_0 observed in guinea pig papillary muscles derives mainly from the suppression of I_{Ca}.

Concerning the prolongation of the plateau phase of action potential, a decrease in outward currents and/or an increase in inward currents could result in the APD prolongation. Since ketamine did not increase I_{Ca} in rat ventricular cells as mentioned below, a decrease in outward current(s) may provide favorable explanation for the prolongation of APD_0 in rat atria. Accordingly, we examined the effect of ketamine on Ca^{2+}-insensitive I_{to}, which is one of the dominant determinants of the plateau duration as mentioned above. In this experiment, we used rat single ventricular cells instead of atrial cells, because of the ease in obtaining single ventricular myocyte. In order to isolate I_{to}, I_{Ca} was blocked by means of adding 2 mM Ca^{2+} to the external solution.

Figure 5A illustrates changes in actual traces of I_{to} in a cell exposed to 10^{-4} M ketamine. A holding potential was kept at −67 mV and test pulses of 300 ms were given in 10 mV steps from −17 to +53 mV. A current–voltage relationship in the same cell is illustrated in figure 5B. I_{to} was activated at potential above −17 mV, and at every potential at which I_{to} was activated, ketamine decreased the peak current of I_{to} with relatively little effect on the current level at the end of the test pulses. Ketamine suppressed I_{to} in a concentration-dependent manner. The current amplitude defined by subtraction of the late current at the end of the test pulse from the initial peak outward current elicited by a test pulse to +32 mV was decreased to 57.5 ± 7.9 and 35.6 ± 5.6% of control after the application of ketamine at 10^{-4} M and 3×10^{-4} M, respectively (n = 5). In addition, it is worth mentioning that the holding outward current at −67 mV was sup-

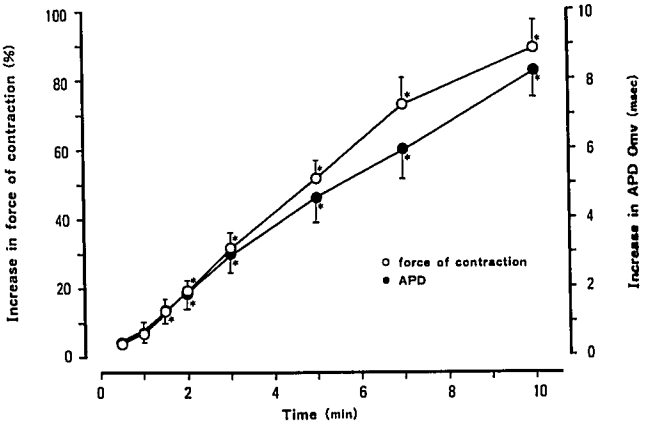

FIG. 3. Time course of changes in force of contraction (O) and action potential duration at 0 mV (●) produced by ketamine at 3×10^{-4} M in rat left atria (mean ± SE; n = 4). Left ordinate: net increase in force of contraction expressed as percentages of the predrug values. Right ordinate: net increase in action potential duration at 0 mV expressed as absolute values (milliseconds). *$P < 0.05$ *versus* the respective baseline values.

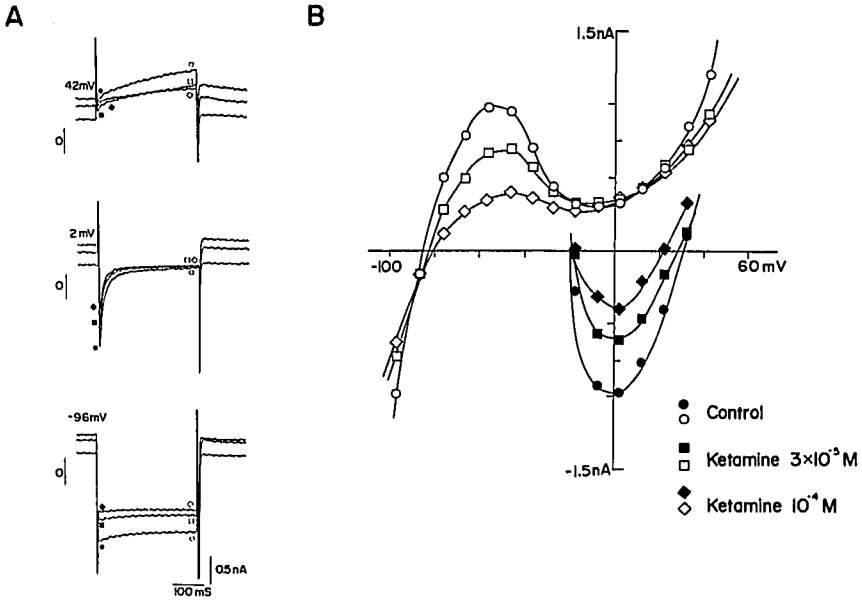

FIG. 4. Effect of ketamine on membrane currents elicited by clamp pulses of 300-ms duration from a holding potential of −37 mV in a guinea pig single ventricular cell. *A*: Typical current traces elicited by voltage-clamp pulses to +42, +2, and −96 mV. The peak current (●, ■, ♦) and steady current at the end of the pulse (O, □, ◇) in the absence and the presence of ketamine (3×10^{-5} M, 10^{-4} M), respectively, were measured, and current–voltage relationships are depicted in *B*.

pressed by ketamine (fig. 5A), indicating its suppressing effect on I_{Krect} in rat ventricular cells as in guinea pig ventricular cells.

We also confirmed the effect of ketamine on I_{Ca} in rat ventricular cells in another set of experiments. In four cells, ketamine at 10^{-4} M decreased I_{Ca} to 89.2 ± 10.8% of control. The inhibitory effect of ketamine on I_{Ca} in rat ventricular cells seems to be less than that in guinea pig cells, although the difference was not statistically significant.

INFLUENCE OF 4-AMINOPYRIDINE ON INOTROPIC RESPONSE OF RAT LEFT ATRIUM TO KETAMINE

4-AP at a concentration of 2 mM increased the force of contraction in rat left atria (fig. 6). After pretreatment with 4-AP, ketamine failed to increase the force of contraction any longer. Instead of the positive inotropic effect, ketamine at 3×10^{-4} M reduced the force of contraction slightly but obviously (fig. 6A). The addition of $CaCl_2$ (1.5 mM) was still able to increase the force of contraction

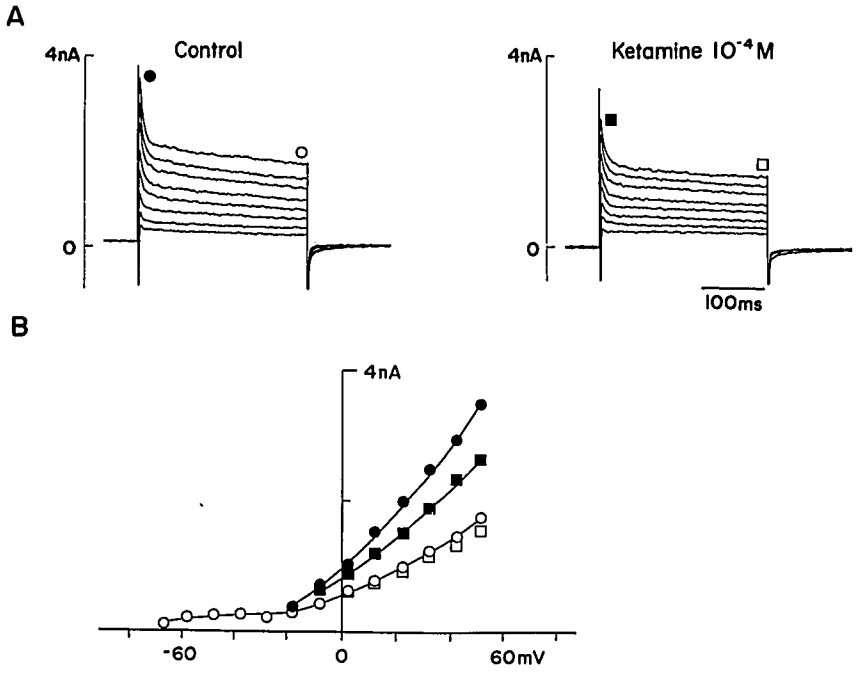

FIG. 5. Effect of ketamine on transient outward current (I_{to}) elicited by 300-ms clamp pulses from a holding potential of −67 mV in a rat single ventricular cell. *A*: Representative family of current traces of I_{to} elicited by voltage-clamp pulses from −17 to +53 mV in 10-mV steps in the absence and presence of ketamine at 10^{-4} M. The respective peak current (●, ■) and the steady current at the end of the pulse (O, □) were measured, and current–voltage relationships are depicted in *B*.

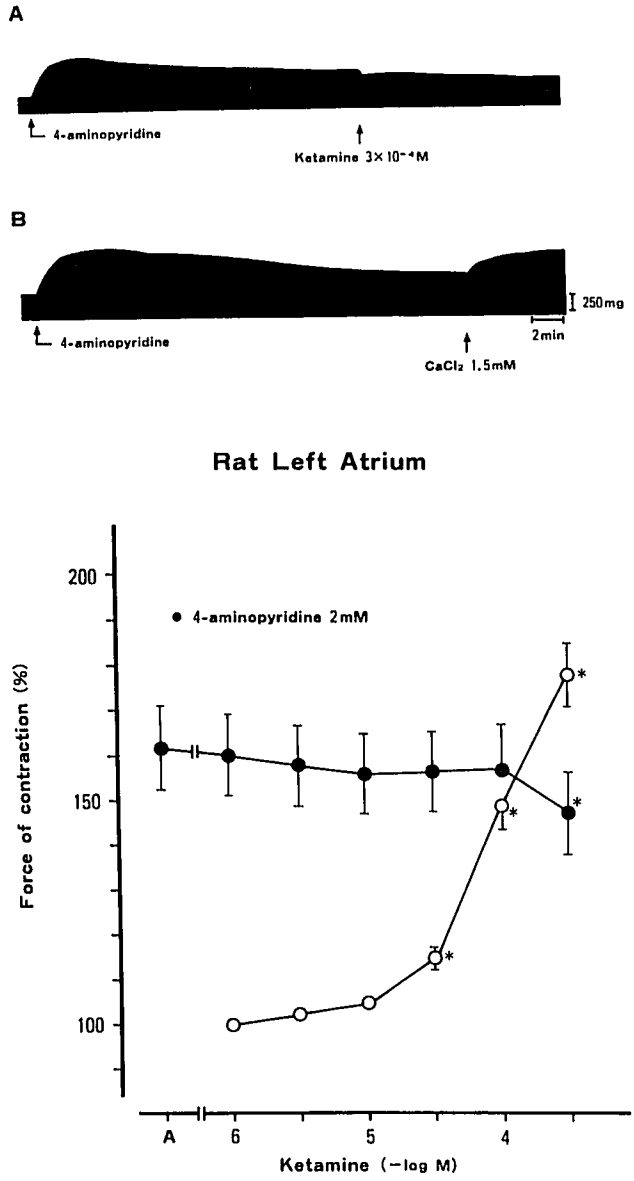

FIG. 6. Influence of 2 mM 4-AP on the inotropic effect of ketamine in rat left atria. *Top*: Typical effects of ketamine at 3×10^{-4} M (*A*) and the addition of $CaCl_2$ at 1.5 mM (*B*) on the force of contraction in the presence of 4-AP. *Bottom*: Concentration–response curves for the inotropic effect of ketamine in the absence (○) and presence (●) of 4-AP. Values after the administration of the drugs are expressed as percentages of predrug control values taken as 100%. Abscissa: A = the point where the force of contraction increased by 4-AP reached a steady level. Points are means ± SE of five or six preparations. *$P < 0.05$ *versus* the respective control values.

of rat left atrium even after the preparation was treated with 4-AP (fig. 6B). The concentration–response curves for the inotropic effect of ketamine in rat atria in the absence and presence of 4-AP is shown in figure 6 (bottom). The application of 4-AP at 2 mM increased the force of contraction by 62 ± 9% of basal level (n = 4), and this increase seems to be almost similar to that induced by ketamine at 3×10^{-4} M.

Inotropic Effects of Ketamine in Guinea Pig Left Atrium and Rat Papillary Muscle

We also examined inotropic effects of ketamine in guinea pig left atria and rat papillary muscles. Action potential recorded from guinea pig left atrium has an early repolarization phase, as depicted in figure 2A. 4-AP at 2 mM suppressed this early repolarization phase and prolonged APD_0 by 27.3 ± 3.0 ms (n = 4), suggesting that Ca^{2+}-insensitive I_{to} is also responsible for the early repolarization phase of action potential in guinea pig left atria. Ketamine at 3×10^{-4} M prolonged APD_0 by about 10 ms in the tissues (table 1).

The concentration–response curves for the inotropic effect of ketamine in electrically driven guinea pig left atria in the absence and presence of 4-AP are shown in figure 7 (left). In the absence of 4-AP, ketamine produced a biphasic inotropic effect. Ketamine at concentrations as great as 3×10^{-5} M increased the force of contraction slightly (7 ± 3% of basal level at 3×10^{-5} M) and then reduced the force of contraction in a concentration-dependent manner. The force of contraction at 3×10^{-4} M was 66 ± 3% of the basal level (n = 6). The application of 4-AP at 2 mM increased the force of contraction by 58 ± 8% of the basal level. After treatment with 4-AP, the small positive inotropic effect of ketamine disappeared, and the negative inotropic effect was obvious at concentrations of 10^{-5} M and higher. Eventually, ketamine at 3×10^{-4} M reduced the force of contraction of guinea pig left atria pretreated with 4-AP to 67 ± 3% of the predrug

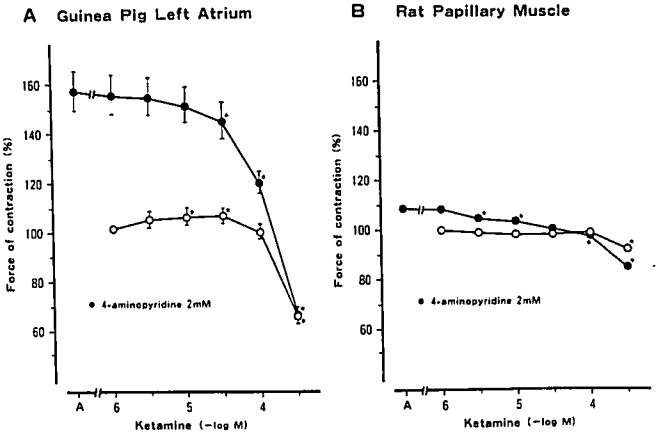

FIG. 7. Effects of ketamine on the force of contraction in guinea pig left atria (*A*) and rat papillary muscles (*B*) in the absence and presence of 2 mM 4-AP. Ordinates and abscissa are the same as in the bottom of figure 6. Points are means ± SE of five or six preparations. *$P < 0.05$ *versus* the respective control values.

basal level (n = 5). This level was almost equal to that obtained from the untreated preparations.

In electrically driven rat papillary muscles, ketamine at concentrations as great as 3×10^{-4} M hardly altered the force of contraction (fig. 7, right), although ketamine increased the APD significantly (table 1). The positive inotropic effect of 4-AP was also small (9 ± 1% of the basal level; n = 4) in contrast to the effects in rat left atria and guinea pig left atria. However, in the presence of the ATP-sensitive K^+ channel opener pinacidil at a concentration of 3×10^{-4} M, which decreased the force of contraction and APD_0 to 66 ± 6% (n = 4) and 74 ± 5% (n = 3) of the basal level, respectively, ketamine (10^{-4} M) and 4-AP (2 mM) clearly produced an increase in the force of contraction (9.5 ± 0.3% (n = 4) and 58.4 ± 3.5% (n = 4) of the predrug level, respectively). The positive inotropic effect of ketamine observed in the presence of pinacidil was completely abolished by pretreatment with 4-AP.

Discussion

In the present study we showed that the establishment of a positive and negative inotropic effect of ketamine is completely dependent on the species and cardiac tissues studied. These diverse effects of ketamine on contractility could be explained by the consistent suppression by ketamine of the transmembrane ionic currents that contribute to the constitution of normal action potential. In other words, the species and tissue differences in membrane ionic current systems seem to play a crucial role in determining the inotropic effects of ketamine.

In mammalian hearts, the normal action potential is composed primarily of five ionic currents: two inward currents (the Na^+ current [I_{Na}] and I_{Ca}) and three outward currents (I_{to}, I_K, and I_{Krect}),[14,21] although pump and transporter currents (Na^+-pump current and Na^+–Ca^{2+} exchange current) have been also suggested to contribute to the constitution of action potential.[22,23] Briefly, I_K plays a crucial role in repolarization of the ventricular cells and in pacemaker activity in sinoatrial cells.[14] In contrast, I_{Krect} plays an important role in generating the resting membrane potential and in modulating the final repolarization phase of the action potentials[16] in ventricular and atrial cells. The differences in action potential configurations among a variety of myocardial tissues, as shown in figure 2A, are believed to originate from the different contributions of these currents to the action potentials.[14] Among the four tissues used in this study, guinea pig papillary muscle is quite different from the other three cardiac tissues in its lack of the early repolarization phase of action potentials. The early repolarization phase is believed to result mainly from the activation of Ca^{2+}-insensitive I_{to},[16–18] suggesting the least contribution, if any, of this current to the action potential in guinea pig papillary muscles. Indeed, to our best knowledge, there is no direct evidence of the existence of this current in guinea pig ventricular cells.

Ketamine exerted various influences on the action potential configurations of the tissues used in this study. First, ketamine depressed the maximum rate of rise ($\dot{V}_{max}$), an approximate index of I_{Na}[20] in all tissues examined. Although the modest depolarization of resting membrane potential by ketamine as mentioned below could in part explain the depression of $\dot{V}_{max}$ because of the increase in inactivated sodium channels, the serious depression of $\dot{V}_{max}$ might indicate the direct inhibitory effect of ketamine on sodium channels. This inhibitory effect of ketamine on sodium channels might, at least in part, be involved in the antiarrhythmic effect of ketamine.[1,24] Second, in rat left atria and papillary muscles and in guinea pig left atria, ketamine prolonged the APD_0 and the APD at the −40-mV (APD_{-40}) and −70-mV levels (APD_{-70}). In contrast, ketamine shortened APD_0 and APD_{-40} in guinea pig papillary muscles. Lastly, the resting membrane potential also was decreased in all of these preparations. These observations suggest that ketamine might affect not only the inward current during the plateau phase, *i.e.*, I_{Ca}, but also outward current(s) in cardiac tissues.

Support for these speculations derived from the whole-cell voltage clamp experiments. In guinea pig single ventricular cells, ketamine suppressed I_{Ca}. In addition, ketamine inhibited the two outward K^+ currents, I_{Krect} and I_K. Because Ca^{2+} influx through Ca^{2+} channels is one of the most important factors in the maintenance of the force of contraction in mammalian hearts,[25] the negative inotropic effect of ketamine observed in guinea pig papillary muscle might be causally related to the decrease in I_{Ca}.

The present observations that ketamine increased the force of contraction and prolonged the APD in rat left atria are consistent with the report of Barrigon *et al.*[10] They also demonstrated that ketamine increased ^{45}Ca uptake and ^{45}Ca efflux, suggesting that ketamine inhibits Ca^{2+} sequestration by sarcoplasmic reticulum, and that the positive inotropic effect of ketamine was antagonized by verapamil. However, because ketamine was found to suppress I_{Ca} in guinea pig and rat ventricular cells, it is highly unlikely that ketamine activated directly the voltage-dependent Ca^{2+} channel in rat atrial cells. It has been shown that enforced prolongation of depolarizing pulse duration of voltage clamp in multicellular or single cellular preparations results in the increase in the force of contraction.[19,20] An increase or decrease in depolarizing pulse duration would indirectly affect Ca^{2+} influx through activated Ca^{2+} channels in opposite direction by changing allowable time for I_{Ca} to flow. Indeed, the shortening of APD induced by an increased outward K^+ current is believed to be the underlying mechanism of muscarinic re-

ceptor-mediated negative inotropic effect in mammalian atrial tissues[26] and the underlying mechanism of the K^+ channel opener-induced negative one.[27] The prolongation of APD resulting from suppression of I_{to} would then be responsible at least in part for the α_1-adrenoceptor–mediated positive inotropic effect in rat ventricle.[11] Therefore, the prolongation of APD at about the 0-mV level resulting presumably from the suppression of outward current(s) might be responsible for the positive inotropic effect of ketamine in rat atria. In fact, the time course of the positive inotropic response of rat left atria to ketamine was identical to the time course of the prolongation of APD_0. Although we did not examine this directly using rat atrial cells, the finding that ketamine suppressed I_{to} elicited in rat ventricular cells strongly suggests that the prolongation of APD_0 could result from the inhibitory effect of ketamine on I_{to} in rat left atria. Supporting this concept is the observation that the specific inhibitor of Ca^{2+}-insensitive I_{to} 4-AP counteracted the positive inotropic effect of ketamine specifically.

The present study showed that 4-AP prolonged APD_0 and increased the force of contraction in guinea pig left atria. Although these data cannot readily be reconciled with the finding by Hume *et al.*[28] that the action potential of guinea pig atrial cell was unaltered by 4-AP, we interpret the present data to indicate the existence of Ca^{2+}-insensitive I_{to} in guinea pig left atrial cells. Thus, the ketamine-induced prolongation of APD_0 appears to be due to the inhibition of Ca^{2+}-insensitive I_{to}. However, we failed to observe any obvious positive inotropic response to ketamine. This may be explained by the idea that the net inotropic response can be expressed as the sum of the positive inotropic effect induced by suppression of I_{to} and the negative one by suppression of I_{Ca}.

The interpretation of the results in rat papillary muscles is more complicated. It should be noted that the application of 4-AP only slightly increased the force of contraction in this tissue despite the presence of I_{to}. It has been shown that the time course of inactivation of I_{Ca} in rat cardiac cells is much faster than that in guinea pig cardiac cells and that the calculated time constants for inactivation of I_{Ca} are about 30 ms in rat cardiac cells and 150 ms in guinea pig ones.[29–31] As listed in table 1, APD_0 in rat left atria was about 7 ms and in rat papillary muscles was about 19 ms. The differences in APD between two tissues of rats might indicate that in rat ventricular cells, the inactivation of I_{Ca} contributes to the repolarization process of the action potential, whereas in rat atrial cells I_{Ca} is terminated by initiation of repolarization, which in turn is induced by outward currents. Therefore, the prolongation of APD induced by suppression of I_{to} could increase Ca^{2+} influx through Ca^{2+} channels in rat left atria, resulting in the increase in force of contraction.

In rat papillary muscles, however, a substantial part of Ca^{2+} channels would be already under process of inactivation, and additional prolongation of APD might not contribute to increase the force of contraction effectively. In order to test this possibility indirectly, we conducted additional experiments using the ATP-sensitive K^+ channel opener pinacidil as a pharmacologic tool. The drug is known to shorten APD and decrease the contraction by increasing the outward current through ATP-sensitive K^+ channels.[27,32] When APD_0 was markedly shortened by pinacidil, 4-AP increased the force of contraction more effectively in rat papillary muscle. Furthermore, ketamine produced a positive inotropic effect more obviously in the presence of pinacidil. Thus, ketamine could increase the force of contraction in rat papillary muscle when action potential prolongation could effectively lead to an increase in Ca^{2+} influx through Ca^{2+} channels.

Ketamine produced a slight negative inotropic effect in rat papillary muscle at concentrations as great as 3×10^{-4} M even in the presence of 4-AP. This is the case in rat left atria and is in marked contrast to the case in guinea pig left atria and papillary muscles. The discrepancy seems to originate in part from the species difference in sensitivity of I_{Ca} to ketamine and in part from the large dependence of rat myocardium on sarcoplasmic reticulum to generate the force of contraction.[33] These properties of rat ventricular muscles could explain the result that ketamine at concentrations as great as 3×10^{-4} M hardly affected the force of contraction in rat papillary muscles.

In conclusion, ketamine consistently suppresses the transsarcolemmal ionic currents that contribute to the constitution of normal action potentials in mammalian myocardial tissues. Inhibition of I_{Ca} and I_{to} may be responsible for the negative and positive inotropic effects of ketamine, respectively, although other potential effects of ketamine upon sarcoplasmic reticulum[34] and myofibrils could contribute to its inotropic responses. Thus, the species and tissue differences in inotropic response to ketamine may be based on the difference of membrane ionic currents in respective tissues. It should be noted, however, that it is difficult to extrapolate the present results to the clinical setting both because of the unphysiologic experimental conditions and because of uncertainty as to exactly what currents are present in human ventricular cells.

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