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Effects of ketamine on voltage-dependent calcium currents and membrane potentials in single bullfrog atrial cells

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Abstract
Purpose. This study was undertaken to assess the effect of ketamine on L-type calcium channel current (I_{Ca}) and membrane action potential in the bullfrog single atrial myocyte.
Methods. Bullfrog single atrial myocytes were prepared by enzymatic dispersion. Whole-cell voltage-clamp technique and current clamp technique were used to monitor I_{Ca}, membrane resting potential, and action potential.
Results. Ketamine (10^{-5}–10^{-3}M) showed dose-dependent inhibition of I_{Ca} in a reversible manner. The 50% inhibitory concentration (IC_{50}) of ketamine on I_{Ca} was estimated to be 0.92×10^{-5}M. Use-dependent block of I_{Ca} was not observed. The resting membrane potential was depolarized at a high concentration (10^{-4}M) of ketamine. Reduction of the plateau phase and prolonged duration of the action potential were observed in the presence of a high concentration of ketamine (10^{-4}M).
Conclusion. Ketamine has an inhibitory effect on I_{Ca} in the bullfrog single atrial myocyte, and a high dose (10^{-4}M) of ketamine prolonges the duration of the action potential. The mechanism of inhibition of I_{Ca} seems to be a direct effect on the L-type calcium channel, not like an open channel blocker.

Key words Ketamine · Calcium current · Action potential · Atrial cell · Bullfrog

Introduction

Ketamine is an intravenous and intramuscular anesthetic that is widely used in both humans and animals. The characteristic point of ketamine is that it produces dose-dependent increases in blood pressure and heart rate [1]. These stimulatory effects on the cardiovascular system are supposed to be due to the sympathomimetic actions of ketamine, primarily excitation of the central nervous system [2,3] and a blocking action on the reuptake of catecholamines at adrenergic nerve endings in a cocaine-like manner [4]. These effects are quite different from those of other intravenous and inhalational anesthetics.

However, once the balance of the sympathetic nervous system fails, in patients with critical cardiovascular conditions, ketamine occasionally causes an unexpected fall in blood pressure [5]. Ketamine has been reported to have a negative inotropic effect in vitro and several papers demonstrated myocardial depression induced by ketamine [6–8]. L-Type calcium channel current (I_{Ca}) was inhibited by ketamine in single smooth muscles of rabbit portal vein [9]. Thus, the inhibition of I_{Ca} is supposed to play a major role in the negative inotropic effect of ketamine on the heart. In addition, a recent paper reported that high concentrations of ketamine had a negative inotropic effect that was accompanied by a decreased intracellular Ca^{2+} transient in human myocardium [10]. However, the mechanisms of the inhibition of I_{Ca}, including use-dependent block, have not yet been fully clarified. The purpose of this study was to investigate the mechanisms of the effect of ketamine on bullfrog single atrial myocytes by monitoring I_{Ca}, resting membrane potential, and action potential.

Materials and methods

Solution

All solutions were made with 18 MΩ purity water and were kept saturated with 95% oxygen / 5% carbon dioxide. Standard Ringer's solution contained (in mM) NaCl 90.6, $NaHCO_3$ 20.0, KCl 2.5, $MgCl_2$ 5.0, $CaCl_2$ 2.5, and glucose 10. Low-calcium Ringer's solution was identical to standard Ringer's solution, except that $CaCl_2$ was reduced to 10 μM. In all the solutions, pH was between 7.2 and 7.4 and was adjusted to 7.4 before use. Ketamine hydrochloride (Sankyo Pharmaceutical,

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Tokyo, Japan) was applied by dissolving into the Ringer's solution.

Cell isolation

The technique for isolation of atrial myocytes from bullfrog heart is a modification of the method previously described by Hume and Giles [11], and it is reported that this preparation is suitable for voltage-clamp studies. Adult bullfrogs (*Rana catesbeiana*) were killed by decapitation under ether anesthesia, and the heart was excised and transferred to a dissecting dish filled with standard Ringer's solution. After the right atrium was cut out, it was placed in a second dish containing low-calcium Ringer's solution. Then it was transferred with forceps into a 5-ml solution of low-calcium Ringer's solution containing 0.15% collagenase (Yakult, Tokyo, Japan) and 0.1% trypsin (Sigma, St. Louis, MO, USA). The right atrium was stirred slowly (60 rpm) with a magnetic stirrer for 45 min. Then the enzyme medium was pipetted off and reincubated with 5 ml of low-calcium Ringer's solution containing 0.1% bovine serum albumin (Sigma) for 5 min, and the medium was pipetted off again and reincubated with 5 ml of low-calcium Ringer's solution containing 0.05% collagenase for approximately 30 min. After these treatments, the incubation medium became quite opaque, and single atrial myocytes were observed under the microscope.

Recording method

The ionic currents and the membrane potentials were recorded with low-resistance glass microelectrodes (1–2 μm, 3–5 MΩ pipette resistance) filled with 150 mM KCl. Glass microelectrodes were fabricated with a two-stage vertical microelectrode puller (PP-83, Narishige, Tokyo, Japan) from glass microtubes (G-1.5, Narishige). The whole-cell voltage-clamp method was used to examine the ionic currents, and the whole-cell current-clamp method was applied to monitor the membrane potentials. A patch-clamp amplifier (Axopatch 1D, Axon, Union City, CA, USA) was used to monitor the ionic current and membrane potentials. Data were stored in an IBM-compatible computer via the analogue-digital converter (Digidata 1200, Axon). Acquisition and analysis of the data were performed by pClamp software (Axon). L-Type calcium currents (I_{Ca}) were evoked by applying depolarizing pulses from a holding potential of −40 mV. I_{Ca} was identified after the current was blocked in the presence of nifedipine (10^{-6} M). Action potentials were elicited by current injection (100 mV, 2 ms). Resting membrane potential, overshoot, duration of action potential, and duration at 20% (APD_{20}) and 90% (APD_{90}) of repolarization were examined. It was supposed that APD_{20} reflected the plateau phase of the action potential, which was carried by calcium influx, and APD_{90} reflected the repolarization phase, which was carried by potassium outward current. All of the experiments were performed at room temperature (22–23°C).

Statistical analysis

Data were expressed as means ± SD. Statistical analyses were performed first by one-way analysis of variance (ANOVA) and, if indicated, the multiple comparison test (Dunnett) was employed to test for significant differences between the groups. A probability value of $P < 0.05$ was considered significant.

Results

Effects of ketamine on I_{Ca}

Ketamine reduced I_{Ca} within the range between 10^{-6} and 10^{-3} M (Table 1). In addition, the inhibition of I_{Ca} was dose-dependent between 10^{-5} and 10^{-3} M ketamine. I_{Ca} was completely blocked in the presence of 10^{-3} M ketamine (Fig. 1A), which was restored to almost 70% of the control after washing out of ketamine. Peak I_{Ca} was observed between 0 and +0 mV (Fig. 1B), and the 50% inhibitory concentration of peak I_{Ca} (IC_{50}) induced by ketamine was estimated as 0.92×10^{-5} M from these results. In the presence of 10^{-4} M ketamine, the reversal potential of I_{Ca} was shifted negatively by about 10 mV.

To further examine the mode of blockade of L-type calcium channels induced by ketamine, we examined whether ketamine showed use-dependent block of I_{Ca}. Cells were held at −40 mV, and text depolarization pulses (100 ms) to 0 mV were applied every 10 s. After the control currents were recorded, the test pulses were stopped and ketamine (10^{-4} M) was applied for 10 min. Then the test pulses were resumed and the amplitude of

Table 1. Inhibition of I_{Ca} by ketamine (mean ± SD; $n = 5$ for each value)

Concentration (M)	10^{-6}	10^{-5}	10^{-4}	10^{-3}
I_{Ca} (% of control)	58.3 ± 12.6[a]	51.7 ± 8.2[a]	37.9 ± 6.3[a,b]	1.1 ± 2.3[a,c]

[a] $P < 0.05$ vs control
[b,c] $P < 0.05$ vs 10^{-5}, 10^{-4}, respectively

I_{Ca} was recorded. Ketamine (10^{-4} M) did not show use-dependent block of I_{Ca} in five cells (Fig. 2A and B).

Effects of ketamine on membrane potentials

Ketamine did not change the resting membrane potential or shorten the duration of the action potential at 10^{-5} M. However, 10^{-4} M ketamine depolarized the resting membrane potential and prolonged the duration of the action potential (Table 2). In addition, a marked reduction of the plateau phase and prolongation of repolarization of the action potential in the presence of ketamine (10^{-4} M) were observed (Fig. 3). These results were based on the shortening of APD_{20} and prolongation of APD_{90} (Table 2). In the presence of a higher concentration of ketamine (10^{-3} M), the membrane potential was depolarized.

Discussion

Ketamine is known to have unique cardiovascular effects. It stimulates the cardiovascular system and increases blood pressure, heart rate, and cardiac output. However, ketamine has negative inotropic effects in isolated hearts [6,7]. Our results showed inhibition of I_{Ca} induced by ketamine between 10^{-6} and 10^{-3} M in bullfrog single atrial myocytes. Inhibition of I_{Ca} in the presence of ketamine has a role in the negative inotropic effect on the myocardium in the guinea pig [12,13] an rat [13]. It is noteworthy that ketamine (10^{-4} M) does not show use-dependent block of I_{Ca}. The presence of use-dependent block of I_{Ca} was reported for thiopental [14], diltiazem [15], and propofol [16], and it is sug-

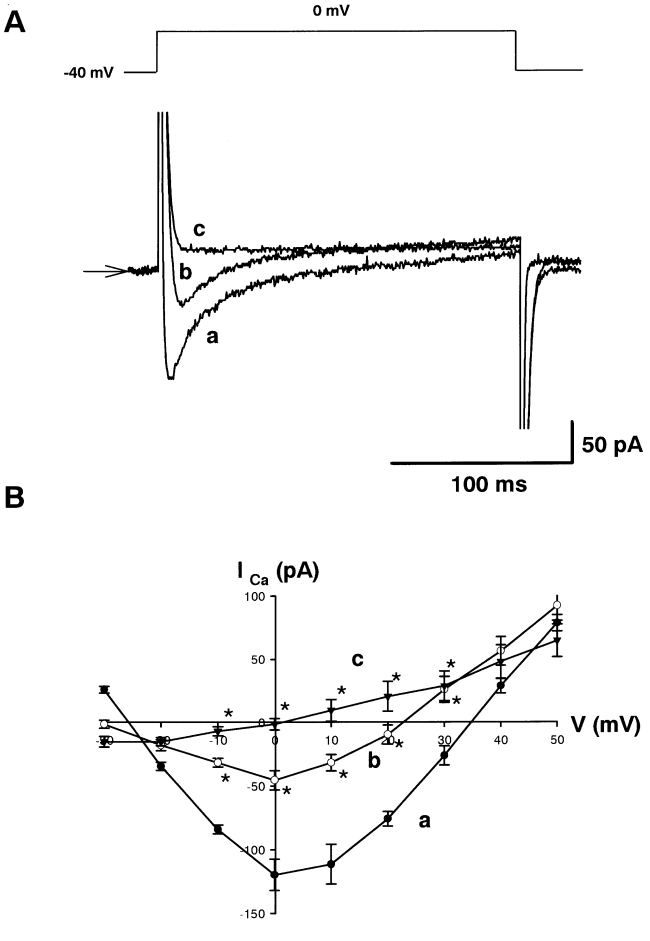

Fig. 1. Effects of ketamine on I_{Ca}. **A** Typical traces of I_{Ca} and inhibition of I_{Ca} in the presence of ketamine (*a* control, *b* 10^{-4} M, *c* 10^{-3} M). I_{Ca} was elicited by a test pulse (200 ms) to 0 mV from a holding potential of −40 mV. *Arrow* shows the zero current level. **B** Current-voltage relationship of I_{Ca}. I_{Ca} was elicited by a test pulse from a holding potential of −40 mV by +10 mV increments to +50 mV (n = 5). $^*P < 0.05$ vs control

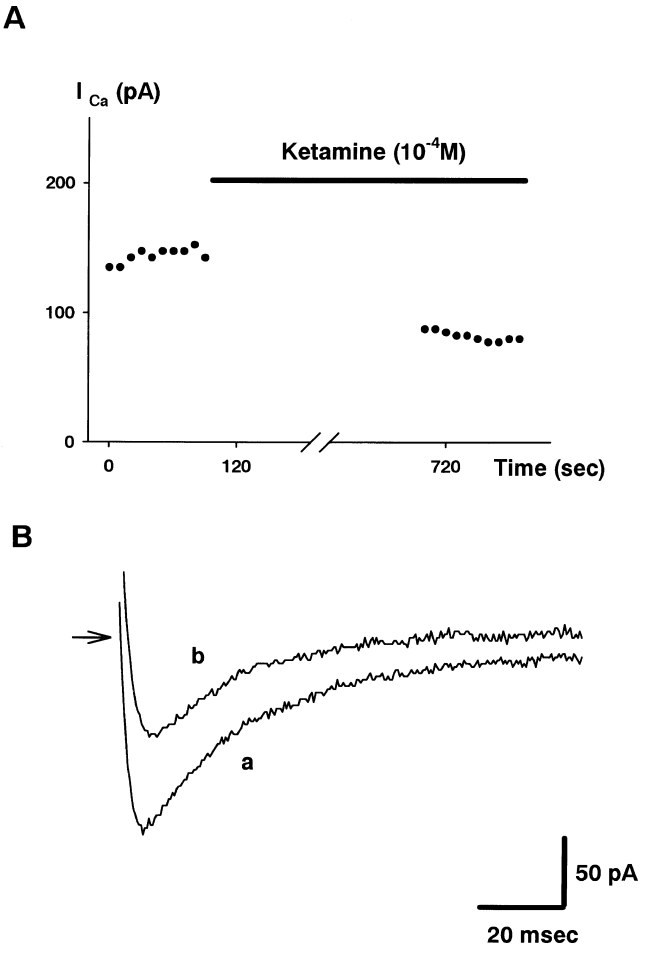

Fig. 2. Lack of use-dependent block of I_{Ca} in the presence of ketamine. **A** Cells were held at −40 mV, and test depolarization pulses to 0 mV were applied every 10 s for 100 ms. After the control currents were recorded, the test pulses were stopped and ketamine (10^{-4} M) was applied for 10 min. On resumption of the test pulse, I_{Ca} showed direct inhibition without use-dependent block. **B** Actual trace of I_{Ca} (*a* control, *b* the first trace in the presence of ketamine 10^{-4} M). *Arrow* shows zero current level. Note that I_{Ca} was inhibited from the first trace in the presence of ketamine

Table 2. Effect of ketamine on membrane potentials

Potential	Control	Ketamine 10^{-5} M	Ketamine 10^{-4} M
RMP (mV)	−88.75 ± 3.2	−91.5 ± 4.3	−85.5 ± 2.3[a]
Overshoot (mV)	41 ± 2.5	39 ± 2.7	34 ± 2.0[a]
Duration (ms)	687 ± 30	657 ± 28	1075 ± 63[a]
APD_{20} (ms)	221 ± 10	210 ± 12	163 ± 25[a]
APD_{90} (ms)	598 ± 32	605 ± 25	1035 ± 120[a]

RMP, Resting membrane potential; APD, duration of action potential; APD_{20}, APD_{90}, APD at 20% and 90% of repolarization, respectively
[a] $P < 0.05$ vs control, $n = 5$

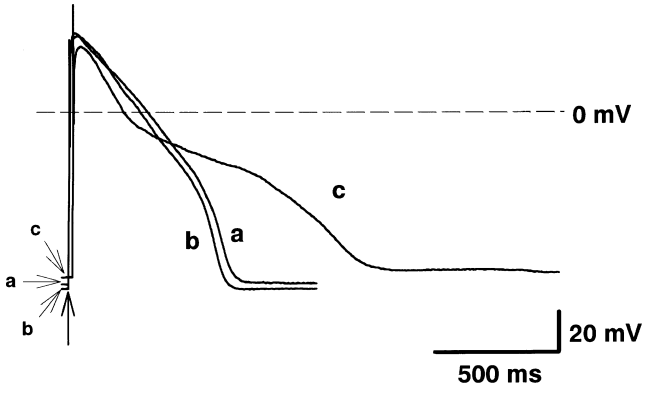

Fig. 3. Effect of ketamine on action potentials. Typical traces of action potentials are shown (*a* control, *b* 10^{-5} M ketamine, *c* 10^{-4} M ketamine). Action potential was elicited by current injection (0.1 Hz, 2 ms, 120 mV) in whole-cell current-clamp mode

gested that a possible mechanism of negative inotropic action induced by these agents is their action as open channel blockers that inhibit L-type Ca^{2+} channels in the open state. In contrast, inhaled anesthetics such as sevoflurane did not demonstrate use-dependent block on I_{Ca} [17]. Our results indicated that ketamine inhibits L-type Ca^{2+} channel in its closed state as does sevoflurane. Furthermore, a role for the participation of the sarcoplasmic reticulum [18] in the production of ketamine-induced negative inotropic effect on the heart should be examined.

Ketamine (10^{-5} M) did not affect the resting membrane potential, but a higher dose (10^{-4} M) of ketamine depolarized it. The resting membrane potential is mostly maintained and controlled by potassium ions, especially regulated by inwardly rectifying potassium channel current (I_{Kir}). This result suggests that ketamine can affect I_{Kir} at higher doses.

Ketamine (10^{-5} M) did not show any effect on action potential, but a higher dose (10^{-4} M) of ketamine reduced overshoot, prolonged total duration and APD_{90}, and shortened APD_{20}. Shortening of APD_{20} reflected the inhibition of the plateau phase of the action potential. Because the plateau phase was supposed to be carried by L-type calcium channel current, shortening of APD_{20} induced by a high dose of ketamine would agree with our result that ketamine inhibited I_{Ca}. APD_{90} reflected the total duration of the action potential. This method of measurement is useful in that artifact and noises at resting level could be disregarded. A high dose of ketamine (10^{-4} M) prolonged APD_{90}, but on the other hand, APD_{20} was shortened. Prolongation of action potentials was reported with thiopental [14] and halothane [19], but not with propofol [16] and sevoflurane [20]. The mechanism of prolongation of the action potential could be due to an inhibition of the repolarizing phase of the action potential, which was carried by delayed rectified potassium current. Ketamine did not alter the conductance of the inward rectifying potassium current [21] in rat ventricular myocytes at 10 μM, nor did it prolong APD_{90} in guinea pig heart [22] at 50 μM. These reports indicate that ketamine does not prolong the duration of the action potential in clinically relevant concentrations, a result that is compatible with our results. However, some other pump system or ionic exchange mechanism is responsible for the repolarization, and further investigation is required.

The therapeutic range of ketamine plasma concentrations in humans is from 2.9 to 9.2×10^{-6} M [23]. Within this range, our results demonstrated some inhibition of I_{Ca}, but negligible changes in membrane potential and action potential. Because some of the ketamine combines with serum protein when it is administered in vivo, the concentration of active ketamine might be lower than when it is administered in vitro. Thus, ketamine is a quite safe general anesthetic for clinical use. However, ketamine reduced I_{Ca} at higher doses and prolonged the duration of the action potential. In addition, ketamine usually stimulates the cardiovascular system, but it was reported that a second dose of ketamine produced hemodynamic effects less than or even opposite to those of the first dose [24]. Thus, we must pay attention to avoid overdose and repeated doses of ketamine, even for clinical use.

In conclusion, ketamine (10^{-5}–10^{-3} M) inhibited I_{Ca} and did not show use-dependent block of I_{Ca}. A high

dose (10^{-4}M) of ketamine depolarized the resting membrane potential, reduced the overshoot and plateau phase of the action potential, and prolonged the total duration.

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