Ketamine attenuates the Na⁺-dependent Ca²⁺ overload in rabbit ventricular myocytes in vitro by inhibiting late Na⁺ and L-type Ca²⁺ currents

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Aim: Intracellular Ca²⁺ ([Ca²⁺]i) overload occurs in myocardial ischemia. An increase in the late sodium current (I_{NaL}) causes intracellular Na⁺ overload and subsequently [Ca²⁺]i overload via the reverse-mode sodium-calcium exchanger (NCX). Thus, inhibition of I_{NaL} is a potential therapeutic target for cardiac diseases associated with [Ca²⁺]i overload. The aim of this study was to investigate the effects of ketamine on Na⁺-dependent Ca²⁺ overload in ventricular myocytes in vitro.

Methods: Ventricular myocytes were enzymatically isolated from hearts of rabbits. I_{NaL}, NCX current (I_{NCX}) and L-type Ca²⁺ current (I_{CaL}) were recorded using whole-cell patch-clamp technique. Myocyte shortening and [Ca²⁺]i transients were measured simultaneously using a video-based edge detection and dual excitation fluorescence photomultiplier system.

Results: Ketamine (20, 40, 80 μmol/L) inhibited I_{NaL} in a concentration-dependent manner. In the presence of sea anemone toxin II (ATX, 30 nmol/L), I_{NaL} was augmented by more than 3-fold, while ketamine concentration-dependently suppressed the ATX-augmented I_{NaL}. Ketamine (40 μmol/L) also significantly suppressed hypoxia or H₂O₂-induced enhancement of I_{NaL}. Furthermore, ketamine concentration-dependently attenuated ATX-induced enhancement of reverse-mode I_{NCX}. In addition, ketamine (40 μmol/L) inhibited I_{CaL} by 33.4%. In the presence of ATX (3 nmol/L), the rate and amplitude of cell shortening and relaxation, the diastolic [Ca²⁺]i, and the rate and amplitude of [Ca²⁺]i rise and decay were significantly increased, which were reverted to control levels by tetrodotoxin (TTX, 2 μmol/L) or by ketamine (40 μmol/L).

Conclusion: Ketamine protects isolated rabbit ventricular myocytes against [Ca²⁺]i overload by inhibiting I_{NaL} and I_{CaL}.

Keywords: ketamine; cardiomyocyte; calcium overload; late sodium current; L-type Ca²⁺ current; NCX current; ATX; myocardial ischemia

Introduction
Cardiomyocyte Ca²⁺ overload occurs in many pathological conditions such as hypoxia, ischemia, oxidative stress, cardiac hypertrophy, and heart failure[11-14]. Intracellular Ca²⁺ ([Ca²⁺]i) overload causes cardiac arrhythmias and myocardial dysfunction[5]. Extensive reports show that the late or persistent sodium current (I_{Na}) in ventricular myocytes is increased in many pathological conditions that lead to [Ca²⁺]i overload[6-10]. An increase in the amplitude of I_{Na} prolongs the action potential duration, increases the transmural dispersion of repolarization, and causes cardiac arrhythmias[11]. An increase in I_{Na} also increases the intracellular sodium concentration and subsequently raises [Ca²⁺], via the reverse-mode Na⁺-Ca²⁺ exchanger (NCX)[7, 12]. Inhibition of I_{Na} was reported to attenuate the increase in [Ca²⁺]i[13-15]. Inhibition of I_{Na} is therefore a potential therapeutic target for the treatment of heart diseases associated with [Ca²⁺]i overload[16, 17].

Ketamine is an intravenous and intramuscular anesthetic that is widely used in both humans and animals. In vitro study data show that ketamine has antiarrhythmic effects and decreases the incidence of reperfusion-induced arrhythmias[18-21] and that it enhances the recovery of force of contraction during reperfusion[22]. Furthermore, ketamine suppresses the activity of neutrophils and decreases their post-ischemic adhesion in the coronary artery[23-28]. In clinical studies, ketamine has reduced the incidence of ventricular arrhythmias and clinical markers of myocardial injury in cardiac surgery patients[26-28]. These results suggest that ketamine may have cardioprotective effects, but the underlying mechanisms

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are still unknown. Ketamine has been reported to inhibit various ionic currents, including the L-type Ca\(^{2+}\) current (\(I_{\text{CaL}}\)) [28-34], peak sodium current (\(I_{\text{Na}}\)) [35], inward rectifier K\(^+\) current (\(I_{\text{KIR}}\)) [36, 37], delayed rectifier K\(^+\) current (\(I_{\text{K}}\)) [38, 39], ATP-sensitive K\(^+\) current (\(I_{\text{KATP}}\)) [36, 37], and human ether-a-go-go-related gene (hERG) channel [38]. However, there is no study regarding the effects of ketamine on \(I_{\text{NaL}}\). In previous studies, we found that ketamine attenuates augmented \(I_{\text{NaL}}\)-induced \([\text{Ca}^{2+}]\) overload [39, 40]. Thus, this study investigated the effects of ketamine on \(I_{\text{NaL}}\), the NCX current (\(I_{\text{NCX}}\)), myocyte shortening and \([\text{Ca}^{2+}]\), transients in the presence of sea anemone toxin II (ATX), an opener of \(I_{\text{NaL}}\) channels.

**Materials and methods**

**Isolation of ventricular myocytes**

This study adheres to the Guidance for Ethical Treatment of Laboratory Animals (the Ministry of Science and Technology of China, 2006) and is approved by the Institutional Animal Care and Use Committee of the Medical College of Wuhan University of Science and Technology (Wuhan, China).

Myocytes were isolated enzymatically from the hearts of rabbits of both sexes (1.7–2 kg; Wuhan Institute of Biological Products Co, Ltd, Wuhan, China), as previously described [41]. In brief, adult New Zealand white rabbits were heparinized (2000 U) and anesthetized with ketamine (iv; 30 mg/kg) and xylazine (im; 7.5 mg/kg). Hearts were quickly excised and retrogradely perfused with Ca\(^{2+}\)-free Tyrode’s solution for 5 min, followed by an enzyme-containing solution (0.1 g/L collagenase type I, 0.01 g/L protease E and 0.5 g/L bovine serum albumin) for a further 40–50 min. The perfusate was finally switched to KB solution for 5 min. All solutions were bubbled with 100% O\(_2\) and maintained at 37 °C. The left ventricle was cut into small chunks and gently agitated in KB solution. The cells were filtered through nylon mesh and stored in KB solution at 4 °C until used.

**Solution**

For cell isolation, the regular Tyrode’s solution contained the following (in mmol/L): 135 NaCl, 0.33 NaH\(_2\)PO\(_4\), 5.4 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 10 glucose, and 10 HEPES (pH 7.4). The KB solution contained the following (in mmol/L): 70 KCl, 40 KCl, 20 KH\(_2\)PO\(_4\), 1 MgCl\(_2\), 20 taurine, 50 glutamic acid, 0.5 EGTA, 10 glucose, and 10 HEPES (pH 7.4). For \(I_{\text{NaL}}\) recordings, the intracellular (pipette) solution contained the following (in mmol/L): 120 CsCl, 1 CaCl\(_2\), 11 EGTA, 5 MgCl\(_2\), 5 Na\(_2\)ATP, 10 TEA-Cl, and 10 HEPES (pH 7.4). The bath solution contained the following (in mmol/L): 135 NaCl, 0.33 NaH\(_2\)PO\(_4\), 5.4 CsCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 0.05 CdCl\(_2\), 0.3 BaCl\(_2\), 10 glucose, and 10 HEPES (pH 7.4). For the hypoxia experiment, the modified bath solution in which glucose was omitted was pre-equilibrated with 100% N\(_2\) for at least 1 h. Hypoxia was induced using a previously described method [42]. For \(I_{\text{NCX}}\) recordings, the pipette solution included the following (in mmol/L): 20 NaCl, 10 CaCl\(_2\), 3 MgCl\(_2\), 5 MgATP, 50 aspartic acid, 20 EGTA, 10 HEPES, and 120 CsOH (pH 7.4). The bath solution contained the following (in mmol/L): 140 NaCl, 2 CsCl, 2 CaCl\(_2\), 1 BaCl\(_2\), 2 MgCl\(_2\), 5 HEPES, and 10 glucose (pH 7.4). In addition, 20 μmol/L ouabain and 1 μmol/L nicardipine were added to block the Na\(^+-\)K\(^+\) pump and \(I_{\text{CaL}}\), respectively. For \(I_{\text{CaL}}\) recordings, the pipette solution contained the following (in mmol/L): 80 CsCl, 60 CsOH, 0.65 CaCl\(_2\), 5 disodium creatine phosphate, 5 MgATP, 40 aspartic acid, 10 EGTA, and 5 HEPES (pH 7.3). The bath solution was the Tyrode’s solution. For cell shortening and \([\text{Ca}^{2+}]\), transient recordings, the bath solution contained the following (in mmol/L): 131 NaCl, 4 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 10 HEPES, and 10 glucose (pH 7.4).

**Current recordings**

All experiments were conducted at 22–25 °C. The electrode resistance (when filled with pipette solution) was 1.5–2 MΩ. Cell capacitance and series resistances were electronically compensated by 60%–80%. Currents were recorded using an EPC-9 amplifier (HEKA Electronic, Lambrecht, Pfulz, Germany), filtered at 2 kHz and sampled at 10 kHz. Current measurements were normalized using the cell capacitance. 

\(I_{\text{NaL}}\) was recorded by a 300-ms depolarizing pulse to −20 mV from a holding potential of −120 at a frequency of 0.2 Hz. The amplitude of \(I_{\text{NaL}}\) was determined from the average current measured during a time interval of 190 to 210 ms after initiation of the depolarizing pulse to eliminate any contribution of \(I_{\text{Na}}\) [35]. To record the current-voltage relationship of \(I_{\text{NaL}}\), 300-ms depolarizing pulses to membrane potentials from −80 to +50 mV were applied at 0.5 Hz from a potential of −120 mV. \(I_{\text{NCX}}\) was elicited by a 10-ms prepulse to +60 mV from a holding potential of −40 mV followed by a 2-s ramp pulse from +60 to −120 mV (with a speed of −90 mV/s). \(I_{\text{NCX}}\) was measured as the current sensitive to 5 mmol/L Ni\(^{2+}\) at +50 and −100 mV. 

\(I_{\text{CaL}}\) was elicited by a 150-ms prepulse to −40 mV from a holding potential of −80 mV followed by a 300-ms depolarizing pulse from −40 mV to 0 mV (0.2 Hz). \(I_{\text{CaL}}\) was measured as the difference between peak inward current and the current remaining at the end of the 300-ms pulse.

**Measurements of myocyte cell shortening and \([\text{Ca}^{2+}]\), transients**

Fura-2 was loaded by incubating cell suspensions with 1 μmol/L Fura-2/AM for 30 min at 25 °C in the dark. Fura-2-loaded myocytes mounted in a chamber situated on the stage of an Olympus IX-70 inverted microscope were field stimulated to contract between platinum electrodes (0.5 Hz, 37 °C). Cardiomyocytes that possessed an appropriate morphological appearance (rod shaped with clean edges, clear striations, and no large blebs), a resting sarcomere length >1.70 μm, and no spontaneous contraction were selected for experimentation. Myocyte shortening and \([\text{Ca}^{2+}]\), transients were measured simultaneously using a video-based edge detection and dual excitation fluorescence photomultiplier system (IonOptix, Milton, MA, USA). A xenon lamp provided the excitation light. Alternating excitation wavelengths of either 340 nm or 380 nm were obtained at a frequency of 250 Hz. A photomultiplier collected the emitted fluorescence signals. The ratio of both Fura-2 fluorescence signals (340/380 ratio) was continuously measured after background fluorescence subtraction.
Ketamine (Ket) inhibited \( I_{\text{NaL}} \) in rabbit ventricular myocytes in a concentration-dependent manner. (A) Representative whole-cell recordings of \( I_{\text{NaL}} \) in the absence of drug (Control) and after the application and washout of Ket (20, 40, or 80 \( \mu \)mol/L). (B) Summary data for the mean current density of \( I_{\text{NaL}} \) under different conditions. The data are expressed as the mean±SD (\( n=11 \)). \( P<0.01 \) vs Control. \( P<0.01 \) vs 20 \( \mu \)mol/L Ket. \( P<0.01 \) vs 40 \( \mu \)mol/L Ket.

**Results**

**Ketamine inhibited \( I_{\text{NaL}} \) in rabbit ventricular myocytes in a concentration-dependent manner**

The whole-cell patch-clamp technique was used to record \( I_{\text{NaL}} \). Ketamine (20, 40, 80 \( \mu \)mol/L) decreased the current density of \( I_{\text{NaL}} \) from 0.34±0.08 to 0.28±0.08, 0.22±0.07, and 0.14±0.06 pA/pF (\( P<0.01 \) vs control for all; \( n=11 \)) in a concentration-dependent manner, respectively (Figure 1). This inhibitory effect of ketamine was reversible upon washout (0.34±0.09 vs 0.33±0.11 pA/pF, \( P>0.05, n=6 \)). Figures 1A and 1B show the representative current records and summary data for the \( I_{\text{NaL}} \) current density.

**Effect of ketamine on ATX-augmented \( I_{\text{NaL}} \)**

The \( I_{\text{NaL}} \) channel opener ATX (30 nmol/L) increased \( I_{\text{NaL}} \) (at –20 mV) from 0.29±0.01 to 1.23±0.05 pA/pF (\( P<0.01; n=8 \)). In the presence of ATX, ketamine (20, 40, 80 \( \mu \)mol/L) decreased it to 1.09±0.08, 0.88±0.08, and 0.72±0.06 pA/pF (\( P<0.01 \) vs ATX for all; \( n=8 \)) in a concentration-dependent manner, respectively (Figure 2). Figures 2A and 2B show the original current records and current-voltage curves of \( I_{\text{NaL}} \) according to the current-voltage relationship protocol, respectively. Figure 2C shows the summary data for the \( I_{\text{NaL}} \) current density recorded at –20 mV.

**Effect of ketamine on the enhanced \( I_{\text{NaL}} \) induced by hypoxia or \( \text{H}_2\text{O}_2 \)**

Previous reports show that \( I_{\text{NaL}} \) is increased under hypoxia and oxidative stress conditions. Thus, we studied the effects of ketamine on \( I_{\text{NaL}} \) after exposure to hypoxia or \( \text{H}_2\text{O}_2 \). Hypoxia (10 min) and 300 \( \mu \)mol/L \( \text{H}_2\text{O}_2 \) increased \( I_{\text{NaL}} \) from 0.33±0.05 to 0.64±0.06 pA/pF (\( P<0.01; n=6 \)) and 0.28±0.03 to 0.64±0.10 pA/pF (\( P<0.01; n=7 \)), respectively. Ketamine (40 \( \mu \)mol/L) decreased it to 0.45±0.06 and 0.43±0.11, respectively (Figure 3). After washing, \( I_{\text{NaL}} \) returned to the predrug level (hypoxia: 0.67±0.05 vs 0.63±0.08 pA/pF, \( n=4 \); \( \text{H}_2\text{O}_2 \): 0.68±0.04 vs 0.67±0.03 pA/pF, \( n=3 \); both \( P>0.05 \)).
ence of ATX, TTX (4 μmol/L) decreased it to the control level (1.04±0.12 pA/pF, \( P>0.05 \) vs Control; Figure 4A–4C). Similarly, ketamine (20, 40, 80 μmol/L) decreased the ATX-stimulated \( I_{\text{NCX}} \) from 2.61±0.22 to 2.21±0.22, 1.71±0.16, and 1.20±0.22 pA/pF in a concentration-dependent manner, respectively (\( n=8, P<0.01 \) vs ATX for all; Figure 4D–4F).

**Effects of TTX and ketamine on enhanced cell shortening and \([\text{Ca}^{2+}]_i\) transients induced by ATX**

ATX (3 nmol/L) enhanced cell shortening and \([\text{Ca}^{2+}]_i\) transients (Figures 5 and 6). PSI, +dL/dt, –dL/dt, diastolic \([\text{Ca}^{2+}]_i\), \( \Delta[\text{Ca}^{2+}]_i \), and \( +d[\text{Ca}^{2+}]_i/dt \) and \( -d[\text{Ca}^{2+}]_i/dt \) were increased to 160%, 180%, 191%, 114%, 165%, and 159%, and 162% of control, respectively, and \( TD_{\text{Ca}} \) was decreased to 82% of control by ATX (P<0.01 vs control for all; \( n=7 \)). In the presence of ATX, TTX (2 μmol/L) reverted these measures to 99%, 97%, 99%, 104%, 98%, 106%, and 98% of control, respectively (\( P>0.05 \) vs control for all; Figure 5). Similarly, ketamine (40 μmol/L) reverted the above parameters from 169% to 99%, 169% to 99%, 224% to 114%, 110% to 99%, 165% to 104%, 159% to 98%, 162% to 106%, and 82% to 98% of control, respectively (\( n=7, P<0.01 \) ATX vs ketamine group for all; Figure 6).

**Effect of ketamine on \( I_{\text{CaL}} \)**

\( I_{\text{CaL}} \) plays an important role in cell shortening and \([\text{Ca}^{2+}]_i\) transients. Previous studies show that ketamine inhibits \( I_{\text{CaL}} \) in the cardiomyocytes of some species\(^{29–34} \). However, no studies have investigated the effect of ketamine on \( I_{\text{CaL}} \) in rabbit ventricular myocytes. The effect of ketamine on \( I_{\text{CaL}} \) could be attributed to ketamine’s suppressive effect on myocyte shortening in this study. Thus, in this study, we examined the effect of ketamine on \( I_{\text{CaL}} \) and observed that ketamine (40 μmol/L) significantly inhibited \( I_{\text{CaL}} \) in rabbit ventricular myocytes. The current density of \( I_{\text{CaL}} \) was decreased from 4.41±1.15 to 2.94±1.06 pA/pF (\( P<0.01, n=9 \); Figure 7). After washing, \( I_{\text{CaL}} \) returned to the predrug control level (4.65±0.74 vs 4.54±0.67 pA/pF, \( P>0.05, n=3 \)).

**Discussion**

Cardiomyocyte \([\text{Ca}^{2+}]_i\) overload occurs in many pathological conditions such as hypoxia, ischemia, oxidative stress, cardiac hypertrophy, and heart failure\(^{21–34} \) and results in cardiac arrhythmias and myocardial dysfunction\(^{3} \). Previous reports show that \( I_{\text{NaL}} \) is an important contributing factor to \([\text{Ca}^{2+}]_i\) overload in many pathological conditions. An increase in \( I_{\text{NaL}} \),
can increase the intracellular sodium concentration and subsequently raise [Ca²⁺], through reverse NCX[7,12]. Extensive studies have reported that inhibition of I_{NaL} attenuates the increase in [Ca²⁺][13–15]. Inhibition of I_{NaL} is therefore a potential therapeutic target for the treatment of heart diseases associated with [Ca²⁺] overload[16,17].

Ketamine is an intravenous and intramuscular anesthetic that is widely used in pediatric and adult cardiac surgery. In clinical studies, the serum concentrations of ketamine reach 60 μmol/L 5 min after an intravenous administration of 2 mg/kg[43]. The protein binding of ketamine is 12%–50% [44,48], and thus, the free concentration of ketamine may reach 30–50 μmol/L at 5 min. However, greater plasma concentrations can be expected at 1–3 min after induction and may reach 100–150 μmol/L. Therefore, our experimental concentrations (20–80 μmol/L) appear likely to be within the clinical range.

Ketamine has been reported to inhibit various ionic currents[29–38]. Hara et al reported that ketamine (30, 100, 300 μmol/L) dose-dependently blocked peak I_{Na} in guinea pig ventricular myocytes[92]. However, no studies have investigated the effects of ketamine on I_{NaL} in cardiomyocytes until now. In this study, we found that ketamine inhibited control and the enhanced I_{NaL} induced by ATX, hypoxia, or H₂O₂ (Figures 1–3). The inhibitory effect of ketamine was reversible (Figures 1, 3). In our present and previous studies[39], the ATX-augmented reverse-mode I_{NCX}, myocyte shortening and [Ca²⁺] transients were reversed completely by TTX (Figures 4–5), which shows that an increase in I_{NaL} causes these changes. The inhibition of I_{NaL} is predicted to reverse the ATX-induced changes described above. Consistent with this hypothesis, ketamine inhibited ATX-augmented reverse I_{NCX}, myocyte shortening and [Ca²⁺] transients by inhibiting I_{NaL}[39,40]. Therefore, the inhibition of I_{NaL} contributes to ketamine’s suppressive effects on [Ca²⁺] transients and myocyte shortening.

In this study, we observed an interesting phenomenon: ketamine partly inhibited ATX-augmented I_{NaL} and I_{NCX} (Figures 2, 4) but completely, although partly not expectedly, reversed the changes in [Ca²⁺] transients and myocyte shortening induced by ATX (Figure 6). This result shows that there are additional mechanisms other than inhibiting I_{NaL} underlying ketamine’s suppressive effects. Previous studies show...
that ketamine inhibits \( I_{\text{CaL}} \) in guinea pig and rat ventricular myocytes, in human right atrial myocytes and bullfrog atrial myocytes\[29–34\]. Hara et al reported that ketamine (30 μmol/L) decreased \( I_{\text{CaL}} \) by 26.1% in guinea pig ventricular myocytes\[32\]. Endou et al reported that ketamine (100 μmol/L) decreased \( I_{\text{CaL}} \) by 10.8% in rat ventricular myocytes\[30\]. However, no study had yet investigated the effect of ketamine on \( I_{\text{CaL}} \) in rabbit ventricular myocytes. In this study, 40 μmol/L ketamine, a concentration used in our myocyte shortening and [Ca\(^{2+}\)]\(i\) transient recordings, inhibited \( I_{\text{CaL}} \) by 33.4% in rabbit ventricular myocytes (Figure 7), which is similar to the findings of Hara et al\[32\] but different from those of Endou et al\[30\]. These discrepancies seem to originate from the species differences. The present result suggested that the inhibition of \( I_{\text{CaL}} \) could also contribute to ketamine’s suppressive effects on [Ca\(^{2+}\)]\(i\) transients and myocyte shortening.

Extensive studies demonstrate that perioperative myocardial ischemia is common and is associated with serious cardiac morbidities and mortality. Its incidence in noncardiac surgery patients at risk of or with known coronary artery disease is 20%–63%\[46\]. The incidence of intraoperative myocardial ischemia in patients undergoing coronary artery bypass grafting surgery is 26%–78%\[47\]. A previous report has shown that hypoxia (8 min) increases \( I_{\text{NaL}} \) and reverses \( I_{\text{NCX}} \) in rabbit ventricular myocytes\[42\], which suggests an increase in [Ca\(^{2+}\)]\(i\) at that time. Furthermore, a burst of H\(_2\)O\(_2\) is generated in cardiomyocytes during ischemia, and H\(_2\)O\(_2\) also increases \( I_{\text{NaL}} \) in cardiomyocytes. As such, cardiomyocyte [Ca\(^{2+}\)]\(i\) overload will probably occur in the perioperative period.

Ketamine is widely used in pediatric and adult cardiac surgery. Previous studies show that ketamine has antiarrhythmic effects and decreases the incidence of reperfusion-induced arrhythmias in animal models\[18–21\]. Hanouz et al reported that ketamine preconditioning human myocardium and enhances the recovery of force of contraction during reperfusion\[22\]. Furthermore, ketamine has been shown to suppress the activity of neutrophils and decrease their postischemic adhesion in the coronary artery in in vitro studies\[23–25\]. In clinical studies, ketamine has reduced the incidence of ventricular arrhythmias and clinical markers of myocardial injury in cardiac surgery patients\[26–28\]. These results suggest that ketamine may have cardioprotective effects. In this study, ketamine inhibited the augmented \( I_{\text{NaL}} \) induced by hypoxia and H\(_2\)O\(_2\) (Figure 3) and attenuated the augmented \( I_{\text{NaL}} \)-induced [Ca\(^{2+}\)]\(i\) overload (Figure 6). Therefore, ketamine could protect the heart against ischemia/reperfusion injury in the perioperative period by preventing [Ca\(^{2+}\)]\(i\) overload and may be a good candidate anesthetic for clinical use.

In summary, ketamine inhibited \( I_{\text{NaL}} \) and \( I_{\text{CaL}} \) and decreased the enhanced reverse \( I_{\text{NCX}} \), myocyte shortening and [Ca\(^{2+}\)]\(i\) transients induced by ATX in rabbit ventricular myocytes. Ketamine could protect ventricular myocytes against increased...

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**Figure 4.** Effects of TTX and ketamine (Ket) on the enhanced \( I_{\text{NCX}} \) induced by ATX. (A) and (D), Representative original currents recorded by a ramp pulse (inset) in the absence (Control) and presence of ATX 30 nmol/L before and after exposure to TTX 4 μmol/L (A) or Ket at 20, 40, or 80 μmol/L (D). (B) and (E), The Ni\(^{2+}\)-sensitive \( I_{\text{NCX}} \) was obtained by subtracting the sweep after the application of 5 mmol/L NiCl\(_2\) (trace d for A; trace f for D) from sweeps before exposure to NiCl\(_2\). (C) and (F), Summary data for the current density of \( I_{\text{NCX}} \) measured at +50 mV and -100 mV. The data are expressed as the mean±SD (n=8). "P<0.01 vs Control; "P<0.01 vs ATX 30 nmol/L (ATX); "P<0.01 vs ATX plus Ket 20 μmol/L (ATX-K20, F); "P<0.01 ATX plus Ket 80 μmol/L (ATX-K80, F) vs ATX plus Ket 40 μmol/L (ATX-K40, F).
I_{NaL}-induced [Ca^{2+}], overload in anesthetic use.

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Author contribution
An-tao Luo, Zhen-zhen Cao, and Ji-hua Ma designed the research; An-tao Luo, Zhen-zhen Cao, Yu Xiang, Shuo...
ZHANG, Chun-ping QIAN, Chen FU, and Pei-hua ZHANG performed the experiments; An-tao LUO, Zhen-zhen CAO, Yu XIANG, Shuo ZHANG, Chun-ping QIAN, and Chen FU analyzed the data; An-tao LUO, Zhen-zhen CAO, and Ji-hua MA wrote the paper.

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Figure 7. Ketamine (Ket) inhibited $I_{Ca,L}$ in rabbit ventricular myocytes. (A) Representative whole-cell recordings of $I_{Ca,L}$ in the absence of drug (Control) and after the application and washout of Ket (40 μmol/L). (B) Summary data for the mean current density of $I_{Ca,L}$. The data are expressed as the mean±SD (n=9). *P<0.01 vs Control.

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