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Original Article

Ketamine attenuates the Na$^+$-dependent Ca^{2+} overload in rabbit ventricular myocytes *in vitro* by inhibiting late Na$^+$ and L-type Ca^{2+} currents

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Aim: Intracellular Ca^{2+} ([Ca^{2+}]$_i$) overload occurs in myocardial ischemia. An increase in the late sodium current (I_{NaL}) causes intracellular Na$^+$ overload and subsequently [Ca^{2+}]$_i$ overload via the reverse-mode sodium-calcium exchanger (NCX). Thus, inhibition of I_{NaL} is a potential therapeutic target for cardiac diseases associated with [Ca^{2+}]$_i$ overload. The aim of this study was to investigate the effects of ketamine on Na$^+$-dependent Ca^{2+} 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 Ca^{2+} current (I_{CaL}) were recorded using whole-cell patch-clamp technique. Myocyte shortening and [Ca^{2+}]$_i$ 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 H$_2$O$_2$-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 [Ca^{2+}]$_i$, and the rate and amplitude of [Ca^{2+}]$_i$ 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 [Ca^{2+}]$_i$ overload by inhibiting I_{NaL} and I_{CaL}.

Keywords: ketamine; cardiomyocyte; calcium overload; late sodium current; L-type Ca^{2+} current; NCX current; ATX; myocardial ischemia

Introduction

Cardiomyocyte Ca^{2+} overload occurs in many pathological conditions such as hypoxia, ischemia, oxidative stress, cardiac hypertrophy, and heart failure[1–4]. Intracellular Ca^{2+} ([Ca^{2+}]$_i$) overload causes cardiac arrhythmias and myocardial dysfunction[5]. Extensive reports show that the late or persistent sodium current (I_{NaL}) in ventricular myocytes is increased in many pathological conditions that lead to [Ca^{2+}]$_i$ overload[6–10]. An increase in the amplitude of I_{NaL} prolongs the action potential duration, increases the transmural dispersion of repolarization, and causes cardiac arrhythmias[11]. An increase in I_{NaL} also increases the intracellular sodium concentration and subsequently raises [Ca^{2+}]$_i$ via the reverse-mode Na$^+$-Ca^{2+} exchanger (NCX)[7, 12]. Inhibition of I_{NaL} was reported to attenuate the increase in [Ca^{2+}]$_i$[13–15]. Inhibition of I_{NaL} is therefore a potential therapeutic target for the treatment of heart diseases associated with [Ca^{2+}]$_i$ 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[23–25]. 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, 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_{CaL}) [29–34], peak sodium current (I_{Na}) [32], inward rectifier K^+ current (I_{K1}) [30, 35], delayed rectifier K^+ current (I_K) [30], ATP-sensitive K^+ current (I_{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_{NaL}. In previous studies, we found that inhibition of I_{NaL} attenuates augmented I_{NaL}-induced $[Ca^{2+}]_i$ overload [39, 40]. Thus, this study investigated the effects of ketamine on I_{NaL}, the NCX current (I_{NCX}), myocyte shortening and $[Ca^{2+}]_i$ transients in the presence of sea anemone toxin II (ATX), an opener of I_{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_2PO_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 KOH, 40 KCl, 20 KH_2PO_4, 1 $MgCl_2$, 20 taurine, 50 glutamic acid, 0.5 EGTA, 10 glucose, and 10 HEPES (pH 7.4). For I_{NaL} recordings, the intracellular (pipette) solution contained the following (in mmol/L): 120 CsCl, 1 $CaCl_2$, 11 EGTA, 5 $MgCl_2$, 5 Na_2ATP, 10 TEA-Cl, and 10 HEPES (pH 7.3). The bath solution contained the following (in mmol/L): 135 NaCl, 0.33 NaH_2PO_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_{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_{CaL}, respectively. For I_{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 $[Ca^{2+}]_i$ 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, Pfalz, Germany), filtered at 2 kHz and sampled at 10 kHz. Current measurements were normalized using the cell capacitance.

I_{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_{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_{Na} [2]. To record the current-voltage relationship of I_{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_{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_{NCX} was measured as the current sensitive to 5 mmol/L Ni^{2+} at +50 and –100 mV.

I_{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_{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 $[Ca^{2+}]_i$ 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 was selected for experimentation. Myocyte shortening and $[Ca^{2+}]_i$ 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. Drugs

were applied after a 10-min stable period. Contractile variables included peak shortening amplitude (PS; µm), maximal rate of shortening (+dL/dt; µm/ms), maximal rate of relaxation (−dL/dt; µm/ms) and time to peak shortening (TPS; s). For $[Ca^{2+}]_i$ transients, the following parameters were measured: diastolic $[Ca^{2+}]_i$ (340/380 ratio), amplitude of the $[Ca^{2+}]_i$ transient (Δ$[Ca^{2+}]_i$; ratio), maximal rate of $[Ca^{2+}]_i$ rise (+d$[Ca^{2+}]_i$/dt; 1/ms), maximal rate of $[Ca^{2+}]_i$ decay (−d$[Ca^{2+}]_i$/dt; 1/ms), time to peak (TP; s) and time to 90% decay (TD$_{90}$; s).

Chemicals

Ketamine, ATX, tetrodotoxin (TTX) and protease E were purchased from Sigma Chemical (Saint Louis, MO, USA). Hydrogen peroxide (H_2O_2) was obtained from Wuhan Zhongnan Chemical Reagent Co (Wuhan, China). Fura-2/AM and collagenase type I were obtained from Tocris (Ellisville, MO, USA) and Gibco (GIBCO™, Invitrogen, Paisley, UK), respectively.

Statistical analysis

The data are expressed as the mean±SD. A paired Student's *t*-test was performed for comparisons between two groups. Statistical analysis was performed using repeated measures analysis of variance (ANOVA), followed by the Scheffé test for multiple comparisons. Statistical analyses were performed using Origin software v7.0 (OriginLab, Northampton, MA). All statistical tests were two-tailed. A value of $P<0.05$ was considered statistically significant.

Results

Ketamine inhibited I_{NaL} in rabbit ventricular myocytes in a concentration-dependent manner

The whole-cell patch-clamp technique was used to record I_{NaL}. Ketamine (20, 40, 80 µmol/L) decreased the current density of I_{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_{NaL} current density.

Effect of ketamine on ATX-augmented I_{NaL}

The I_{NaL} channel opener ATX (30 nmol/L) increased I_{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 µmol/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_{NaL} according to the current-voltage relationship protocol, respectively. Figure 2C shows the summary data for the I_{NaL} current density recorded at −20 mV.

Effect of ketamine on the enhanced I_{NaL} induced by hypoxia or H_2O_2

Previous reports show that I_{NaL} is increased under hypoxia and oxidative stress conditions. Thus, we studied the effects of ketamine on I_{NaL} after exposure to hypoxia or H_2O_2. Hypoxia (10 min) and 300 µmol/L H_2O_2 increased I_{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 µmol/L) decreased it to 0.45±0.06 and 0.43±0.11, respectively (Figure 3). After washing, I_{NaL} returned to the predrug level (hypoxia: 0.67±0.05 *vs* 0.63±0.08 pA/pF, *n*=4; H_2O_2: 0.68±0.04 *vs* 0.67±0.03 pA/pF, *n*=3; both $P>0.05$).

Effects of ketamine on the increased reverse-mode I_{NCX} induced by ATX

ATX (30 nmol/L) increased the reverse-mode I_{NCX} (at +50 mV) from 0.88±0.08 to 2.76±0.21 pA/pF ($P<0.01$; *n*=8). In the pres-
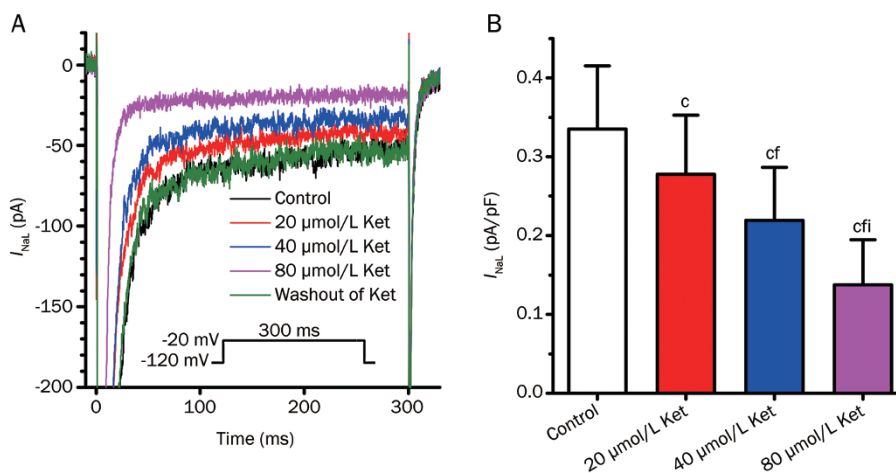

Figure 1. Ketamine (Ket) inhibited I_{NaL} in rabbit ventricular myocytes in a concentration-dependent manner. (A) Representative whole-cell recordings of I_{NaL} in the absence of drug (Control) and after the application and washout of Ket (20, 40, or 80 µmol/L). (B) Summary data for the mean current density of I_{NaL} under different conditions. The data are expressed as the mean±SD (*n*=11). $^c P<0.01$ *vs* Control. $^f P<0.01$ *vs* 20 µmol/L Ket. $^i P<0.01$ *vs* 40 µmol/L Ket.
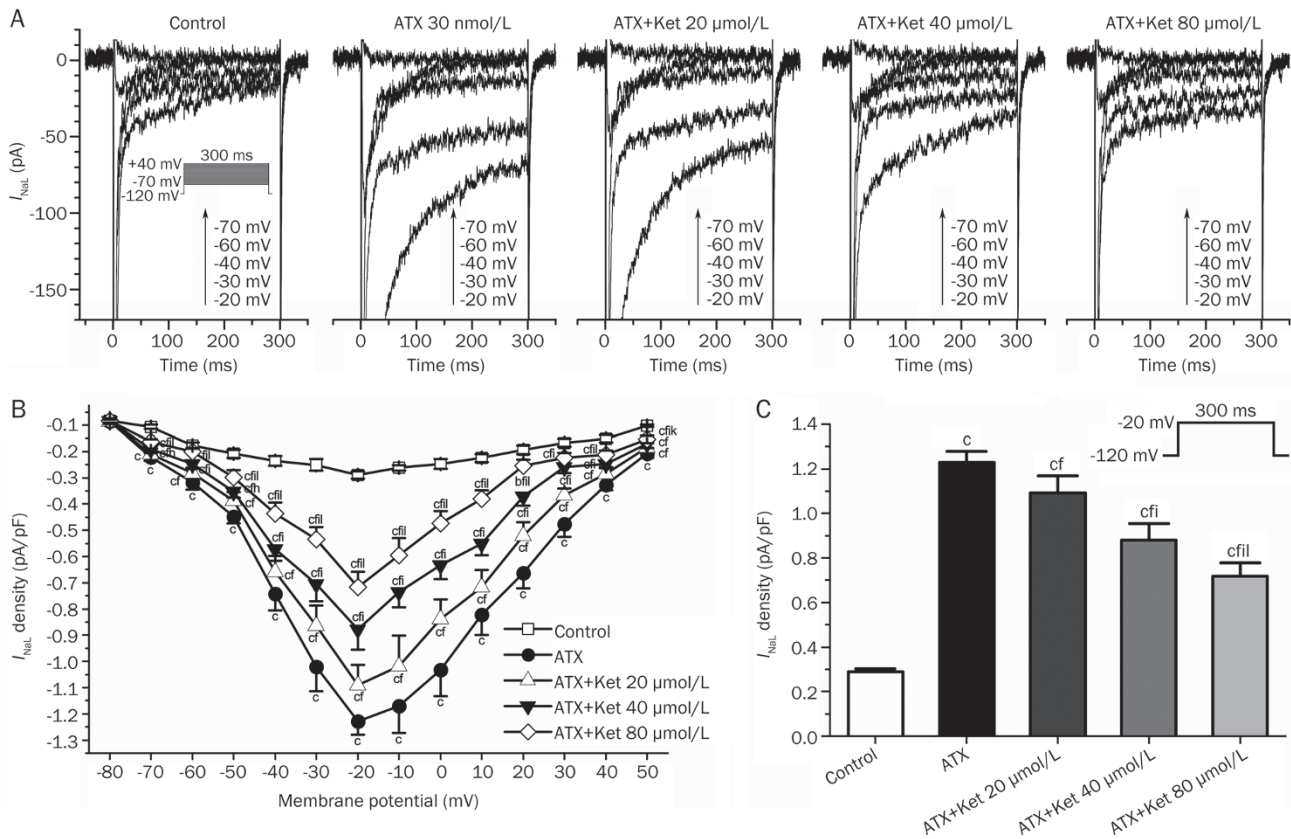
Figure 2. Ketamine (Ket) inhibited ATX-augmented I_{NaL} in a concentration-dependent manner. (A) Representative whole-cell recordings of I_{NaL} in the absence of drug (Control) and in the presence of ATX (30 nmol/L) before or after the application of Ket (20, 40, or 80 µmol/L). (B) The current-voltage relationship for I_{NaL}. (C) Summary data for the mean current density of I_{NaL} (at –20 mV) in the absence (Control) and presence of ATX and ATX plus Ket. The data are expressed as the mean±SD (n=8). ^{c}P<0.01 *vs* Control; ^{f}P<0.01 *vs* 30 nmol/L ATX; ^{i}P<0.01 *vs* ATX plus Ket 20 µmol/L; ^{l}P<0.01 *vs* ATX plus Ket 40 µmol/L.

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_{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 [Ca²⁺]ᵢ transients induced by ATX

ATX (3 nmol/L) enhanced cell shortening and [Ca²⁺]ᵢ transients (Figures 5 and 6). PS, +dL/dt, −dL/dt, diastolic [Ca²⁺]ᵢ, Δ[Ca²⁺]ᵢ, and +d[Ca²⁺]ᵢ/dt, and −d[Ca²⁺]ᵢ/dt were increased to 160%, 180%, 191%, 114%, 165%, 159%, and 162% of control, respectively, and TD₉₀ 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%, 101%, 100%, 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_{CaL}

I_{CaL} plays an important role in cell shortening and [Ca²⁺]ᵢ transients. Previous studies show that ketamine inhibits I_{CaL} in the cardiomyocytes of some species[29–34]. However, no studies have investigated the effect of ketamine on I_{CaL} in rabbit ventricular myocytes. The effect of ketamine on I_{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_{CaL} and observed that ketamine (40 µmol/L) significantly inhibited I_{CaL} in rabbit ventricular myocytes. The current density of I_{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_{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 [Ca²⁺]ᵢ overload occurs in many pathological conditions such as hypoxia, ischemia, oxidative stress, cardiac hypertrophy, and heart failure[1–4] and results in cardiac arrhythmias and myocardial dysfunction[5]. Previous reports show that I_{NaL} is an important contributing factor to [Ca²⁺]ᵢ overload in many pathological conditions. An increase in I_{NaL}
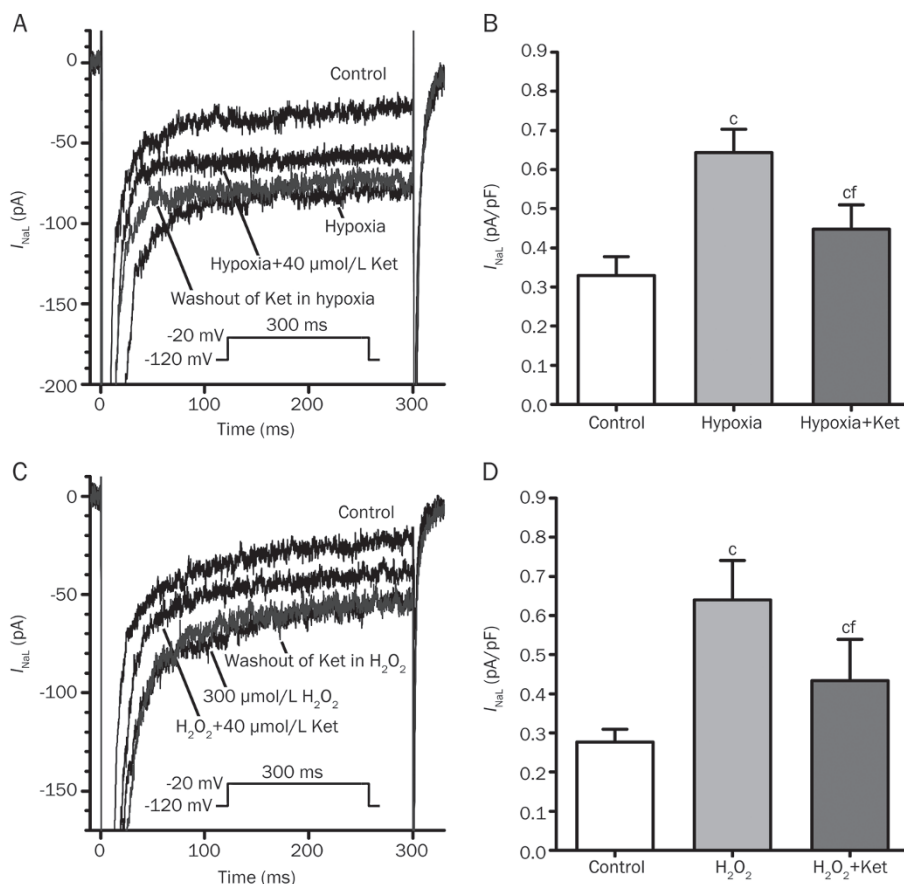
Figure 3. Ketamine (Ket) inhibited the enhanced I_{NaL} induced by hypoxia (A, B) or H_2O_2 (C, D). (A) and (C), Representative whole-cell recordings of I_{NaL} in the absence of drug (Control) and before and after the application and washout of Ket (40 µmol/L) after treatment with 10 min of hypoxia (A) or 300 µmol/L H_2O_2 (C), respectively. (B) and (D), Summary data for the mean current density of I_{NaL} under different conditions. The data are expressed as the mean±SD (*n*=6 or 7). $^c P<0.01$ *vs* Control; $^f P<0.01$ *vs* hypoxia (B) or H_2O_2 (D).

can increase the intracellular sodium concentration and subsequently raise $[Ca^{2+}]_i$ through reverse NCX[7, 12]. Extensive studies have reported that inhibition of I_{NaL} attenuates the increase in $[Ca^{2+}]_i$[13–15]. Inhibition of I_{NaL} is therefore a potential therapeutic target for the treatment of heart diseases associated with $[Ca^{2+}]_i$ 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[32]. 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 H_2O_2 (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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ overload (Figures 4, 6). This result is similar to that obtained in our previous reports, which describe the inhibition of reverse I_{NCX} by sophocarpine and resveratrol, cell shortening and $[Ca^{2+}]_i$ overload by inhibiting I_{NaL}[39, 40]. Therefore, the inhibition of I_{NaL} contributes to ketamine's suppressive effects on $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ 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
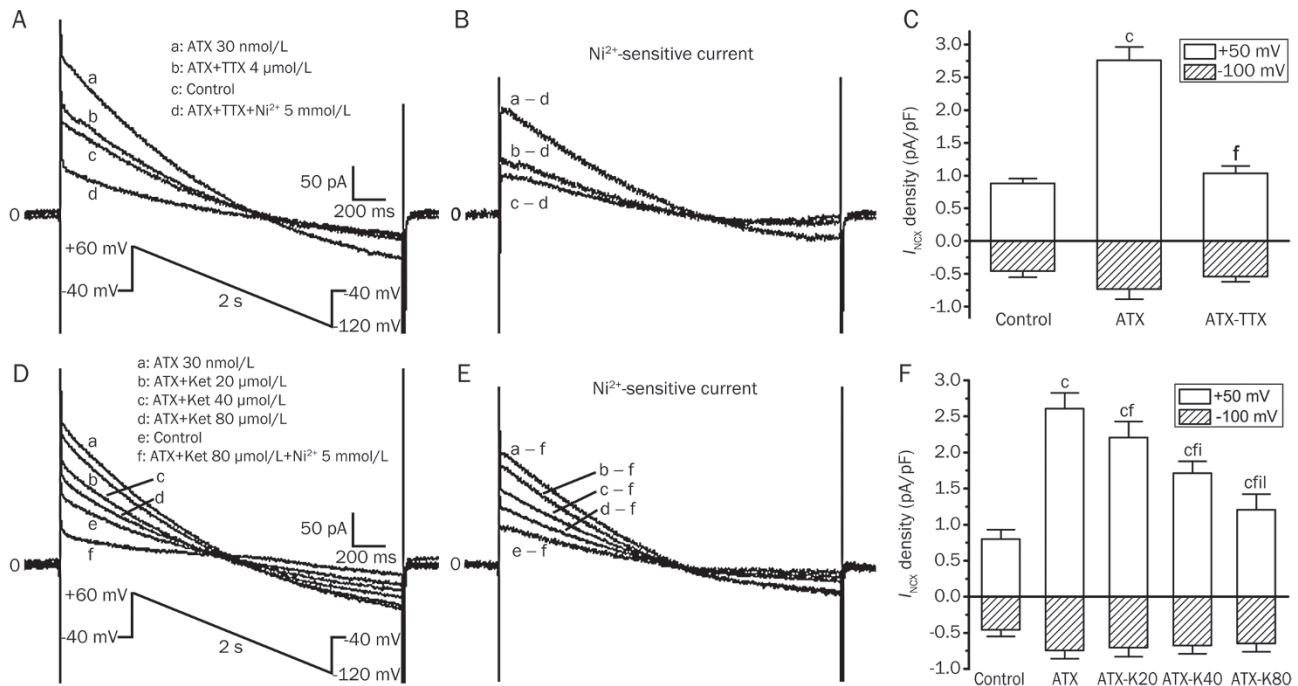
Figure 4. Effects of TTX and ketamine (Ket) on the enhanced I_{NCX} induced by ATX. (A) and (D), Representative original currents recorded by a ramp pulse (*inset*) in the absence (Control) and presence of ATX 30 nmol/L before and after exposure to TTX 4 μmol/L (A) or Ket at 20, 40, or 80 μmol/L (D). (B) and (E), The Ni^{2+}-sensitive I_{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_{NCX} measured at +50 mV and –100 mV. The data are expressed as the mean±SD (*n*=8). $^c P<0.01$ *vs* Control; $^f P<0.01$ *vs* ATX 30 nmol/L (ATX); $^i P<0.01$ *vs* ATX plus Ket 20 μmol/L (ATX-K20, F); $^l P<0.01$ ATX plus Ket 80 μmol/L (ATX-K80, F) *vs* ATX plus Ket 40 μmol/L (ATX-K40, F).

that ketamine inhibits I_{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_{CaL} by 26.1% in guinea pig ventricular myocytes [32]. Endou *et al* reported that ketamine (100 μmol/L) decreased I_{CaL} by 10.8% in rat ventricular myocytes[30]. However, no study had yet investigated the effect of ketamine on I_{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_{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_{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_{NaL} and reverses I_{NCX} in rabbit ventricular myocytes[42], which suggests an increase in $[Ca^{2+}]_i$ at that time. Furthermore, a burst of H_2O_2 is generated in car-

diomyocytes during ischemia, and H_2O_2 also increases I_{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 preconditions 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_{NaL} induced by hypoxia and H_2O_2 (Figure 3) and attenuated the augmented I_{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 clinic use.

In summary, ketamine inhibited I_{NaL} and I_{CaL} and decreased the enhanced reverse I_{NCX}, myocyte shortening and $[Ca^{2+}]_i$ transients induced by ATX in rabbit ventricular myocytes. Ketamine could protect ventricular myocytes against increased
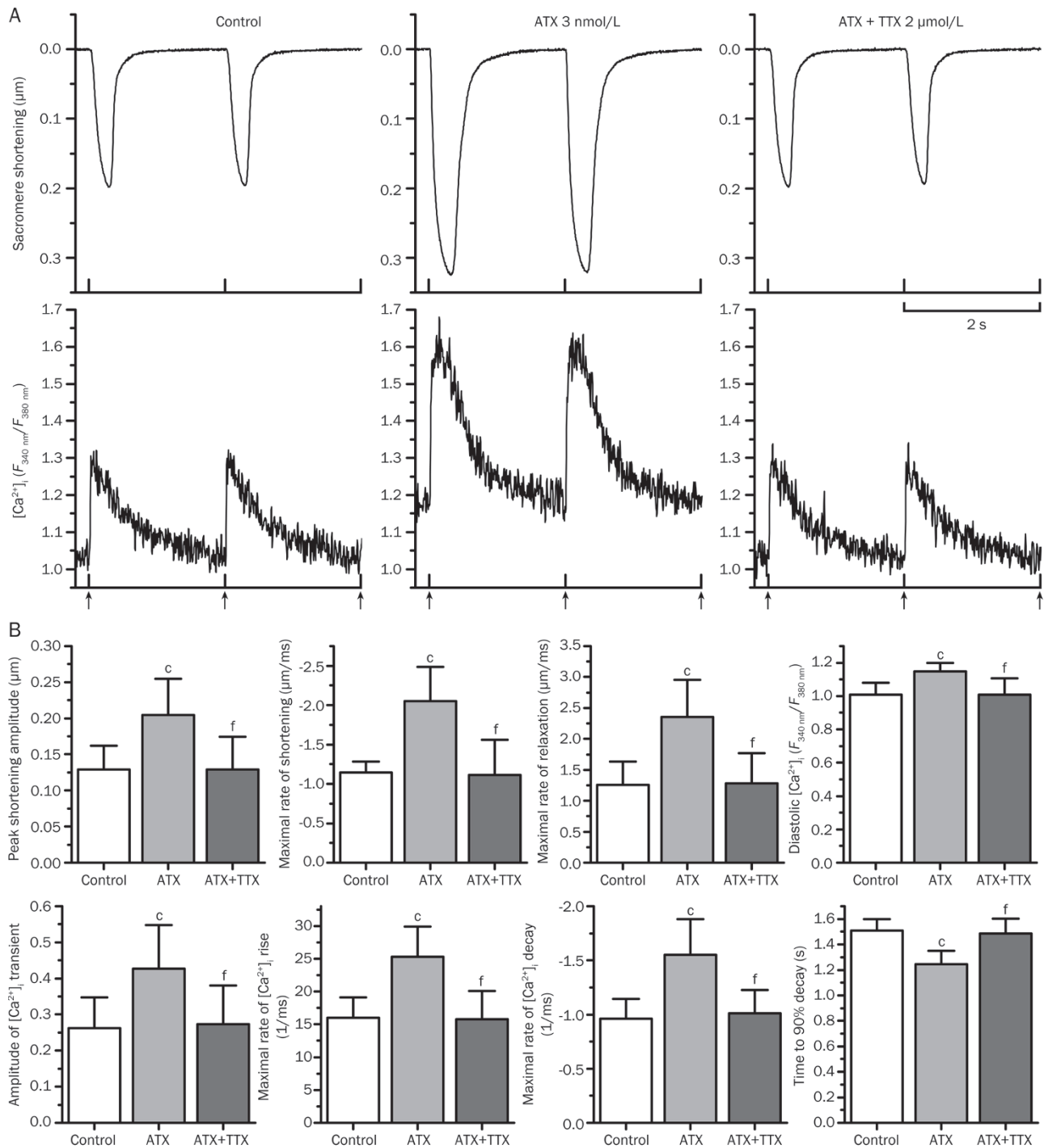
Figure 5. Effects of TTX on enhanced myocyte shortening and $[Ca^{2+}]_i$ transients induced by ATX. (A) Representative recordings of cell shortening (upper) and $[Ca^{2+}]_i$ transients (lower) in the absence (Control) and presence of 3 nmol/L ATX before and after exposure to TTX 2 μmol/L. (B) Summary data for representative parameters of cell shortening and $[Ca^{2+}]_i$ transients. The data are expressed as the mean±SD (n=7). [c]P<0.01 *vs* Control; [f]P<0.01 *vs* ATX group.

I_{NaL}-induced $[Ca^{2+}]_i$ overload in anesthetic use.

tion of Wuhan University of Science and Technology of China.

Author contribution
An-tao LUO, Zhen-zhen CAO, and Ji-hua MA designed the research; An-tao LUO, Zhen-zhen CAO, Yu XIANG, Shuo
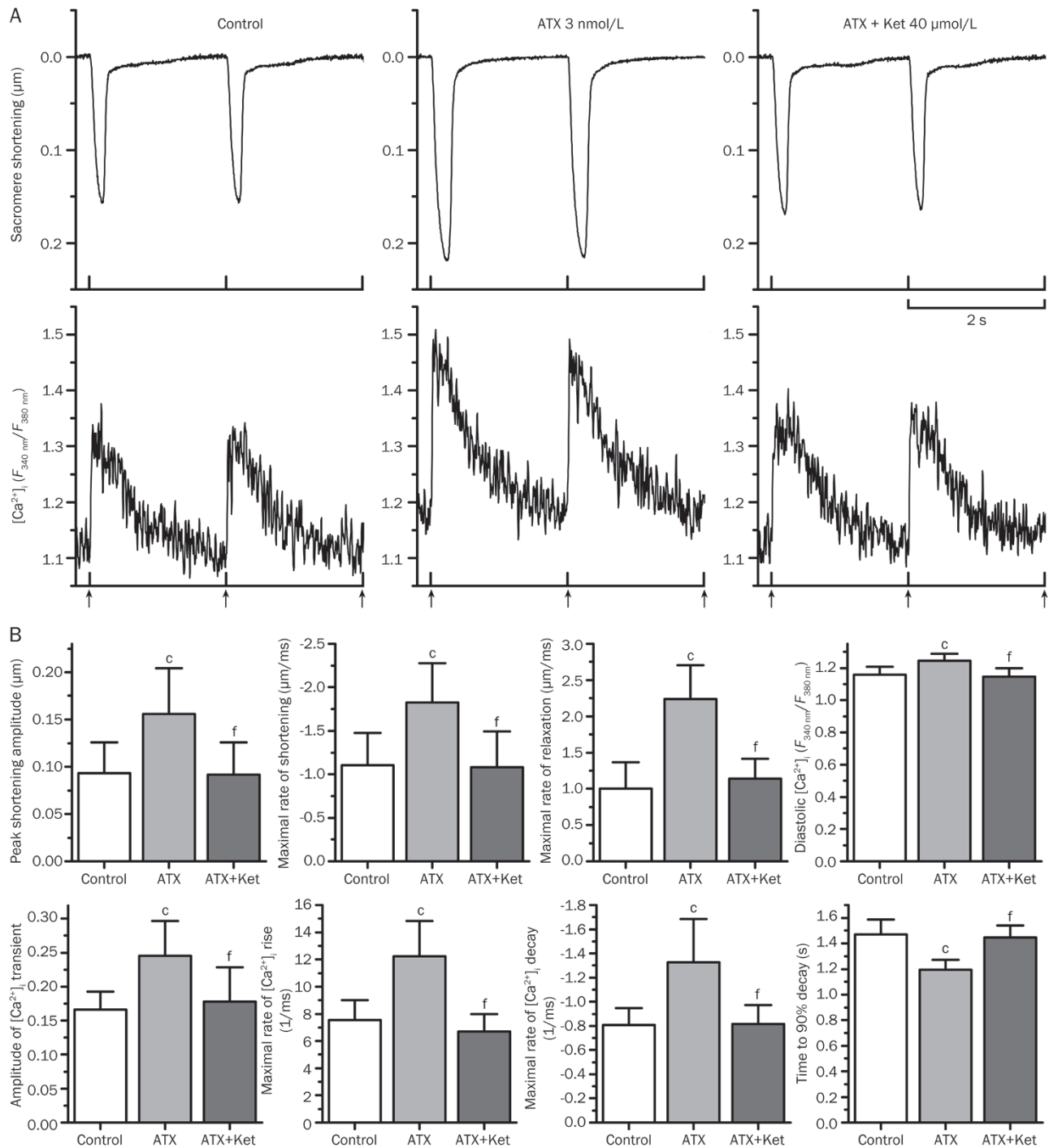
Figure 6. Effects of ketamine (Ket) on enhanced myocyte shortening and $[Ca^{2+}]_i$ transients induced by ATX. (A) Representative recordings of cell shortening (upper) and $[Ca^{2+}]_i$ transients (lower) in the absence (Control) and presence of ATX 3 nmol/L before and after exposure to Ket 40 µmol/L. (B) Summary data for representative parameters of cell shortening and $[Ca^{2+}]_i$ transients. The results are expressed as the mean±SD (n=7). [c]$P<0.01$ *vs* Control; [f]$P<0.01$ *vs* ATX group.

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.

References

1 Bers DM, Barry WH, Despa D. Intracellular Na⁺ regulation in cardiac myocytes. Cardiovasc Res 2003; 57: 897–912.

2 Maltsev VA, Sabbah HN, Higgins RS, Silverman N, Lesch M, Undrovinas AI. Novel, ultraslow inactivating sodium current in human ventricular
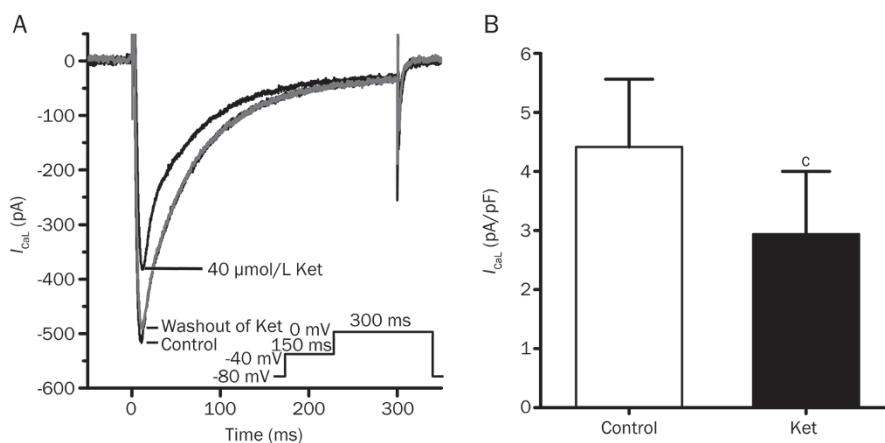
Figure 7. Ketamine (Ket) inhibited I_{CaL} in rabbit ventricular myocytes. (A) Representative whole-cell recordings of I_{CaL} in the absence of drug (Control) and after the application and washout of Ket (40 µmol/L). (B) Summary data for the mean current density of I_{CaL}. The data are expressed as the mean±SD (n=9). [c]$P<0.01$ vs Control.

cardiomyocytes. Circulation 1998; 98: 2545–52.

3 Pieske B, Houser SR. [Na$^+$]$_i$ handling in the failing human heart. Cardiovasc Res 2003; 57: 874–86.

4 Undrovinas AI, Fleidervish IA, Makielski JC. Inward sodium current at resting potentials in single cardiac myocytes induced by the ischemic metabolite lysophosphatidylcholine. Circ Res 1992; 71: 1231–41.

5 Vassalle M, Lin CI. Calcium overload and cardiac function. J Biomed Sci 2004; 11: 542–65.

6 Ahern GP, Hsu SF, Klyachko VA, Jackson MB. Induction of persistent sodium current by exogenous and endogenous nitric oxide. J Biol Chem 2000; 275: 28810–5.

7 Hammarström AK, Gage PW. Hypoxia and persistent sodium current. Eur Biophys J 2002; 31: 323–30.

8 Luo A, Ma J, Zhang P, Zhou H, Wang W. Sodium channel gating modes during redox reaction. Cell Physiol Biochem 2007; 19: 9–20.

9 Ma JH, Luo AT, Zhang PH. Effect of hydrogen peroxide on persistent sodium current in guinea pig ventricular myocytes. Acta Pharmacol Sin 2005; 26: 828–34.

10 Undrovinas AI, Maltsev VA, Kyle JW, Silverman N, Sabbah HN. Gating of the late Na$^+$ channel in normal and failing human myocardium. J Mol Cell Cardiol 2002; 34: 1477–89.

11 Antzelevitch C. Electrical heterogeneity, cardiac arrhythmias, and the sodium channel. Circ Res 2000; 87: 910–4.

12 Imahashi K, Kusuoka H, Hashimoto K, Yoshioka J, Yamaguchi H, Nishimura T. Intracellular sodium accumulation during ischemia as the substrate for reperfusion injury. Circ Res 1999; 84: 1401–6.

13 Haigney MC, Lakatta EG, Stern MD, Silverman HS. Sodium channel blockade reduces hypoxic sodium loading and sodium-dependent calcium loading. Circulation 1994; 90: 391–9.

14 Undrovinas AI, Belardinelli L, Undrovinas NA, Sabbah HN. Ranolazine improves abnormal repolarization and contraction in left ventricular myocytes of dogs with heart failure by inhibiting late sodium current. J Cardiovasc Electrophysiol 2006; 17 Suppl 1: 169–77.

15 Van Emous JG, Nederhoff MG, Ruigrok TJ, Van Echteld CJ. The role of the Na$^+$ channel in the accumulation of intracellular Na$^+$ during myocardial ischemia: consequences for post-ischemic recovery. J Mol Cell Cardiol 1997; 29: 85–96.

16 Undrovinas NA, Maltsev VA, Belardinelli L, Sabbah HN, Undrovinas A. Late sodium current contributes to diastolic cell Ca^{2+} accumulation in chronic heart failure. J Physiol Sci 2010; 60: 245–57.

17 Ver Donck L, Borgers M, Verdonck F. Inhibition of sodium and calcium overload pathology in the myocardium: a new cytoprotective principle. Cardiovasc Res 1993; 27: 349–57.

18 Aya AG, Robert E, Bruelle P, Lefrant JY, Juan JM, Peray P, et al. Effects of ketamine on ventricular conduction, refractoriness, and wavelength: potential antiarrhythmic effects: a high-resolution epicardial mapping in rabbit hearts. Anesthesiology 1997; 87: 1417–27.

19 Baczkó I, Leprán I, Papp JG. Influence of anesthetics on the incidence of reperfusion-induced arrhythmias and sudden death in rats. J Cardiovasc Pharmacol 1997; 29: 196–201.

20 D'Amico M, Di Filippo C, Rossi F, Rossi F. Arrhythmias induced by myocardial ischaemia-reperfusion are sensitive to ionotropic excitatory amino acid receptor antagonists. Eur J Pharmacol 1999; 366: 167–74.

21 Hanouz JL, Repesse Y, Zhu L, Lemoine S, Rouet R, Sallé L, et al. The electrophysiological effects of racemic ketamine and etomidate in an in vitro model of "border zone" between normal and ischemic/reperfused guinea pig myocardium. Anesth Analg 2008; 106: 365–70.

22 Hanouz JL, Zhu L, Persehaye E, Massetti M, Babatasi G, Khayat A, et al. Ketamine preconditions isolated human right atrial myocardium: roles of adenosine triphosphate-sensitive potassium channels and adrenoceptors. Anesthesiology 2005; 102: 1190–6.

23 Szekely A, Heindl B, Zahler S, Conzen PF, Becker BF. S(+)-ketamine, but not R(–)-ketamine, reduces postischemic adherence of neutrophils in the coronary system of isolated guinea pig hearts. Anesth Analg 1999; 88: 1017–24.

24 Weigand MA, Schmidt H, Zhao Q, Plaschke K, Martin E, Bardenheuer HJ. Ketamine modulates the stimulated adhesion molecule expression on human neutrophils in vitro. Anesth Analg 2000; 90: 206–12.

25 Szekely A, Heindl B, Zahler S, Conzen PF, Becker BF. Nonuniform behavior of intravenous anesthetics on postischemic adhesion of neutrophils in the guinea pig heart. Anesth Analg 2000; 90: 1293–300.

26 Hess WC, Ohe A. Does ketamine/propofol anesthesia possess antiarrhythmogenic quality? A perioperative study in aortocoronary bypass patients. Eur J Med Res 2001; 17: 543–50.

27 Neuhäuser C, Preiss V, Feurer MK, Müller M, Scholz S, Kwapisz M, et al. Comparison of S-(+)-ketamine- with sufentanil-based anaesthesia for elective coronary artery bypass graft surgery: Effect on troponin T

levels. Br J Anaesth 2008; 100: 765–71.

28 Ríha H, Kotulák T, Brezina A, Hess L, Kramár P, Szárszoi O, *et al*. Comparison of the effects of ketamine-dexmedetomidine and sevoflurane-sufentanil anesthesia on cardiac biomarkers after cardiac surgery: an observational study. Physiol Res 2012; 61: 63–72.

29 Baum VC, Tecson ME. Ketamine inhibits transsarcolemmal calcium entry in guinea pig myocardium: direct evidence by single cell voltage clamp. Anesth Analg 1991; 73: 804–7.

30 Endou M, Hattori Y, Nakaya H, Gotoh Y, Kanno M. Electrophysiologic mechanisms responsible for inotropic responses to ketamine in guinea pig and rat myocardium. Anesthesiology 1992; 76: 409–18.

31 Sekino N, Endou M, Hajiri E, Okumura F. Nonstereospecific actions of ketamine isomers on the force of contraction, spontaneous beating rate, and Ca^{2+} current in the guinea pig heart. Anesth Analg 1996; 83: 75–80.

32 Hara Y, Chugun A, Nakaya H, Kondo H. Tonic block of the sodium and calcium currents by ketamine in isolated guinea pig ventricular myocytes. J Vet Med Sci 1998; 60: 479–83.

33 Hatakeyama N, Yamazaki M, Shibuya N, Yamamura S, Momose Y. Effects of ketamine on voltage-dependent calcium currents and membrane potentials in single bullfrog atrial cells. J Anesth 2001; 15: 149–53.

34 Deng CY, Yu XY, Kuang SJ, Rao F, Yang M, Shan ZX, *et al*. Electrophysiological effects of ketamine on human atrial myocytes at therapeutically relevant concentrations. Clin Exp Pharmacol Physiol 2008; 35: 1465–70.

35 Baum VC. Distinctive effects of three intravenous anesthetics on the inward rectifier (I_{K1}) and the delayed rectifier (I_K) potassium currents in myocardium: implications for the mechanism of action. Anesth Analg 1993; 76: 18–23.

36 Ko SH, Lee SK, Han YJ, Choe H, Kwak YG, Chae SW, *et al*. Blockade of myocardial ATP-sensitive potassium channels by ketamine. Anesthesiology 1997; 87: 68–74.

37 Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Garcia C, Schaub MC. Differential effects of anesthetics on mitochondrial K_{ATP} channel activity and cardiomyocyte protection. Anesthesiology 2002; 97: 15–23.

38 Zhang P, Xing J, Luo A, Feng J, Liu Z, Gao C, *et al*. Blockade of the human ether-a-go-go-related gene potassium channel by ketamine. J Pharm Pharmacol 2013; 65: 1321–8.

39 Zhang S, Ma JH, Zhang PH, Luo AT, Ren ZQ, Kong LH. Sophocarpine attenuates the Na^+-dependent Ca^{2+} overload induced by *Anemonia sulcata* toxin-increased late sodium current in rabbit ventricular myocytes. J Cardiovasc Pharmacol 2012; 60: 357–66.

40 Qian C, Ma J, Zhang P, Luo A, Wang C, Ren Z, *et al*. Resveratrol attenuates the Na^+-dependent intracellular Ca^{2+} overload by inhibiting H_2O_2-induced increase in late sodium current in ventricular myocytes. PLoS One 2012; 7: e51358.

41 Ma J, Luo A, Wu L, Wan W, Zhang P, Ren Z, *et al*. Calmodulin kinase II and protein kinase C mediate the effect of increased intracellular calcium to augment late sodium current in rabbit ventricular myocytes. Am J Physiol Cell Physiol 2012; 302: C1141–51.

42 Tang Q, Ma J, Zhang P, Wan W, Kong L, Wu L. Persistent sodium current and Na^+/H^+ exchange contributes to the augmentation of the reverse Na^+/Ca^{2+} exchange during hypoxia or acute ischemia in ventricular myocytes. Pflügers Arch 2012; 463: 513–22.

43 Idvall J, Ahlgren I, Aronsen KR, Stenberg P. Ketamine infusions: pharmacokinetics and clinical effects. Br J Anaesth 1979; 51: 1167–73.

44 Wieber J, Gugler R, Hengstmann JH, Dengler HJ. Pharmacokinetics of ketamine in man. Anaesthesist 1975; 24: 260–3.

45 Dayton PG, Stiller RL, Cook DR, Perel JM. The binding of ketamine to plasma proteins: emphasis on human plasma. Eur J Clin Pharmacol 1983; 24: 825–31.

46 Priebe HJ. Triggers of perioperative myocardial ischaemia and infarction. Br J Anaesth 2004; 93: 9–20.

47 Mangano DT. Perioperative cardiac morbidity. Anesthesiology 1990; 72: 153–84.