Tonic Block of the Sodium and Calcium Currents by Ketamine in Isolated Guinea Pig Ventricular Myocytes

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ABSTRACT. Effects of ketamine on the sodium (\(I_{Na}\)) and L-type calcium currents (\(I_{Ca}\)) were examined by using whole-cell patch clamp techniques in guinea pig single ventricular myocytes. The mode of action of ketamine was compared with those of quinidine, a sodium channel blocker, and verapamil, a calcium channel blocker. Ketamine (30–300 µM) inhibited both \(I_{Na}\) and \(I_{Ca}\) in a concentration-dependent manner. Quinidine (30 µM) and verapamil (0.1 µM) produced use-dependent depression of \(I_{Na}\) and \(I_{Ca}\), respectively. The amplitude of \(I_{Na}\) elicited by the first depolarizing pulse after a long quiescent period was slightly decreased by quinidine. During a train of depolarizing pulses the current amplitude decreased gradually, and reached a steady state level in the quinidine-treated cells (use-dependent block, UDB). Verapamil produced a similar mode of inhibition of \(I_{Ca}\), i.e., UDB. In contrast, ketamine produced significant decrease in \(I_{Na}\) and \(I_{Ca}\) elicited by the first depolarizing pulses and the decreases of both currents were not augmented during a train of depolarizing pulses. From these results, it can be concluded that ketamine produces tonic block of the cardiac sodium and calcium channels and the mode of inhibition is clearly different from UDB by quinidine and verapamil. — KEY WORDS: calcium current, cardiac myocyte, ketamine, sodium current, tonic block.

Ketamine affects the contractile tension and the action potential configuration in isolated mammalian myocardial preparations [1, 2, 24]. It has been demonstrated that ketamine inhibits several potassium currents and L-type calcium current (\(I_{Ca}\)) in isolated single cardiomyocytes of guinea pigs and rats [6]. We have recently reported that ketamine decreased the maximum rate of rise of action potential upstroke (\(\Delta \text{max}\)) in isolated guinea pig papillary muscles, suggesting that ketamine also exerts an inhibitory action on cardiac sodium (Na) channels [12]. It is well-known that Na channel blockers such as lidocaine and quinidine produce inhibition of \(\Delta \text{max}\) in a use-dependent manner (use-dependent block, UDB) where its inhibition is enhanced with repetition of evoking action potential [13, 16]. In our previous study [12] ketamine markedly depressed \(\Delta \text{max}\) of the first action potential after a long pulse-free period and failed to produce a further decrease in \(\Delta \text{max}\) during the subsequent train of stimuli. However, \(\Delta \text{max}\) of the action potential is an indirect estimate of the Na current (\(I_{Na}\)) and accurate measurement of \(I_{Na}\) may be needed. Accordingly, the first aim of the present study was to evaluate the mode of inhibitory action of ketamine on cardiac Na channels in enzymatically dissociated guinea pig ventricular myocytes and to compare it with that of the Na channel blocker quinidine. Calcium (Ca) channel blockers including verapamil are known to suppress \(I_{Ca}\) in a use-dependent manner [19, 20]. However, it remains undetermined whether ketamine produces a use-dependent block of the Ca channel. Therefore, the second aim of this study was to investigate the mode of inhibitory action of ketamine on \(I_{Ca}\) in comparison with that of verapamil.

MATERIALS AND METHODS

Cell preparations: Guinea pigs, weighing 200–350 g, were used in this study. Single ventricular cells were isolated by an enzymatic dispersion, as previously described [11]. Briefly, the heart was removed from the open-chest guinea pig anesthetized with pentobarbital Na, and mounted on a modified Langendorff perfusion system for retrograde perfusion of the coronary circulation with a normal HEPES-Tyrode solution. The perfusion medium was then changed to a nominally Ca-free HEPES-Tyrode solution and then to a solution containing 0.02% w/v collagenase (Wako, Osaka, Japan). After digestion, the heart was perfused with a high K+ and low Cl– solution (modified Kraftbrühe (KB) solution) [17, 22]. Ventricular tissue was cut into small pieces in the modified KB solution and gently shaken to isolate cells. Dispersed cells were stored in the KB solution at 4°C and used over the next 7–10 hr.

Recording techniques: Whole-cell membrane currents were recorded by patch clamp method [9]. Single ventricular cells were placed in a recording chamber (1-ml volume) attached to an inverted microscope (Olympus IMT-2, Tokyo, Japan) and superfused with the HEPES-Tyrode solution at a rate of 3 ml/min. The temperature of the external solution was kept constant at 36 ± 1°C. Glass patch pipettes with a diameter of 1.5 mm were filled with a pipette solution. The resistance of the patch pipette filled with the pipette solution was 2–3 MΩ. The tight-seal, whole cell voltage clamp technique was used. After the gigaseal between the tip of the electrode and the cell membrane was established, the membrane patch was disrupted by...
applying more negative pressure to make the whole cell voltage-clamp mode. \( I_{\text{Ca}} \) was recorded by applying 300-ms depolarizing pulses from a holding potential of -40 mV to 0 mV at 1 Hz. In order to record \( I_{\text{Na}} \) the cells were depolarized to -30 mV from a holding potential of -120 mV by depolarizing pulses of 50 ms at 1 Hz. The experiments to record \( I_{\text{Na}} \) were performed at room temperature.

The electrode was connected to a patch-clamp amplifier (Nihon Kohden CEZ-2300, Tokyo, Japan). Command pulses were generated by a 12-bit digital-to-analog converter controlled by pClamp software (Axon Instruments, Inc., Foster City, CA, U.S.A.). Current signals were digitized and stored on the hard disk of an IBM compatible computer.

**Solutions:** The normal HEPES-Tyrode solution was composed of (mM): NaCl 143, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 0.5, NaH\(_2\)PO\(_4\) 0.33, glucose 5.5 and HEPES-NaOH buffer (pH 7.4) 5.0. The composition of modified KB solution was (mM): KOH 70.0, L-glutamic acid 50.0, KCl 40.0, taurine 20.0, KH\(_2\)PO\(_4\) 20.0, MgCl\(_2\) 3.0, glucose 10.0 and HEPES-KOH buffer (pH 7.4) 10.0. In order to record \( I_{\text{Na}} \) the following external and pipette solutions were used. The external solution contained (mM): NaCl 30.0, tetramethylammonium chloride 110.0, CoCl\(_2\) 1.0, CsCl 5.0, MgCl\(_2\) 1.2, glucose 11.0, HEPES 20.0, CaCl\(_2\) 1.8 (pH 7.3 adjusted by tetramethylammonium hydroxide). The pipette solution was composed of (mM): NaF 5.0, CsF 125.0, ATP-K\(_2\) 5.0, phosphocreatine-K\(_2\) 5.0, EGTA 10 and HEPES-KOH buffer (pH 7.2 adjusted with CsOH). The normal HEPES-Tyrode solution was used as an external solution to record \( I_{\text{Ca}} \). The composition of the pipette solution (pCa 8) for \( I_{\text{Ca}} \) recording was (mM): K-aspartate 110, KCl 20, MgCl\(_2\) 1.0, ATP-K\(_2\) 5.0, phosphocreatine-K\(_2\) 5.0, EGTA 10 and HEPES-KOH buffer (pH 7.4) 5.0.

**Drugs:** Ketamine HCl (Warner-Lambert, Tokyo, Japan), quinidine (Wako, Osaka, Japan) and verapamil HCl (Eisai, Tokyo, Japan) were dissolved in the HEPES-Tyrode solution, 0.1 N HCl and water, respectively, as 10\(^{-2}\) M stock solution.

**Statistics:** All values are presented in terms of mean ± S.E.M. ANOVA were used for statistical analysis of the data. P value of less than 0.05 was considered significant.

**RESULTS**

**Effects of ketamine and quinidine on \( I_{\text{Na}} \):** Effects of ketamine and quinidine inhibited \( I_{\text{Na}} \), and a steady state inhibition was achieved within 5 to 10 min. At that time, the mode of inhibition of \( I_{\text{Na}} \) by these drugs, i.e., tonic block and UDB, were evaluated by applying a train of 30 depolarizing pulses of 50-ms duration from a holding potential of -120 mV to -30 mV at 1 Hz after a 60-s pulse-free period. Tonic (or resting) block was designated as a percentage decrease in \( I_{\text{Na}} \) of the first depolarizing pulse of the stimulus train after the pulse-free period. UDB was defined as a percentage decrease in the current from that of the first pulse to a new steady-state current level during a stimulus train of 30 depolarizing pulses after the quiescent period.

In the absence of any drug the peak \( I_{\text{Na}} \) did not change after a train of 30 depolarizing pulses (0.6 ± 0.3%, n=27), as shown in Fig. 1. Ketamine (300 \( \mu \)M) markedly depressed the peak \( I_{\text{Na}} \) at the first depolarizing pulses and no further decrease in the amplitude of \( I_{\text{Na}} \) was observed with successive pulses, as shown in Fig. 1A. Five to 10 min after treatment with ketamine, tonic block and UDB were evaluated. Tonic block of \( I_{\text{Na}} \) produced by ketamine 30, 100 and 300 \( \mu \)M was 16.3 ± 5.4% (n=6), 19.1 ± 2.6% (n=9), and 36.2 ± 11.1% (n=6), respectively. UDBs observed in the presence of 30, 100 and 300 \( \mu \)M ketamine
Inhibitory effects of ketamine (100 µM) (A) and verapamil (0.1 µM) (B) on I_Ca in single ventricular myocytes. I_Ca was elicited by applying 300-ms depolarizing pulses from a holding potential of -40 mV to 0 mV at 1 Hz. A train of 30 pulses was applied to the cell after a pulse-free period of 60 s before (Control) and 5 min after the treatment with ketamine or verapamil. Actual current traces induced by the first (a and c) and the 30th (b and d) depolarizing pulses are shown in the lower parts. Note that ketamine, unlike quinidine, produced a use-independent block (tonic block) of I_Ca.

Effects of ketamine and verapamil on I_Ca: Effects of ketamine on I_Ca were examined and compared with those of a Ca antagonist verapamil. Both ketamine and verapamil inhibited I_Ca, and a steady state inhibition was achieved within 5 to 10 min. Tonic block and UDB were evaluated 5 to 10 min after treatment of the drugs by applying a train of 30 depolarizing pulses of 300 ms duration from a holding potential of -40 mV to 0 mV at 1 Hz after a 60-s pulse-free period. Ketamine (100 µM) depressed the peak I_Ca elicited by the first depolarizing pulse and no further decrease in I_Ca was observed with a successive train of pulses, as shown in Fig. 2A. Tonic block of I_Ca by 30, 100 and 300 µM ketamine were 26.1 ± 20.0% (n=3), 52.8 ± 4.6% (n=8), and 49.5 ± 12.1% (n=6), respectively. UDBs produced by 30, 100 and 300 µM ketamine were 16.7 ± 5.4%, 22.3 ± 3.7% and 12.3 ± 5.0%, respectively. Because small reduction of I_Ca (13.2 ± 2.3%, n=22) was observed during a train of 30 depolarizing pulses in the absence of any drug, UDB of ketamine appeared to be negligible.

After the exposure to verapamil (0.1 µM), significant tonic block and UDB were observed (Fig. 2B). Tonic block and UDB produced by 5 to 10 min exposure to verapamil (0.1 µM) were 56.7 ± 9.0% (n=5) and 77.2 ± 10.3%, respectively. The decline of the peak I_Ca during the delivery of repetitive depolarizing pulses followed a single exponential, and the onset rate of UDB at 1 Hz was calculated as 0.162 ± 0.019 pulse⁻¹ in 5 cells.

DISCUSSION

Ketamine has been reported to inhibit various ionic currents including I_Na, the inward rectifier K⁺ current (I_k), the delayed rectifier K⁺ current (I_k) [3, 6, 26]. In addition, we have recently reported that ketamine also decreased \( I_{\text{max}} \), an indirect estimate of I_Na, in isolated guinea-pig papillary muscles [12]. The present study was focused on the mode of inhibition of I_Na and I_Ca by ketamine. In the present study quinidine but not ketamine produced UDB of I_Na. It is well-established that class I antiarrhythmic drugs inhibit \( I_{\text{max}} \) in a use-dependent manner [5, 16, 25], and several models have been proposed to explain the interaction between the class I antiarrhythmic drugs and the cardiac Na channel [15, 16, 27]. According to the modulated receptor hypothesis [16], the affinity of the Na channel blocker to the binding site is determined by the electrophysiologic state of the Na channel, i.e., resting, activated, and inactivated states, and the drug-associated channels do not conduct Na ion even when activated. Quinidine produced a slight resting block and a significant UDB of I_Na in the present study. Ketamine, however, elicited a tonic block without producing any appreciable UDB of I_Na. In addition, the ketamine-induced depression of I_Na was not relieved by delivering repetitive depolarizing pulses. These findings indicate that ketamine blocks the Na channels irrespective of the channel states.

Using an experimental protocol similar to that used in studying the Na channel blockade by local anesthetics [8, 11], effects of verapamil and ketamine on I_Ca were evaluated. Organic Ca channel blockers are reported to produce a little tonic block and significant UDB of I_Ca due to higher affinity
for open Ca channels [14, 19–21]. Consistent with these reports, verapamil showed tonic block and UDB of I_{Ca} in the present study. Ketamine, similar to other general anesthetics [4, 7], inhibited I_{Ca} of cardiac myocytes. Ketamine produced tonic block and concentration-independent UDB of I_{Ca}. This UDB observed was not significantly different from the small reduction of I_{Ca} during the train of 30 pulses in drug-free condition. Thus, the mode of inhibition of I_{Ca} by ketamine is tonic block but not UDB. In other words, ketamine may block Ca channels in a state-independent manner.

It has been reported that peak plasma concentrations of ketamine during anesthesia are 20–40 µM in animals [10, 18, 28, 29]. The range of concentrations of ketamine used in the present study was not far from the concentrations encountered in the clinical setting. Because the cardiac function of a critically ill animal seems to be more susceptible to a cardiodepressant, it is likely that the direct cardiodepression caused by a clinical dose of ketamine is related to an unexpected decrease in cardiac function. Caution should be used when ketamine is administered to animals with preexisting cardiac disorder.

It is concluded that ketamine produces inhibitions of I_{Na} and I_{Ca} in a mode of action different from that of quinidine and verapamil; ketamine inhibits both the Na and Ca channels in a state-independent manner. This suppression of the Na and Ca channels may contribute to the negative inotropic effects of ketamine [23] in the heart.

REFERENCES


Cir. Res. 65: 723–739.

