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Effects of the venom of the spider *Ornithoctonus hainana* on neonatal rat ventricular myocytes cellular and ionic electrophysiology

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ABSTRACT

Cardiac ion channels are membrane-spanning proteins that allow the passive movement of ions across the cell membrane along its electrochemical gradient, which regulates the resting membrane potential as well as the shape and duration of the cardiac action potential. Additionally, they have been recognized as potential targets for the actions of neurotransmitters, hormones and drugs of cardiac diseases. Spider venoms contain high abundant of toxins that target diverse ion channels and have been considered as a potential resource of new constituents with specific pharmacological properties. However, few peptides from spider venoms were detected as cardiac channel antagonists. In order to explore the effects of the venom of *Ornithoctonus hainana* on the action potential and ionic currents of neonatal rat ventricular myocytes (NRVMs), whole cell patch clamp technique was used to record action potential duration (APD), sodium current (I_{Na}), L calcium current (I_{CaL}), rapidly activating and inactivating transient outward currents (I_{to1}), rapid (I_{Kr}) and slow (I_{Ks}) components of the delayed rectifier currents and the inward rectifier currents (I_{K1}). Our results showed that 100 μg/mL venom obviously prolonged APDs. Significantly, the venom could inhibit I_{Na} and I_{CaL} effectively, while no evident inhibitory effects on cardiac K^+ channels (I_{to1}, I_{ks}, I_{kr} and I_{k1}) were observed, suggesting that the venom represented a multifaceted pharmacological profile. The effect of venom on Na^+ and Ca^{2+} currents of ventricular myocytes revealed that the *hainan* venom as a rich resource of cardiac channel antagonists might be valuable tools for the investigation of both channels and drug development.

1. Introduction

Cardiac ion channels play a pivotal role in maintaining normal cardiac electrical activity. They regulate the resting membrane potential and excitability, participate in the repolarization, and determine the shape and duration of cardiac action potential (Tamargo et al., 2004). Malfunction of ion channels, due to either congenital encoded gene mutations or drug blockade, alters not only the cardiomyocyte excitability, but also the electrical balance of

Abbreviations: NRVMs, neonatal rat ventricular myocytes; APD, action potential duration; I_{Na}, cardiac sodium current; I_{CaL}, cardiac L-type calcium current; I_{to1}, cardiac rapidly activating and inactivating transient outward current; I_{Kr}, cardiac rapid components of the delayed rectifier current; I_{Ks}, cardiac slow components of the delayed rectifier current; I_{K1}, cardiac inward rectifier current; LQTS, long QT syndrome.

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depolarization and repolarization, and thus causes a long QT syndrome (LQTS) or short QT syndrome (Kannankeril and Roden, 2007; Sarganas et al., 2014; Schimpf et al., 2010; Zareba and Cygankiewicz, 2008), and underlies different types of cardiac arrhythmias (Koval et al., 2012) or other cardiac conduction disorders. Therefore, cardiac ion channels are important targets of anti-arrhythmic drugs.

It is well recognized that the shape and duration of cardiac action potential are determined by a balance (i.e., sequential activation and inactivation) of inward currents and outward currents. Six prominent voltage-gated ion currents are expressed in cardiac ventricular myocytes: voltage-gated Na^+ current (I_{Na}), transient outward K^+ current (I_{to1}), delayed rectifier K^+ current (I_{Kr} and I_{Ks}) and inward rectifier K^+ currents (I_{K1}) and L-type Ca^{2+} currents (I_{CaL}). The cardiac ion channel antagonists are important resource of anti-arrhythmic drugs in clinic. Procainamide and lidocaine, as Na^+ channel blockers that may prolong ventricular repolarization, are effective antiarrhythmic drugs (Osadchii, 2013). Blockers of cardiac K^+ channels prolong the cardiac action potential duration (APD) and refractoriness without slowing impulse conduction (Roepke and Abbott, 2006), for example, Amiodarone (Amio) has been used clinically in the treatment of ventricular arrhythmias, being effective in recurrent ventricular tachycardia and fibrillation (Armon, 2007; London, 2007; Roomi et al., 2014). Calcium channel blocking by antagonists such as verapamil may prevent cardiac arrhythmias due to intracellular calcium overload (Stams et al., 2012).

Animal venoms contain a mixture of compounds with different biological activities. The venom, secreted by the venom gland of the spider *Ornithoctonus hainana*, contains abundant of peptide toxins targeted on diverse ion channels, making it a valuable resource with potential therapeutic applications. For example, hainantoxin-I and hainantoxin-VI selectively inhibit insect sodium channels (Li et al., 2003; Wang et al., 2010), hainantoxin-III, hainantoxin-IV and hainantoxin-V are neurotoxins selectively inhibiting TTX-S sodium channels (Liu et al., 2013). However, few peptides from spider venoms were identified as cardiac channel antagonists. In this study, we tested the inhibition of *O. hainana* venom on the action potential duration (APD) and ion channels in NRVMs. Our results showed that 100 μg/mL venom of *O. hainana* prolonged APDs by inhibiting Na^+ and L-type Ca^{2+} currents effectively, implying that the venom might be a potential resource for treating cardiac diseases.

2. Materials and methods

2.1. Ventricular myocyte isolation

Neonatal rat ventricular myocytes (NRVMs) cell were dissociated from ventricles of 1–2 day old neonatal Sprague–Dawley rats using a previously reported method with some modifications (Sung et al., 2012). Ventricular parts of neonatal rats were excised and ventricular tissues were minced on ice and treated with trypsin (US Biochemicals) and collagenase (Worthington). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/F-12 culture medium containing 10% fetal bovine serum (Bursac et al., 2002). The cell pellets were incubated to attach to non-cardiac myocytes at 37 °C in a 95% O_2 incubator for 1.5 h. Then, the cells were cultured for 2–5 d for ion current recordings.

2.2. Collection of the venom

Adult female *O. hainana* spiders were kept in plastic pails covered with plastic net and given water daily. The venom was collected by using an electro-pulse stimulator descripted previously (Liang, 2004).

2.3. Electrophysiological recording

Whole-cell patch-clamp recordings were performed by an Axon 700B patch-clamp amplifier (Axon Instruments, Irvine, CA, USA) as described previously (Rong et al., 2011). Patch pipettes with DC resistance of 2–3 MΩ were fabricated from borosilicate glass tubing (VWR micropipettes; VWR Co., West Chester, PA, USA).

To record the action potentials (APs) from differentiated NRVMs, the Giga-Ohm seal was achieved under the voltage clamp mode and the APs were collected under the current clamp configuration using an Axon 700B patch-clamp amplifier (Axon Instruments, Irvine, CA, USA), Cardiomyocyte cells with spontaneous contractions were selected for recordings. Perforated patch was used to prolong recording stability. Pipette solution contained 120 mM KCl, 1 mM $MgCl_2$, 10 mM EGTA, 10 mM Hepes, and 3 mM MgATP at pH 7.2 adjusted with KOH. Amphotericin B (Sigma) at 500 μg/mL was included in the pipette solution. The extracellular buffer is the modified Tyrode's solution containing 140 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM Hepes, and 5.5 mM glucose at pH 7.4 adjusted with NaOH. Recordings were performed at 30 °C (Yu et al., 2013).

For I_{to1} recordings, $CdCl_2$ (200-μmol/L) were added in external solutions to block Ca^{2+}-currents. Na^+-current contamination was avoided by using a holding potential (HP) of −40 mV or by substitution of equimolar choline for external NaCl (Gomez et al., 2008). I_{to1} current was elicited by 300-ms depolarizing steps from a holding potential of −40 mV to potentials ranging from −50 mV to +100 mV in 10-mV increments.

To examine compound effects on native I_{Ks} currents, external Na^+ was replaced by equimolar choline (126 mM) and the solution was supplemented by 4-AP (5 mM), $BaCl_2$ (0.5 mM), $CdCl_2$ (0.2 mM), dofetilide (1 μM) and glibenclamide (1 μM) to suppress potential interference of I_{Na}, I_{to1}, I_{K1}, I_{CaL}, I_{Kr} and ATP-dependent K^+ channels (K_{ATP})., respectively (Yu et al., 2013). I_{Ks} current was defined as the chromanol 293B–sensitive (10 μM) current and was elicited by 3-s depolarizing steps from a holding potential of −50 mV to potentials ranging from −50 mV to +100 mV in 10-mV increments.

For I_{K1} currents recording, external Na^+ was replaced by equimolar choline (126 mM) and the solution was supplemented by 4-AP (5 mM), chromanol 293B–sensitive (10 μM), $CdCl_2$ (0.2 mM), dofetilide (1 μM) and glibenclamide (1 μM) to suppress potential interference of I_{Na}, I_{to1},