

Different Effects of Capsaicin on I_A and I_K in Pain-conduct Neurons of Rats*

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Summary: The different effects of capsaicin on I_A and I_K currents in pain-conduct neurons of trigeminal ganglia (TG) were investigated. In cultured TG neurons of rats, whole-cell patch clamp techniques were used to record the I_A and I_K before and after capsaicin perfused. Results revealed that 1 $\mu\text{mol/L}$ capsaicin could inhibit the amplitude of I_A by 48.2 % ($n=10$, $P<0.05$), but had no inhibitory effect on I_K ($n=7$, $P>0.05$). Ten $\mu\text{mol/L}$ capsaicin could significantly inhibit the amplitude of I_A by 93.2 % ($n=8$, $P<0.01$), but only slightly inhibit the amplitude of I_K by 13.2 % ($n=7$, $P<0.05$). Neither 1 $\mu\text{mol/L}$ nor 10 $\mu\text{mol/L}$ capsaicin had effects on the active curve of I_A and I_K . It was concluded that capsaicin could selectively inhibit the I_A current, and this effect might involve in the analgesic mechanisms of capsaicin.

Key words: capsaicin; pain-conduct neurons; I_A current; I_K current; rat

Capsaicin, the major ingredient in chili peppers and responsible for its pungent taste, is a specific activator of nociceptor on pain-conduct neurons^[1]. Now capsaicin and its analogue have been used in clinic as a new analgesic^[1, 2].

The mechanism of capsaicin on pain-conduct neurons hasn't been understood clearly now. The earlier work of our laboratory discovered that capsaicin could significantly inhibit the action potentials (APs) and the voltage-gated sodium channels (VGSCs) in pain-conduct neurons at low concentration (1 $\mu\text{mol/L}$)^[3]. The purpose of this study was to compare the different effects of capsaicin on I_A and I_K in pain-conduct neurons so as to understand the analgesic mechanism of capsaicin.

1 MATERIALS AND METHODS

1.1 Cell Culture

Trigeminal ganglia (TG) neurons from adult Sprague-Dawley (SD) rats were cultured according to the methods described previously^[3]. TG were dissected aseptically and collected in 0.1 % collagenase (Type XI-S) in F-14 medium. After incubation for 30–50 min at 37 °C, individual cells were dissociated by a fire-polished glass pipette, followed by a 10 min incubation at 37 °C in 10 $\mu\text{g/mL}$ DNase I in F-14 medium. After being washed 3 times with F-14 medium, the cells were cultured in DMEM/F-14 supplemented with 10 % fetal bovine serum. The cells were plated on poly-D-lysine and laminin-coated glass coverslips and cultured for 12–24 h at 37 °C in a water saturated atmosphere with 5 % CO_2 . All animals were provided by the

Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology (Certificate No. 19-020).

1.2 Patch-clamp Recording

The signal was measured using an Axopatch-200B patch-clamp amplifier, and the output was digitized with a Digidata 1322A converter (Axon Instruments). The sampling rate and filter rate were 10 kHz and 2 kHz, respectively. In the experiments, the neurons whose diameter ranges from 10 to 45 μm were chosen. The liquid-junction potential, capacitance and series resistance (≥ 90 %) were compensated. Recording was obtained 5 min after the rupturing of the membrane. The measurement of I_A and I_K : The holding potential was -80 mV, sequent with 250 ms depolarization pulses from -80 mV to $+60$ mV in 10 mV steps. The amplitude of I_A and I_K was defined as the maximum of the outward currents. All experiments were carried out at room temperature. The chamber's volume was 370 μL and was controlled by MPS-1 perfusion system (Yibo Instruments, Huazhong University of Science and Technology, China).

1.3 Drugs and Solution

To record I_A and I_K , the external solution was (in mmol/L): Choline-Cl 80, TEA-Cl 80, KCl 5, MgCl_2 1, CaCl_2 2, D-glucose 10, HEPES 10, CdCl_2 0.1, and Choline-Cl 137, KCl 5, MgCl_2 1, CaCl_2 2, D-glucose 10, HEPES 10, CdCl_2 0.1, 4-aminopyridine (4-AP) 3, respectively. PH was adjusted to 7.4 with 1 mmol/L NaOH. The pipette internal solution was (in mmol/L) K-aspartate 120, KCl 20, CaCl_2 1, MgCl_2 2, EGTA 10, HEPES 10, Tris-ATP 4, Tris-GTP 1, with the pH being adjusted to 7.3 with 1 mmol/L KOH.

Capsaicin and other chemicals including Choline-Cl, TEA-Cl, CdCl_2 , 4-AP, EGTA, Tris-ATP, Tris-GTP and collagenase, DNase I were

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purchased from Sigma Chemical (USA). Cell culture materials were purchased from GIBCO (Life Technologies, USA). Other reagents were of analytical grade.

1.4 Data Analysis

Data were analyzed using the Student's *t*-test, and expressed as $\bar{x} \pm s$. The active curve was fitted to the Boltzmann relation: $X = C + \{X_{\max} / [1 + \exp(V_{0.5} - V_m/k)]\}$, where $V_{0.5}$ was the membrane potential at which 50 % of activation was observed, *k* was the slope of the function^[3] and *C* was a constant (=0 in the active relation).

2 RESULTS

2.1 Effect of Capsaicin on I_A Current

Capsaicin could inhibit the I_A in a concentration-dependant manner (fig. 1): 1 $\mu\text{mol/L}$ capsaicin could inhibit the I_A by $(48.2 \pm 4.2) \%$ ($n=10$, $P<0.05$) and 10 $\mu\text{mol/L}$ capsaicin could inhibit the I_A by $(93.2 \pm 3.9) \%$ ($n=8$, $P<0.01$). After washing with the external solution for 3 min, the current partially recovered. The active curve of I_A (not shown) was fit to the Boltzmann equation and yielded the data shown in table 1. It was found that capsaicin had no effect on the active course of I_A .

Table 1 Effects of capsaicin on the amplitude and the active curve of I_A

Groups	I_A (nA)	$V_{0.5}$ (mV)	<i>k</i>
Control	7.9 ± 2.8	-8.1 ± 2.3	18.3 ± 1.7
1 $\mu\text{mol/L}$ capsaicin	$4.1 \pm 3.0^*$	-9.5 ± 2.1	18.1 ± 1.3
Control	8.5 ± 3.3	-7.2 ± 4.3	17.8 ± 1.6
10 $\mu\text{mol/L}$ capsaicin	$0.58 \pm 0.3^{**}$	-9.4 ± 2.7	17.9 ± 1.4

* $P<0.05$, ** $P<0.01$ as compared with control groups

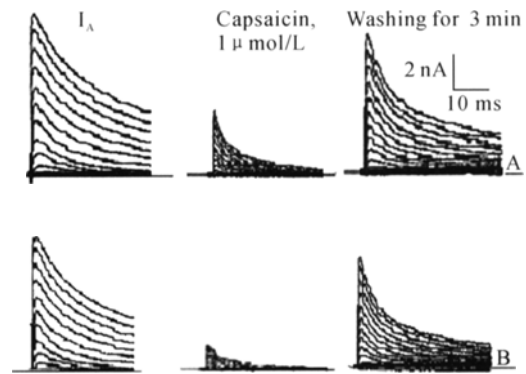


Fig. 1 Effects of capsaicin on I_A

A: Upon 1 $\mu\text{mol/L}$ capsaicin perfusion, the I_A current was reduced from its control value of 6.9 nA to 3.1 nA. After washing for 3 min, the I_A current recovered to 5.1 nA; B: Upon 10 $\mu\text{mol/L}$ capsaicin perfusion, the I_A current was reduced from 7.8 nA to 1.3 nA. After washing for 3 min, the I_A current recovered to 6.2 nA

2.2 Effect of Capsaicin on I_K

When perfusing with 1 $\mu\text{mol/L}$ capsaicin, the amplitude of I_K had no significant change ($n=7$, $P>0.05$). When perfusing with 10 $\mu\text{mol/L}$ capsaicin, the amplitude of I_K was reduced from 6.3 ± 3.3 to 5.5 ± 3.7 nA with the inhibitory rate being $(13.2 \pm 4.7) \%$ ($n=7$, $P<0.05$). After washing with the external solution for 3 min, the current partially recovered. The active curve of I_K current was fit to the Boltzmann equation and yielded the following parameters: $V_{0.5} = -4.9 \pm 1.8$ mV, $k = 17.8 \pm 0.5$ (precapsaicin); $V_{0.5} = -2.2 \pm 2.5$ mV, $k = 17.7 \pm 0.5$ (after 10 $\mu\text{mol/L}$ capsaicin), as same to I_A , capsaicin had no effect on the activation course of I_K (fig. 2).

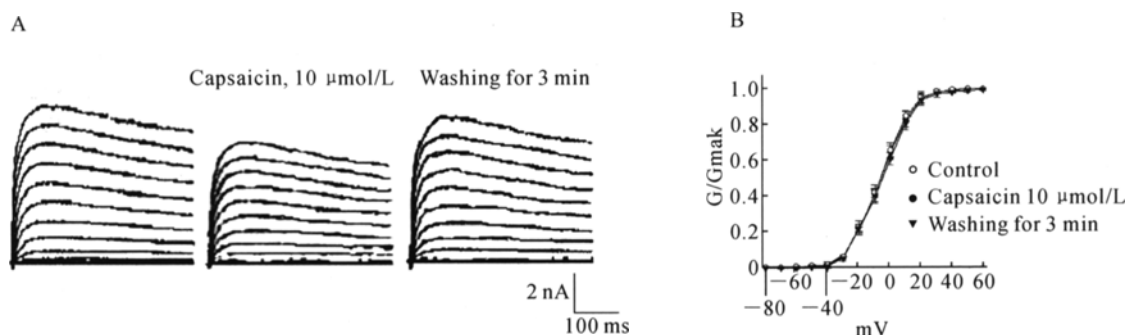


Fig. 2 Effects of capsaicin on I_K

A: 10 $\mu\text{mol/L}$ capsaicin decreased the I_K current from 8.2 nA to 6.7 nA. After washing for 3 min, the current partially recovered;

B: 10 $\mu\text{mol/L}$ capsaicin had no significant effect on the active curve of I_K

3 DISCUSSION

Capsaicin mainly acts selectively on medium and small neurons responsible for pain sense. On primary application to these pain-conduct neurons, capsaicin activates the pain-conduct neurons and acts as an irritant producing protective reactions,

however, when applied repeatedly or used for a long-term, it reversibly inhibits pain conduction and causes analgesic effect^[4]. It had been reported that the inhibitory effect of capsaicin on VGSCs might involve in the analgesic effect of capsaicin.

The duration and frequency of APs can be evidently affected by the voltage-gated potassium channels (VGPCs)^[5]. The pain-conduct neurons

contain several types of VGPCs, including two major types: I_A and I_K . It has been reported that capsaicin could inhibit VGPCs with different CD_{50} s in different animals and tissues, for example, in rat DRG neurons, capsaicin inhibited both I_A and I_K currents with a CD_{50} of $8 \mu\text{mol/L}$ ^[6], but in recording from cardiac muscle, capsaicin blocked I_K with a CD_{50} of $11.5 \mu\text{mol/L}$ ^[7].

In our study, it was found that $1 \mu\text{mol/L}$ capsaicin could selectively inhibit I_A but had no special inhibitory effect on I_K . Only at high concentration ($10 \mu\text{mol/L}$), the I_K was slightly inhibited (13.2%). And the active curve of I_A and I_K were not affected by $1 \mu\text{mol/L}$ or $10 \mu\text{mol/L}$ capsaicin, suggesting the opening course of the channel was not affected.

The I_A was the main outward current in the early phase of the repolarization, capsaicin could inhibit the repolarization, elongate the duration of APs and decrease the frequency of APs through its effect on I_A . So we thought that the inhibitory effect of capsaicin on I_A might involve in the anal-

gesic effect of capsaicin.

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pine-enhanced reperfusion arrhythmia. On the other hand, atropine partially suppressed the role of EA.

The above data indicate that the mechanism of EA resistance reperfusion arrhythmia involved the regulation of β -adrenoceptor and M-receptor activation for maintaining the balance of the sympathetic and parasympathetic system.

It has long been recognized that the benefits using anesthesia of EA for operative patients, particularly for old, weak patients. In our studies we also found that EA can reduced the impairment of ultrastructure after reperfusion in rats with electron microscope (data not shown). The introduction and cure effects of EA before and in ischemia elevated the heart's tolerance to reperfusion damage, thus can enhance the safety in surgery and thrombolytic treatment. EA could be an adjunctive measure of cardiovascular intervention therapies.

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