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# Effect of Capsaicin on Delayed Rectifier $K^+$ Current in Adult Rat Dorsal Root Ganglion Neurons

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$K^+$ currents play multiple roles in the excitability of dorsal root ganglion (DRG) neurons. Influences on these currents change the shape of the action potential, its firing threshold and the resting membrane potential. In this study, whole cell configuration of patch clamp technique had been applied to record the blocking effect of capsaicin, a lipophilic alkaloid, on the delayed rectifier $K^+$ current in cultured small diameter DRG neurons of adult rat. Capsaicin reduced the amplitude of $K^+$ current in dose dependent manner, and the concentration-dependence curve was well described by the Hill equation with $K_D$ value of 19.1 $\mu$M. The blocking effect of capsaicin was reversible. Capsaicin (10 $\mu$M) shifted the steady-state inactivation curve in the hyperpolarizing direction by about 15 mV and increased the rate of inactivation. The voltage dependence of activation was not affected by capsaicin. These multiple effects of capsaicin may suggest that capsaicin bind to the region of $K^+$ channel, participating in inactivation process.

Key Words: Capsaicin, $K^+$ current, Dorsal root ganglion, Patch clamp

## INTRODUCTION

Dorsal root ganglion (DRG) neurons can be divided into several groups on the basis of their size, morphology, function, peripheral nerve conduction velocity and duration of somatic action potential (Harper et al, 1985a, b). It is thought that these properties are determined by the set of ionic channels specifically expressed in the DRG neurons of each group. The $K^+$ currents of mammalian sensory neurons have been described in various preparation, such as neonatal/adult cells (Kostyuk et al, 1981; Akins et al, 1993) and large/small diameter cells (Gold et al, 1996; Safronov et al, 1996). Some of these voltage-dependent $K^+$ currents are specifically expressed in a small diameter DRG neuron (Safronov et al, 1996), which is known to conduct pain, suggesting that these $K^+$ currents may participate in the determination of response properties of nociceptive neurons. Adult rat DRG neurons heterogeneously express transient, delayed rectifier $K^+$ currents and $Ca^{2+}$-dependent $K^+$ current (Akins et al, 1993). Among these currents the delayed rectifier $K^+$ current determines the threshold for action potential firing, contributes to the repolarizing phase of the action potential, sets the resting potential in small DRG neurons (Safronov et al, 1996). Therefore, modulation of delayed rectifier $K^+$ current of sensory neurons would have important functional implications.

Capsaicin is the active component of hot peppers, *Capsicum*. It is useful for the study of pain and analgesia because it specifically excites subset of C-type sensory neurons in mammals (Bevan et al, 1990). When applied in low micromolar concentration capsaicin increases the permeability of the plasma membrane to cation by binding to specific receptor, vanilloid receptor (Caterina et al, 1997), and consequently depolarizes DRG cells (Bevan et al, 1990). However, at higher concentration capsaicin affects many different kinds of voltage-dependent currents from various cells. Capsaicin blocks $Na^+$ current as well as fast-inactivating $K^+$ current in DRG neurons of guinea-pig and chicken (Petersen et al, 1987),

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fast-inactivating $K^+$ current in the frog node of Ranvier (Dubois, 1982) and the voltage-dependent $Ca^{2+}$ currents in sensory neurons (Bleakman et al, 1990; Docherty et al, 1991). However, the effect of capsaicin on the delayed rectifier $K^+$ current of small diameter DRG neuron is not known.

In this study we report that micromolar concentration of capsaicin blocks delayed rectifier $K^+$ current in cultured small diameter DRG neurons of adult rat, and that capsaicin may change the intrinsic inactivation process of the channel.

## METHODS

### *Preparation of cells*

Dissociated DRG neurons from 3 weeks-old Sprague-Dawley rats were prepared by enzymatic and mechanical dissociation. Rats were rapidly decapitated and DRG neurons from all levels of thoracic spinal cord were carefully collected and half dissected in sterile cold PBS (phosphate buffer saline; Sigma). DRG were washed with culture media, and incubated for 15 min in warm (37°C) culture media containing 0.125% collagenase (Worthington type II). The culture medium was a mixture of Dulbecco's Modified Eagle Media (DMEM; Sigma) and F-12 solution (Sigma) with 10% FBS (fetal bovine serum; Gibco). The DRG were washed twice with PBS. The DRG were incubated in the warm PBS (37°C) containing 0.25% trypsin (Sigma type IX) and shaken gently for 10 min. They were then transferred to the culture media containing 10% FBS and 100 μg/ml DNase (Boehringer DNase I). Cells were isolated by gentle triturating of the ganglia using fire polished Pasteur pipettes with progressively finer tips. Isolated single cells were washed with the culture media containing 10% FBS, 100 ng/ml nerve growth factor (NGF 7s) and plated on poly-L-lysine coated glass coverslips. Cells were placed in a 37°C incubator and gassed with 5% $CO_2$ atmosphere.

### *Electrophysiology*

Experiments were performed on dissociated cell 6~48 hrs after plating on coverslips. Gigaseal was formed with borosilicate glass pipette (TW150F-4, World Precision Instrument, Inc). Patch pipettes were prepared a micropipette puller (Type PP-83, Narishige). The pipettes were fire-polished and had a final resistance of 3~5 MΩ. Whole-cell currents were recorded using a patch-clamp technique with an Axopatch 200B amplifier and digidata 1200B interface. To obtain whole-cell configuration, cell attached patches were formed, and the cell membrane under the patch pipette was ruptured by gentle suction. After forming the whole-cell configuration, the capacitive transients were canceled and series resistance compensation (at least 70%) was routinely employed at the beginning of each recording. The recordings were obtained from DRG neurons with less than 25 μm diameter. Data acquisition and analysis were performed using pClamp 6.0. All experiments were done at room temperature (20~23°C).

### *Solutions*

For whole-cell studies, the two types of bath solution were used. Normal external solution contained (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.3). $MnCl_2$ containing external solution was prepared by replacing $CaCl_2$. The pipette solution contained 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES, and 3 $Na_2ATP$ (pH 7.3). Capsaicin (LC Laboratory) was dissolved in absolute ethanol.

### *Statistical analysis*

Data were expressed as mean standard error with n representing the number of patches. Student's *t* test was used and $p<0.05$ was considered to be statistically significant for the test.

## RESULTS

In order to eliminate $Ca^{2+}$-dependent component from voltage-dependent $K^+$ current, $CaCl_2$ was replaced by $MnCl_2$ in all of external solutions (Kim et al, 1998). From the fact that 100 pM charybdotoxin did not block the outward $K^+$ current in $Ca^{2+}$-free, $Mn^{2+}$-containing external solution, we confirmed that the use of $Mn^{2+}$ containing external solutions effectively eliminated $Ca^{2+}$-dependent $K^+$ currents (data not shown). The recordings were obtained from small DRG neurons with less than 25 μm diameter.

The depolarizing voltage pulses from −70 mV to +50 mV activated large outward currents. Fig. 1A

shows that outward $K^+$ current is reduced by the addition of 10 $\mu$M and 30 $\mu$M capsaicin to the external solution. Because capsaicin evoked inward currents through non-selective cation channel (Bevan et al, 1990), small change in holding current was observed in Fig. 1A. Fig. 1B shows the time dependent blocking effect of 10 $\mu$M capsaicin. We measured the current levels at 300 ms after the start of voltage pulses to exclude the fast-inactivating component (Kim et al, 1998). Each point represents the current level at every 15 s. The blocking effect of capsaicin developed rapidly, and was reversible as shown in the Fig. 1B. The degree of recovery from block was 94.1±2.7% (n=5) at +30 mV. The concentration-dependence of capsaicin block of $K^+$ current is shown in Fig. 1C. The current amplitudes were normalized compared to the control current, and presented as % values. The smooth line in the figure represents the best fit of Hill equation in which the coefficient is 1.14 and $K_D$ is 19.1 $\mu$M. This affinity of capsaicin is similar to that of delayed rectifier $K^+$ currents in melanotrophs of the rat pituitary (17.4 $\mu$M) (Kehl, 1994) and *Xenopus* embryonic spinal neurons (21 $\mu$M) (Kuezi et al, 1996), but lower than that of $K^+$ currents in large DRG neurons of adult rat (8 $\mu$M) (Akins, 1993). The Hill coefficient suggests that at least one capsaicin molecule bind to the channel to exert its effect. The blocking effect of capsaicin was voltage-independent. At any different voltage pulses, capsaicin reduced the current by the same extent. For example, the remaining current after the treatment of 10 $\mu$M capsaicin was 67.5±3.5% (n=6) at 0 mV and 62.5±3.8% (n=6) at +50 mV of control currents. The remaining current with 30 $\mu$M capsaicin was 38.0±2.1% (n=6) and 33.3±2.3% (n=6) at 0 mV and +50 mV, respectively.

Upon closer inspection of Fig. 1A, we found that capsaicin not only reduced the current amplitude but it produced an apparent 'fast inactivation' of the current. The rate of this fast inactivation increased with increasing concentrations of capsaicin (10, 30 $\mu$M) as reported before (Dubois, 1982; Kuenzi et al,

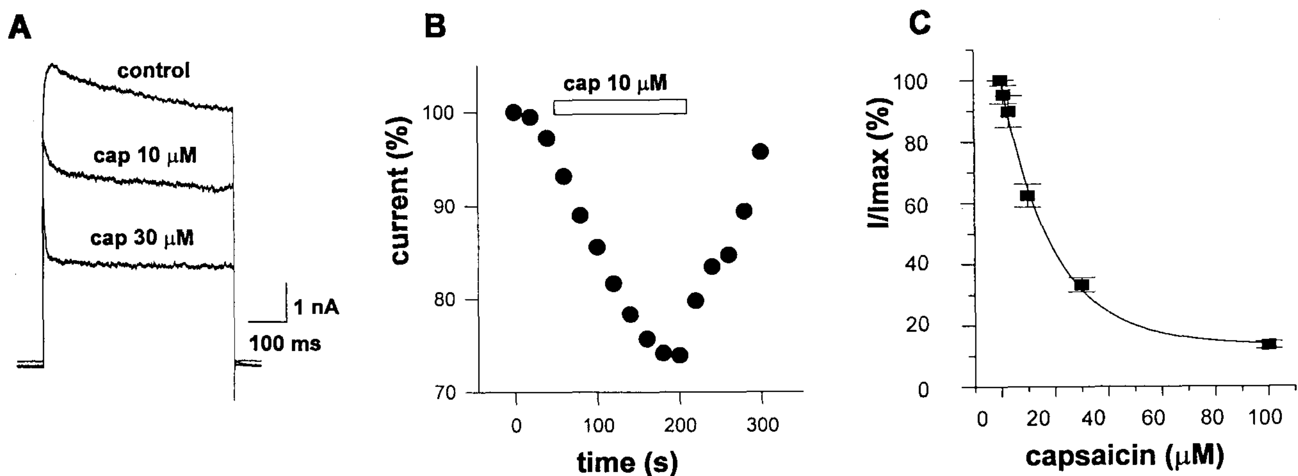


**Fig. 1.** Capsaicin blocks delayed rectifier $K^+$ currents. (A) Block of $K^+$ currents by 10 $\mu$M, and 30 $\mu$M capsaicin. The amplitude of the outward $K^+$ current decreased and the rate of inactivation was accelerated. Whole cell $K^+$ current was evoked by depolarization from a holding potential of −70 mV to +50 mV. (B) Time dependent change of current amplitude by capsaicin. Open box indicates superfusion with 10 $\mu$M capsaicin. Outward $K^+$ currents were evoked by voltage pulses to +30 mV from a holding potential of −70 mV, applied every 15's and measured at 300 ms after the start of voltage pulses. (C) Concentration dependence of capsaicin block on $K^+$ current. The data were pooled from 6 different cells (mean±SE). The currents were evoked by step depolarization to +50 mV from a holding potential of −70 mV and the normalized current was defined as the percentage of the steady-state current in the presence of capsaicin to that of control ($I_{capsaicin}/I_{control}$). The solid line is the best fit with the Hill equation:

$$\frac{I_{capsaicin}}{I_{control}} = \frac{1}{1+([\text{capsaicin}])/K_D)^H}$$

The Hill coefficient (*H*) is 1.14 and the $K_D$ is 19.1 $\mu$M.

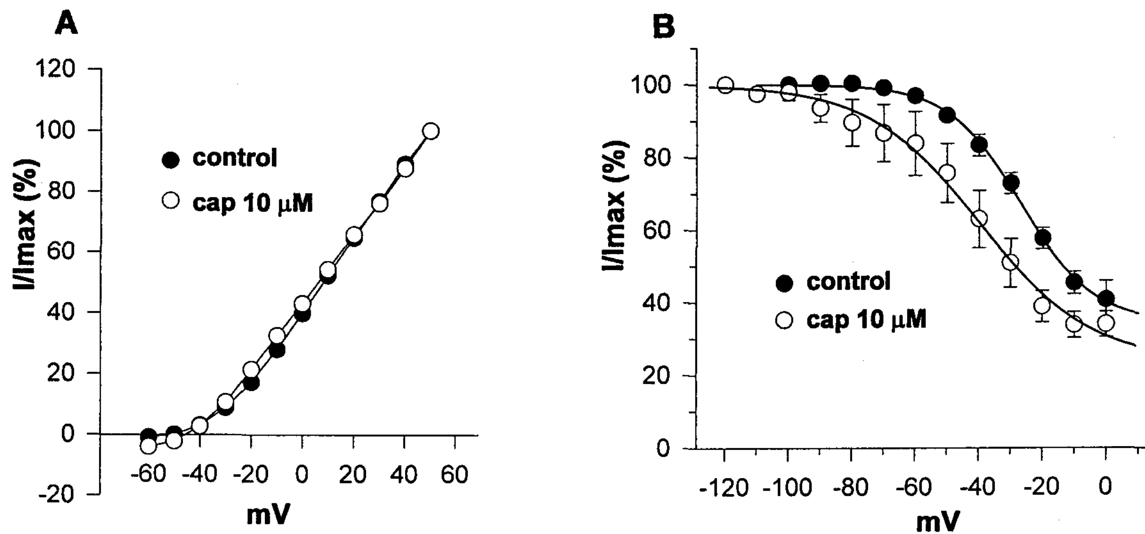


**Fig. 2.** Capsaicin changes the steady-state inactivation curve but not the activation curve of delayed rectifier $K^+$ current. (A) The activation curve of $K^+$ current. Membrane was held at $-70$ mV. Currents were evoked by voltage steps from $-60$ mV to $+50$ mV given every 30's interval. The outward $K^+$ currents in control (●) and 10 $\mu$M capsaicin (○) were measured at 300 ms after the start of test pulse and then normalized by maximum current (n=5, mean±SE). The standard error bars were smaller than the symbols. (B) The steady-state inactivation curve of $K^+$ current. The steady-state inactivation curves at control (●) and 10 $\mu$M capsaicin (○) were determined using a double-pulse protocol. The holding potential was $-120$ mV, voltage pulses were applied for 30's between $-120$ mV and 0 mV in 10 mV increment and then test pulse were applied to 0 mV for 500 ms. The resultant current amplitudes were fit by a Boltzmann function

$$I= \frac{\{I_{max}-(I_{max}\times c)\}}{1+\exp\{V_{1/2}-V_m)/k\}}+(I_{max}\times c)$$

where I is the observed current, $I_{max}$ is the maximal current, $V_{1/2}$ is the membrane potential for half-inactivation, $V_m$ is the holding potential, $k$ is the slope factor, and $c$ is a the fraction of non-inactivating current (n=5, mean±SE).

1996). A 100 ms pre-pulse, to $-30$ mV, which was routinely used to eliminate the fast-inactivating $K^+$ current (Gold et al, 1996), did not removed the 'inactivating' component induced by capsaicin (data not shown), indicating that the new component was different from the fast-inactivating $K^+$ current.

To characterize the capsaicin block of delayed rectifier $K^+$ currents, its effect on the activation and steady-state inactivation was examined. The activation curves in the absence and presence of 10 $\mu$M capsaicin were almost identical (Fig. 2A), suggesting that capsaicin does not affect the voltage-dependence of channel activation. However, upon the addition of 10 $\mu$M capsaicin the steady-state inactivation curve was shifted to hyperpolarizing direction by about 15 mV compared to the control as shown in Fig. 2B.

## DISCUSSION

Primary afferents are functionally diverse population of neurons that transduce and encode a variety of stimuli. Some of this diversity may reflect the differential distribution of voltage-dependent $K^+$ currents, which play an integral role in the regulation of a number of neuronal response properties including spike repolarization, interspike interval and burst adaptation (Rudy, 1988). Adult rat DRG neurons express fast-inactivating and delayed rectifier $K^+$ currents. A $Ca^{2+}$-dependent $K^+$ current was also known to be present (Akins et al, 1993). Furthermore, five types of $K^+$ currents, one type of fast inactivating A current and four types of delayed rectifier $K^+$ currents, have been identified under voltage-gated $K^+$ conductance in small DRG neurons. Four types of delayed rectifier $K^+$ channels were found at

a high density. These channels activated in the same voltage range between 60 mV and 10 mV (Safronov et al, 1996). Moreover, $I_{aht}$, $I_{as}$ and $I_{Ki}$ appear to be selectively expressed in neurons with a small cell body diameter. They are potential targets of modulation by hyperalgesic inflammatory mediators or analgesics and may serve to dynamically regulate nociceptor function (Gold et al, 1996).

The effects of capsaicin on the outward $K^+$ currents were observed in small diameter DRG neurons investigated in this study. These results show that capsaicin may affect the delayed rectifier $K^+$ current of rat small DRG neurons in three different ways: (1) it decreased the maximum current, (2) induced 'inactivation', and (3) shifted the steady-state inactivation curve to the left. Capsaicin may block homogenous population of $K^+$ current from many different types of delayed rectifier $K^+$ currents in DRG neurons (Kostyuk et al, 1981; Akins et al, 1993), or it may block all types of the delayed rectifier $K^+$ currents with similar potency. Although a similar effect of capsaicin on $K^+$ currents has been reported in other preparations (Dubois et al, 1982; Baker et al, 1994; Kuenzi et al, 1996) there has not been a detailed analysis of the mechanism of action of capsaicin.

There are two general mechanisms by which capsaicin can produce channel blocking. First, it may occlude the pore by binding to a site that is only exposed when the channel is in the open conformation. In melanotrophs of the rat pituitary, capsaicin acts as an open channel blocker (Kehl, 1994). Second, it may bind to the channel and allosterically increase the rate of intrinsic inactivation. Interestingly, verapamil, which is structurally similar to capsaicin, blocks $Ca^{2+}$ channels by binding to a site that includes the S6 region (Striessing et al, 1990), and it also induces rapidly inactivating component from delayed rectifier $K^+$ current without effect on activation (Rampe et al, 1993). Since $K^+$ channels and $Ca^{2+}$ channels share S6 region, it is proposed that capsaicin may bind at or near the S6 region, where it may cause an increase in the rate of inactivation (Kuenzi et al, 1996). If capsaicin were to act by affecting the inactivation state of the channel, it should shift the steady-state inactivation curve in a hyperpolarizing direction.

Our results show that capsaicin reduces the current amplitude and causes the left shift of steady-state inactivation curve as well as the increase in the inactivation rate of delayed rectifier $K^+$ current from small DRG neurons. The blockade of $K^+$ currents would explain the depolarization and repetitive firing of the majority of cultured sensory neurons of neonatal rats (Baccaglini et al, 1983) and the depolarization of small and big neurons in in vitro DRG preparations (Williams et al, 1982) observed after superfusion with capsaicin. These results suggest that capsaicin can excite sensory neuron through not only activation of the vanilloid receptor but inhibition of the delayed rectifier $K^+$ current.

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