Published in final edited form as:

Cell Mol Neurobiol. 2014 May ; 34(4): 565–576. doi:10.1007/s10571-014-0041-1.

Inhibitory effects of capsaicin on voltage-gated potassium channels by TRPV1-independent pathway

Rong Yang,

Department of Physiology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Department of Physiology, Medical College, Jiangnan University, Wuhan 430056, China

Zhe Xiong,

Department of Physiology, Medical College, Jiangnan University, Wuhan 430056, China

Changjin Liu, and

Department of Physiology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Lieju Liu

Department of Physiology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Department of Anesthesiology, Duke University, Durham, NC 27708, USA

Abstract

Previously we observed that capsaicin, a transient receptor potential vanilloid 1 (TRPV1) receptor activator, inhibited transient potassium current (I_A) in capsaicin-sensitive (CS) and capsaicin-insensitive (CIS) trigeminal ganglion (TG) neurons from rats. It suggested that the inhibitory effects of capsaicin on I_A have two different mechanisms: TRPV1-dependent and TRPV1-independent pathways. The main purpose of this study is to further investigate the TRPV1 independent effects of capsaicin on voltage-gated potassium channels (VGPCs). Whole cell patch clamp technique was used to record I_A and sustained potassium current (I_K) in cultured TG neurons from *trpv1* knockout (TRPV1^{-/-}) mice. We found that capsaicin reversibly inhibited I_A and I_K in a dose-dependent manner. Capsaicin (30 μ M) did not alter the activation curve of I_A and I_K but shifted the inactivation-voltage curve to hyperpolarizing direction, thereby increasing the number of inactivated VGPCs at the resting potential. Administration of high concentrations capsaicin, no use-dependent block and delay of recovery time course were found on I_K and I_A . Moreover, forskolin, an adenylate cyclase agonist, selectively decreased the inhibitory effects of I_K by capsaicin, whereas no influenced the inhibitions of I_A . These results suggest that capsaicin inhibits the VGPCs through TRPV1-independent and PKA-dependent mechanisms, which may contribute to the capsaicin-induced nociception.

Keywords

capsaicin; voltage-gated potassium channels; trigeminal ganglion; TRPV1

Introduction

Voltage-gated potassium channels (VGPCs) are important physiological regulators of membrane potentials. They are widely expressed in excitable tissues including sensory ganglia. Patch-clamp studies in trigeminal ganglion (TG) neurons have identified two types of potassium currents, a transient current (I_A) and a sustained current (I_K) (Liu and Simon 2003). Recently VGPCs have been received extensive attention as drug targets for the treatment of a variety of pain, such as neuropathic pain and inflammatory pain (Brederson et al. 2013; Takeda et al. 2006; Xu et al. 2006; Scultoreanu et al. 2004; Malykhina et al. 2013; Hakim et al. 2009; Stewart et al. 2003; Nie et al. 2009), due to their essential role in stabilizing the resting membrane potential and controlling the frequency of nociceptor firing (Maljevic and Lerche 2013).

Capsaicin, a major pungent constituent of red pepper, is used in various medicinal treatments to alleviate pain, but its initial application causes pain and inflammation by activating and sensitizing nociceptors (Calixto et al. 2005; Palazzo et al. 2012; Premkumar and Abooj 2013; Fischer et al. 2013). It is generally accepted that the initial painful sensation after capsaicin application arises from the selective activation of TRPV1 (transient receptor potential ion channel of vanilloid subtype-1), which is mainly expressed in sensory neurons. It is reported that the inhibition of I_A by inflammatory mediators increases the excitability of nociceptor and leads to pain and hyperalgesia (Liu et al. 2001; Jeub et al. 2011). To determine whether the VGPCs might contribute to the pain induced by capsaicin, we investigated how capsaicin modulated I_A in rat TG neurons previously. We found that low concentration of Capsaicin blocked I_A mainly in the capsaicin-sensitive neurons, while at high concentration ($> 10 \mu\text{M}$), and the block of capsaicin on I_A exists in both CS and capsaicin-insensitive (CIS) neurons. The results show that there are two mechanisms, TRPV1 dependent and TRPV1 independent effects that may involve in the modulation of capsaicin on I_A . Moreover, mounting evidence also suggests that capsaicin exert its effects through TRPV1 at sub-micromolar concentrations. However, capsaicin at micro- to millimolar concentrations seem to have effects beyond TRPV1-dependent actions (Lundbaek et al. 2005; Boudaka et al. 2007; Hibino et al. 2011; Cao et al. 2007). In accordance with this, it has been shown the inhibitory effects of capsaicin on I_A , even at $30 \mu\text{M}$, were small relative to CS neurons at $1 \mu\text{M}$ capsaicin in CIS neurons; this further indicated that the inhibition of TRPV1 independent pathway occurred at concentrations much larger than the inhibition of TRPV1 dependent (Liu and Simon 2003). In TRPV1 positive nociceptors, the initial painful sensation after capsaicin application arouses from the inhibition of I_A via TRPV1. However, little has been documented regarding the effects of the capsaicin and its analogues producing pain in TRPV1 negative nociceptors. Thus, the aim of this study is to further explore the TRPV1 independent effects of capsaicin on VGPCs in the primary sensory neurons by using *trpv1* knock-out mice ($\text{TRPV1}^{-/-}$). Our

findings have implications for understanding a TRPV1-independent mechanism for initial hyperalgesia effects of capsaicin in animals and human beings when TRPV1 is absent.

Materials and methods

Cell culture

TG neurons from C57BL6 wild type and TRPV1 knock-out mice (Caterina et al. 2000), the generous gift from Dr David Julius, were cultured using the similar method for adult rats (Liu and Simon 2003). TG were dissected aseptically and collected in Hank's Balanced Salt Solution (HBSS). After washing three times with HBSS, the ganglia were diced into small pieces and incubated for 30 – 50 min at 37°C in 0.1% collagens (Type XI-s, Sigma) in HBSS. Individual cells were dissociated by triturating the tissue through a fire-polished glass pipette, followed by 10 min incubation at 37°C in 10 µg/ml DNase I in F-12 medium (Life Technologies, Gaithersburg, MD). After washing three times with F-12 medium, the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. The cells were plated on poly-D-lysine coated glass coverslips (15 mm diameter) and cultured for 12 – 24 h at 37 °C in a water saturated atmosphere with 5% CO₂. At the beginning of each experiment, neurons were placed for 10 min in a chamber containing the external solution for measuring I_A or I_K.

Care of animals conformed to standards established by the National Institutes of Health. All efforts were made to minimize animal suffering, and to reduce the number of animals used. All animal protocols were approved by the Duke University Institutional Animal Care and Use Committee.

Patch clamp recording

In this experiment, we used glass pipettes (R-6 borosilicate, Drummond Scientific Company, Broomall, PA.) with resistances 1.5 MΩ according to our previous study (Xiong et al. 2013). The pipette solution contained (in mM) 116 KF, 20 KCl, 1.0 CaCl₂, 2.0 MgCl₂, 10 EGTA, 10 HEPES, and 5 Tris-ATP, adjusted to pH 7.3 for both I_A and I_K. Neurons having projected soma diameters ranging between 18–25 µm were used.

The signals were measured using an Axopatch-1D patch clamp amplifier (Axon instruments, Foster City, CA) and the amplifier output was digitized with a Digidata 1322A converter (Axon instruments). The sampling rate was 10 kHz. The capacitance and series resistance was compensated, the latter by about 90%. The leak current was subtracted from the potassium currents using Clampfit programs. All experiments were carried out at room temperature (22 – 25 °C).

I_A were obtained from the total outward current using tetraethyl ammonium chloride (TEA-Cl) to block I_K; I_K were obtained from the total outward current using 4-aminopyridine (4-AP) to block I_A (Liu et al. 2004). The voltage-dependent activation curve (G–V curve) was measured by a series of depolarizing pulses (500 ms) from –80 mV to +40 mV stepping by 10 mV with interval time of 2 s. The voltage-dependent inactivation curve (inactivation–voltage curve) was measured by double pulses: precondition pulses (400 ms) ranging from –100 mV to +40 mV by stepping 10 mV and following +40 mV test pulse (400 ms) with

internal of 4 s. Use-dependent protocol was composed of 16 continually depolarizing pulses (+40 mV) with duration of 500 ms at different frequency from 0.5 to 1.7 Hz. The recovery time was measured by double pulses, first condition pulse of +40 mV (500 ms) caused the current completed inactivation, as the interval time was increased (from 0.002 to 2 s), and the current evoked by +40 mV test pulse (500 ms) recovered gradually. For all experiments the holding potential was -80 mV.

Drugs and solution

Cell culture materials were purchased from GIBCO (Life Technologies, Rockville, MD). Capsaicin and capsazepine were obtained from RBI (Natick, MA), unless otherwise stated, all other chemicals were purchased from Sigma (St. Louis, MO). Capsaicin was prepared as stock solutions in DMSO. The final concentration of DMSO in external solution was 0.5%. No detectable effect of the vehicles was found in our experiments. The recording chamber had a volume of 360 μ l and was perfused with the external solution at a rate of 3 ml/min. External solution for I_A contained (in mM): choline-Cl, 80; KCl, 5.0; $CaCl_2$, 2.0; $MgCl_2$, 1.0; HEPES, 10; d-glucose, 10; TEA-Cl, 80; $CdCl_2$, 1; for I_K contained: choline-Cl, 137; KCl, 5.0; $CaCl_2$, 2.0; $MgCl_2$, 1.0; HEPES, 10; d-glucose, 10; 4-AP, 3.0; $CdCl_2$, 1.

Data analysis

Data were analyzed and fitted using Clampfit (Axon instruments, Foster City, CA) and Sigmaplot (SPSS inc., Chicago IL) software. Dose-response curve was fitted by a modified Hill equation, which $Y = Y_0 + A \text{Cap}^n / (K_{1/2}^n + \text{Cap}^n)$, with $K_{1/2}$ as the concentration producing 50% effect, n as the cooperative index and where the constants $A + Y_0 = 1$. G-V curve and inactivation curves (h-infinity curve) were fitted by Boltzmann functions, which $G = (G_{\max} - G_{\max} * a) / (1 + \exp((V_{0.5} - V_m) / k)) + (G_{\max} * a)$, where $V_{0.5}$ is the membrane potential (V_m) at which 50% of activation or inactivation was observed, k is the slope of the function and a is a constant ($a = 0$ in the G-V relation). Both the use dependent curve and the recovery time were fitted by single exponential function: $Y = Y_0 + a(1 - e^{-bx})$, where rate constancy b was represented by TAU. I_A and I_K were measured at the peak outward current (I_p). All of data were presented as mean $\pm$ SEM and the significance was indicated as $P < 0.05$ and $P < 0.01$ tested by paired or unpaired Student's t tests.

Results

Blockage of capsaicin on VGPCs in the TRPV1^{+/+} mice

As shown in our previous experiment, in cultured rat TG neurons, capsaicin had different effects on I_A in CS and CIS neurons. I_A was reduced about 49% in CS neuron by applying 1 μ M capsaicin for 3 min, while only 9% were inhibited in CIS neurons (Liu and Simon 2003). Similar results were found in the present experiments using TRPV1^{+/+} mice. After exposure to 1 μ M capsaicin for 3 min, I_A and I_K were reduced by $52.3 \pm 17.2\%$ ($n = 7$) and $43.4 \pm 16.9\%$ ($n = 6$) in CS neurons respectively, while only decreased by $7.2 \pm 1.3\%$ ($n = 6$) and $8.2 \pm 1.5\%$ ($n = 8$) in CIS neurons respectively. The inhibitory effects of 1 μ M capsaicin on I_A and I_K were reversed by capsazepine (a TRPV1 receptor antagonist) in CS neurons. A classical case was shown in Fig. 1.

Capsaicin reduced I_A and I_K in TRPV1^{-/-} mice

Since the TRPV1 dependent inhibitory effects of capsaicin on I_A in CS neurons had been examined in our previous study (Liu and Simon 2003), here we focused on studying the TRPV1 independent effects of capsaicin on VGPCs using TG neurons from TRPV1^{-/-} mice. In TRPV1^{-/-} mice, the effects of capsaicin on I_A were dose-dependent and reversible, as shown in Fig. 2a, in the presence of 1, 10, 30, 100 μ M capsaicin, I_A were reduced by $2.7 \pm 6.6\%$ ($n = 6$), $29.7 \pm 11.9\%$ ($n = 13$), $57.5 \pm 14.5\%$ ($n = 7$) and $81.6 \pm 8.8\%$ ($n = 7$) respectively. The dose-response curve was fitted to modified Hill equation with $K_{1/2}$ value of 21 μ M. 30 μ M capsaicin had no markedly effects on the G-V curve of I_A (control: $V_{0.5} = -3.1 \pm 2.2$ mV, $k = 18.0 \pm 0.6$, $n = 11$; capsaicin: $V_{0.5} = -3.9 \pm 1.8$ mV, $k = 19.8 \pm 0.7$, $n = 12$, $P > 0.05$) (Fig. 2b), while significantly shifted the inactivation curve to hyperpolarizing potential (Control: $V_{0.5} = -41.6 \pm 3.2$ mV, $k = -7.3 \pm 0.8$, $n = 10$; capsaicin: $V_{0.5} = -58.8 \pm 3.1$ mV, $k = -8.2 \pm 0.9$, $n = 9$; for $V_{0.5}$, $P < 0.05$) (Fig. 2c).

The response of I_K to capsaicin was presented in Fig. 3. The effects of capsaicin on I_K also were dose-dependent and reversible. In the presence of 1, 10, 30 and 100 μ M capsaicin, the amplitude of I_K was reduced by $9.4 \pm 4.8\%$ ($n = 5$), $33.1 \pm 7.6\%$ ($n = 9$), $55.1 \pm 6.4\%$ ($n = 8$) and $80.5 \pm 7.2\%$ ($n = 8$) respectively with $K_{1/2}$ value of 23 μ M. 30 μ M capsaicin did not significantly alter the G-V parameters of I_K (Control: $V_{0.5} = -5.2 \pm 2.7$ mV, $k = 17.3 \pm 0.8$, $n = 9$; capsaicin: $V_{0.5} = -5.8 \pm 3.1$ mV, $k = 17.0 \pm 0.9$, $n = 8$; for $V_{0.5}$, $P > 0.05$), but shifted the inactivation curve to hyperpolarizing direction (Control: $V_{0.5} = -72.99 \pm 1.48$ mV, $k = -9.6 \pm 1.28$, $n = 9$; capsaicin: $V_{0.5} = -83.00 \pm 1.22$ mV, $k = -15.19 \pm 1.22$, $n = 12$; for $V_{0.5}$, $P < 0.05$).

Use-dependent block of capsaicin on I_A and I_K in TRPV1^{-/-} mice

The use-dependent block was measured by a series depolarizing pulses at different frequencies. Even in the normal solution, the reduction of I_A became stronger with frequency of stimulation increasing from 0.5 to 1.7 Hz. This reduction could be explained by the slow recovery of I_A from the inactivation state. When exposed to 30 μ M capsaicin, the amplitude of I_A was decreased in all stimulative frequencies (tonic block) but no use-dependent block was found even at 1.7 Hz stimuli (Fig. 4). The amplitude of currents evoked by the n th impulse was normalized to the current evoked by the first impulse. Like I_A , no use-dependent block was found for I_K in the presence of capsaicin either (Fig. 5).

Effects of capsaicin on the I_A and I_K recovery time in TRPV1^{-/-} mice

To explore the modulation of capsaicin on the transition of the VGPCs from the inactivated state to the resting state, the recovery time course was measured in the absence and presence of capsaicin. TG neurons were stimulated with a double-pulses protocol with intervals varying between 2 ms and 2 s. In the presence of 30 μ M capsaicin, the amplitude of I_A and I_K were reduced (Fig. 6a), but the recovery time (TAU) course of I_A (Control: TAU = 0.007 ± 0.002 ms, $n = 9$; Capsaicin: TAU = 0.006 ± 0.001 ms, $n = 7$, $P > 0.05$) and I_K ($n = 9$) did not significantly change (Fig. 6b).

Role of forskolin in capsaicin-mediated inhibition of VGPCs in TRPV1^{-/-} mice

It is known that VGPCs are modulated by a variety of intracellular second messengers. It is reported that capsaicin (100 μ M) attenuates transepithelial currents in human airway epithelial Calu-3 cells through TRPV1-independent and cAMP-dependent mechanisms (Hibino et al. 2011). To address whether the PKA pathway contributed to capsaicin-mediated inhibition of VGPCs in TRPV1^{-/-} TG neurons, we studied the effects of capsaicin in the presence of forskolin, an adenylate cyclase agonist. After pre-application of 10 μ M forskolin for 5 min, I_K and I_A were decreased by 8.21 ± 2.25 % (n=7, paired t test, $P < .05$) and 9.36 ± 3.72 % (n=8, paired t test, $P < 0.05$), respectively. As shown in Fig. 7, pre-application of forskolin, significantly decreased the inhibition of I_K by capsaicin, whereas unaffected the inhibitions of I_A by capsaicin. These results suggested the selective involvement of PKA pathway in the inhibition of I_K by capsaicin.

Discussion

TG neurons are primary afferent neurons that carry sensory signals from the face, oral cavity, and nasal cavity to the medulla. Many nociceptive stimuli is known to change the neuronal excitability at peripheral nerve endings of the TG neurons, so in the present study, TG neurons were used as a pain model to study the capsaicin induced tuning of VGPCs. Capsaicin was reported to block VGPCs in different kinds of cells and species at concentrations ranging from less than 1 μ M to more than 100 μ M (Petersen et al. 1987; Castle 1992; Kuenzi and Dale 1996; Kang et al. 2010; Chieng et al. 2006; Kinch et al. 2012; Kehl 1994). This immense variation in both cell kind and concentration of capsaicin implicates that different mechanisms may involve in the block of capsaicin on VGPCs. Our previous study shows that there are two mechanisms, selective effects dependent on capsaicin receptors and non-selective effects independent of capsaicin receptors, which may be involved in the blockages of capsaicin on I_A in rat TG neurons. However, it is possible that different effects of capsaicin on I_A result from heterogeneous expression of VGPCs subunits in CS and CIS neurons; the CS neurons are mainly of small- and medium-sized neurons in TG and DRG. Here we compared the effects of capsaicin in TRPV1^{+/+} with TRPV1^{-/-} mice using neurons of the same size ranging from 15 to 22.5 μ m. Neurons in this size range have long-duration action potentials, TTX-resistant sodium currents and other receptors that indicate that they are nociceptors in our previous study (Cao et al. 2007).

Firstly, In CS neurons of TRPV1^{+/+} mice, I_A and I_K were reduced by 52.3 ± 17.2 % and 43.4 ± 16.9 % respectively by 1 μ M capsaicin and the effects could be reversed by capsazepine. Secondly, in CIS neurons of TRPV1^{+/+} mice, or TG neurons of TRPV1^{-/-} mice, both I_A and I_K only were blocked less than 10% by 1 μ M capsaicin and the effects were not reversed by capsazepine. These data further confirm that the vast majority of the capsaicin-induced inhibition of I_A and I_K in CS neurons occurs as a consequence of the activation of TRPV1.

In the present experiment, we demonstrated that VGPCs, including I_K and I_A , were inhibited by capsaicin via TRPV1 independent pathway in TRPV1^{-/-} TG neurons. TRPV1 independent inhibitory effects of high concentration capsaicin on voltage-gated sodium channels (VGSCs), including total and tetrodotoxin-resistant sodium currents, are also found in TRPV1^{-/-} mice (Cao et al. 2007) and rat cultured TG neurons (Liu and Simon 2003).

Similarity to VGSCs, being accompanied the amplitude reduced, h-infinity curve of VGPCs also significantly shift to hyperpolarizing direction but without a shift of G-V curve. Different from VGSCs, even with very high concentration of capsaicin (30 μ M) and very high frequency stimuli (1.7 Hz), use-dependent blockage is not yet induced for I_A and I_K . It is generally accepted that a drug-induced use-dependent block is attributed to the drug binding to a specific channel state (to the channel open stage, or to the inactivated state, thereby stabilizing the nonconducting channel state) (Savio-Galimberti et al. 2012; Takeda et al. 2006). Use-dependent block of capsaicin (30 μ M) in neuronal Na^+ channels is related to direct capsaicin binding via non-TRPV1 receptor within the open Na^+ channel (Wang et al. 2007). It is not known whether there is a non-TRPV1 receptor of capsaicin within open K^+ channels. And the newer hypothesis suggests that drug-induced stabilization of slow inactivation of channels should be partly responsible for use dependence block (Silva and Goldstein 2013). In the present study, we found that the recovery time courses of I_A and I_K were not delayed, even at very high capsaicin concentration (30 μ M), that might interpret why use dependent blockage was not induced. Otherwise, we found dose-dependent inhibition effects of capsaicin on I_A and I_K were similar to linear in TRPV1^{-/-} mice, but the linear inhibition did not necessarily mean non-specific effects. Regarding the inhibition of I_A by capsaicin in CS neurons, the dose-dependent inhibition curves are different in our previous study (Liu and Simon 2003) and in the present study, it is possible that the species (rat versus mice) and dose of capsaicin differences may play role here.

It should be noted that the capsaicin-sensitive TRPV1 independent effects in TG neuron are complicated. On one hand, capsaicin inhibits VGSCs which decreases the excitability of neurons, resulting in analgesic effects. On the other hand, capsaicin inhibits VGPCs which increases the excitability of neurons and leads to the algetic effects. As is known that the abnormal excitability of nociceptor is mediated by changes in function of many ion channels including voltage-gated channels and receptors, therefore the nociception induced by capsaicin is very likely an integrative result.

Capsaicin-sensitive mechanisms are composed of both TRPV1 dependent and independent pathways. The TRPV1 dependent mechanism for the action of capsaicin is well known. There is increasing evidence that some pharmacological roles of capsaicin are TRPV1 independent pathway (Surh and Kundu 2011). It has been shown that Peroxisome proliferator-activated receptor γ (Kim et al. 2004), TRPV6 (Chow et al. 2007) and cAMP-dependent mechanisms (Hibino et al. 2011) are involved in TRPV1 independent effects of capsaicin. The PKA pathway has an important role in the modulation of ion channels involved in neuronal and nociceptor excitability. Here we found that forskolin reduced the inhibitory effects of I_K by capsaicin. Thus it appears that PKA pathway is involved in the inhibition of I_K by capsaicin via TRPV1 independent mechanism. In contrast to the situation observed in I_K , in the presence of forskolin, the decrease of I_A by capsaicin was unaffected. This differential regulation suggest that the effects of capsaicin on VGPCs in TRPV1^{-/-} TG neurons are unlikely to arise from a non-specific effect on channel activity as a consequence of the adsorption of lipophilic capsaicin molecules to alter the cell membrane properties. Nevertheless, we have no sufficient evidence to indicate that VGPCs are inhibited after capsaicin induction of the PKA pathway via TRPV1 independent mechanism. Further experiments will be required to test this possibility.

In summary, in the present study by using TRPV1^{+/+} and TRPV1^{-/-} mice, we confirm that capsaicin inhibits VGPCs in TG neurons through its action on TRPV1 dependent as well as TRPV1 independent pathway and PKA pathway is selectively involved in the inhibition of I_K by capsaicin. Moreover, inhibitory effects of capsaicin on I_A and I_K will cause nociceptors to become more excitable and in this manner may contribute to its sensitizing effects.

Acknowledgments

This work was supported by NNSF grant 30571537, 30271500, NIH grant GM-63577 and by Education Department grant of Hubei province B2013148.

Abbreviations

VGPCs	Voltage-gated potassium channels
TRPV1	Transient receptor potential vanilloid 1
I_A	transient potassium currents
I_K	sustained potassium currents

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Fig. 1.

Effects of capsaicin (Cap) on VGPCs in TG neurons from TRPV1^{+/+} mice. **a** The application of $1 \mu\text{M}$ Cap induced an inward current in CS neuron. The typical recordings show that, exposed to $1 \mu\text{M}$ Cap, I_A was reduced from 3.8 nA to 1.4 nA and I_K was decreased from 3.3 nA to 1.8 nA respectively. Pre-incubation for 3 min with the TRPV1 antagonist, CPZ ($10 \mu\text{M}$), inhibited Cap-induced I_A and I_K decreases. **b** $1 \mu\text{M}$ Cap almost had no effects on I_A and I_K in CIS neuron. The peak amplitude of I_A was 2.91 nA , 2.81 nA

and 2.88 nA before, during and after capsaicin application, respectively. The amplitude of I_K was 3.06 nA, 2.94 nA and 2.90 nA before, during and after Cap application, respectively

Fig. 2.

Effects of capsaicin on I_A in TG neuron from $\text{TRPV1}^{-/-}$ mice. **a** The typical recordings show that I_A was reduced from 4.0 nA to 3.5, 2.7, 1.1 and 0.8 nA in the presence of 1, 10, 30, and 100 μM Cap, respectively. The inhibition was reversible after a 3 min wash. The plot showed the percentage of inhibition of I_A after application of capsaicin (1–100 μM). **b** Application of 30 μM Cap had no effect on G–V curve of I_A . **c** The typical recordings show that the amplitude of I_A was reduced in the presence of Cap and inactivation–voltage curve shifted to the hyperpolarizing direction

Fig. 3.

Effects of capsaicin on I_K in TG neurons from TRPV1^{-/-} mice. **a** The typical recordings show that I_K was reduced from 5.1 nA to 4.7, 2.7, 1.1 and 0.6 nA in the presence of 1, 10, 30, and 100 μ M capsaicin, respectively. After a 3 min washout, these effects were reversible. The plot indicated the percentage of inhibition of I_K after application of capsaicin (1 – 100 μ M). **b** The G - V curve of I_K did not shift in the absence and presence of capsaicin. **c** After application of 30 μ M capsaicin, inactivation-voltage curve of I_K significantly shifted to the hyperpolarizing direction

Fig. 4.

The use-dependent block of capsaicin on I_A in TG neurons from $TRPV1^{-/-}$ mice. **a** The typical recordings show that in the presence of 30 μ M Cap, I_A was reversibly inhibited in different stimulation frequencies. **b** Exposed to 30 μ M Cap, the amplitudes of I_A were reduced in all stimulation frequencies. **c** The plots were normalized which set the amplitudes of I_A triggered by first depolarizing pulse as 1, to compare use-dependent block at different frequency under the control and capsaicin-application condition. As shown in the plots, in the presence of capsaicin the block for I_A was tonic block, but not use-dependent block

Fig. 5.

The use-dependent block of capsaicin on I_K in TG neurons from $TRPV1^{-/-}$ mice. **a** The typical recordings were shown in the presence of 10 and 30 μ M capsaicin for 3 min. **b** The amplitudes of I_K were reduced in all stimulation frequencies in the presence of capsaicin (10 and 30 μ M). **c** The plots were normalized which set the amplitudes of I_K triggered by first depolarizing pulse as 1, to compare use-dependent block at different frequency under the control and capsaicin-application condition. As shown in the plots, in the presence of capsaicin the block for I_K was tonic block, but not use-dependent block

Fig. 6.

The effects of capsaicin on recovery time of I_A and I_K in cultured TG neuron from $TRPV1^{-/-}$ mice. **a** Plots showed that amplitude of both I_A and I_K recovered as interval time increased in the absence and presence of 30 μ M capsaicin. **b** Plots were normalized to compare time courses of recovery in the absence and presence of capsaicin

Fig. 7.

Modulation of PKA pathway on the effects of VGPCs induced by capsaicin in TG neurons from TRPV1^{-/-} mice. **a** The typical recordings show that the amplitude of I_A was 12.85 nA, 5.91 nA, 5.95 nA and 5.98 nA before, during application of Cap (30 μ M), After a 5 min pre-incubation with forskolin (10 μ M) and subsequent addition of Cap (30 μ M) and after 3 min wash, respectively. The histogram shows that pre-incubation with forskolin failed to affect the inhibition of I_A by Cap (unpaired t test, $P > 0.05$). **b** Forskolin markedly decreased the inhibitions of I_K by capsaicin. The amplitude of I_K was 10.35 nA, 4.80 nA, 6.41 nA and 4.92 nA before, during application of Cap (30 μ M), After a 5 min pre-incubation with forskolin (10 μ M) and subsequent addition of Cap (30 μ M) and after 3 min wash, respectively. The histogram shows the distinct difference in relative amplitude of I_K between Cap ($40.7 \pm 4.4\%$, $n=8$) experiments and Cap + Forskolin ($63.2 \pm 5.6\%$, $n=10$) experiments. * $P < 0.05$ compared with the Cap experiments