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ORIGINAL PAPER

Inhibitory effects of honokiol on the voltage-gated potassium channels in freshly isolated mouse dorsal root ganglion neurons

Anqi Sheng[1]·Yan Zhang[1] ·Guang Li[2]·Guangqin Zhang[1]

[1] Department of Clinical Pharmacy, China Pharmaceutical University, Nanjing 210009, China

[2] Key Laboratory of Medical Electrophysiology, Ministry of Education, Institute of Cardiovascular Research, Southwest Medical University, Luzhou 646000, China

Corresponding author: Guangqin Zhang

E-mail: njzhanggq@163.com **Tel.:** +8625 83360221

ORCID iD: 0000-0002-0282-3807

Abstract Voltage-gated potassium (K_V) currents, subdivided into rapidly inactivating A-type currents (I_A) and slowly inactivating delayed rectifier currents (I_K), play a fundamental role in modulating pain by controlling neuronal excitability. The effects of Honokiol (Hon), a natural biphenolic compound derived from *Magnolia officinalis*, on K_V currents were investigated in freshly isolated mouse dorsal root ganglion (DRG) neurons using the whole-cell patch clamp technique. Results showed that Hon inhibited I_A and I_K in concentration-dependent manner. The IC_{50} values for block of I_A and I_K were 30.5 μM and 25.7 μM, respectively. Hon (30 μM) shifted the steady-state activation curves of I_A and I_K to positive potentials by 17.6 mV and 16.7 mV, whereas inactivation and recovery from the inactivated state of I_A were unaffected. These results suggest that Hon preferentially interacts with the active states of the I_A and I_K channels, and has no effect on the resting state and inactivated state of the I_A channel. Blockade on K^+ channels by Hon may contribute to its antinociceptive effect, especially anti-inflammatory pain.

Keywords Honokiol· Dorsal root ganglion neurons· Patch clamp· Voltage-gated potassium channels

Introduction

Honokiol (Hon - see Fig. 1), a biphenolic compound extracted from the Chinese medicinal herb *Magnolia officinalis*, has been used as an anti-bacterial, anti-neoplastic, anti-allergic, anti-spasmodic and anti-asthmatic compound [1-5]. Hon has been shown to be able to inhibit platelet-activating factor production in human neutrophils [6], scavenge hydroxyl radicals *in vitro* [7], decrease ventricular arrhythmia induced by coronary ligation [8], and suppress the aggregation and release reaction of rabbit platelets induced by collagen and arachidonic acid through the inhibition of thromboxane formation [9]. Recently, Hon has displayed a neuroprotective effect. It has therapeutic potential in anxiety, pain, epilepsy, cerebrovascular injury , and cognitive disorders including Alzheimer's disease [10-15]. The neuroprotective effects of Hon may be attributed to a wide range of mechanisms , but the effects of Hon on ion channels in neuroprotection, especially in pain, are unclear.

Peripheral nerve damage elicits hyperactivity of dorsal root ganglion (DRG) neurons to contribute toward peripheral neuropathic pain. Increased excitability of DRG neurons is attributable to alterations in the expression and biophysical properties of voltage-gated Na^+, K^+ and Ca^{2+} channels [16, 17]. Voltage-gated potassium (K_V) channel currents are crucial determinants of neuronal excitability for spike threshold and firing frequency control and are subdivided into rapidly inactivating A-type K^+ current (I_A) and slowly inactivating delayed-rectifier K^+ current (I_K) [18-21]. Recent reports have demonstrated that both I_A and I_K were significantly reduced in the chronic constriction injury (CCI) rat [22]. The changes of I_K currents in the chronic compression (CCD) model obtained from different studies are inconsistent [23, 24]. These discrepancies may be because of differences in cell-size. But a reduction in I_K and/or I_A currents is observed in DRG neurons under various pathological conditions, including chronic bladder inflammation, spinal cord injury and chronic pancreatitis [25-27]. These findings

indicate that the hyperexcitability is associated with a decreased density of K_V current.

In the present study, we investigated the effects of Hon on K_V current in DRG neurons using the whole-cell patch clamp technique and determined whether Hon affected the activity and kinetic properties of K_V channels. We first found that Hon inhibited I_A and I_K in a concentration- dependent manner in DRG neurons. In addition, Hon shifted the steady-state activation curves of I_A and I_K to more positive potentials, without altering inactivation and recovery from the inactivated state of I_A.

Materials and Methods

Animals

Kunming mice (25-30g), obtained from the Qinglongshan Animal Center of Nanjing, were caged with a 12 h natural light-dark cycle with proper food and water. All procedures involving animals and their care were approved by the Institutional Animal Care and Use Committee of China Pharmaceutical University and were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize the suffering and number of animals used during the experiments.

Cell Preparation

Dorsal root ganglion cells were freshly isolated on the basis of the method described previously [28]. Briefly, mice were killed by decapitation and ganglia were dissected from the vertebral column. Ganglia

were submerged in oxygenated Tyrode's solution (containing in mM: NaCl 137, $MgCl_2$ 1.2, KCl 5.4, NaH_2PO_4 1.2, $CaCl_2$ 1, HEPES 10, glucose 10, pH 7.4) and minced with iridectomy scissors. Minced ganglia were incubated at 37℃ for 60 min in Tyrode's solution containing collagenase (1 mg/ml, type Ⅱ; Worthinton, USA). Then the DRGs were dissociated by triturating through a fire-polished Pasteur pipette. The isolated DRGs were washed for 3 times to suspend digestion using extracellular solutions and then plated on Poly-D-lysine coated 35 mm culture dishes containing standard external solution (containing in mM: NaCl 137, $MgCl_2$ 1.2, KCl 5.4, NaH_2PO_4 0.33, HEPES 10, glucose 10, $CaCl_2$ 1, Nifedipine 0.005, TTX 0.001, pH 7.4).

Electrophysiological recordings

Whole-cell K^+ currents were recorded from DRG neurons with a patch-clamp amplifier (HEKA EPC 10, HEKA Instruments, Germany). Stimulation protocols and data acquisition were controlled with Pulse software (HEKA Pulse, HEKA Instruments). Pipettes were fabricated from 1.5 mm out diameter borosilicate glass capillaries (Institute of Biophysics, Chinese Academy of Sciences, China) using a Sutter P-97 puller (Sutter Instrument, USA). Fire-polished pipette resistance was 3~5 MΩ when filled with the intracellular solution (containing in mM: KCl 140, $CaCl_2$ 1, $MgCl_2$ 1, Na_2ATP 2, EGTA 10, HEPES 5, adjusted to pH 7.2 with KOH). Current signals were filtered at 3 kHz, digitized at 10 kHz. Series resistance was compensated (>70%). The capacitances (c-fast and c-slow) were compensated automatically by the software online during recording. When needed, linear leak current artifacts were removed by online leak subtraction. To block Ca^{2+} and TTX-sensitive Na^+ currents, nifedipine and TTX were added to the extracellular solution, respectively. Recordings were obtained from small neurons with

diameters less than 30 μm. All experiments were performed at room temperature (22~25℃).

Chemicals

TTX, Tetraethylammonium Chloride (TEA-Cl), 4-Aminopyridine (4-AP), EGTA and HEPES were purchased from Sigma (St Louis, MO, USA), and others were analytical reagents made in China. Stock solution of TTX was prepared in distilled water. Hon (purity 98%) was purchased from the National Institute for Food and Drug Control (Beijing, China). A stock solution of Hon was prepared in DMSO (Sigma, USA) and then diluted in extracellular solution to attain the desired working solutions. Drug was applied to the bath through the perfusion system.

Data Analyses

All data were analyzed by pulse (HEKA electronic, Germany), Igor pro 6.3 (WaveMetrics, USA) and Sigmaplot (Jandel Scientific, USA) softwares. Data were expressed as mean ± S.E.M. Statistical analysis was performed using paired Student's t-test to evaluate the statistical differences between two groups, and one-way ANOVA was used for multiple groups. A P value < 0.05 was considered to be statistically significant.

Results

Effects of Hon on K_V currents

Cells were held at –100 mV, voltage-gated potassium (K_V) channel currents were elicited by a series of 400 ms test pulses ranging from–60 mV to +80 mV in 10 mV increments at 0.5 Hz. Activated K_V currents include transient current (I_A), sensitive to 4-AP (Fig. 2a, b), and sustained outward current (I_K), sensitive to TEA (Fig. 2c, d). In this experiment, 5 mM 4-AP and 50 mM TEA suppressed K_V currents by 57.0 ± 3.3% and 64.7 ± 2.2% (n = 5), respectively.

Upon the administration of Hon , blocking action of Hon on K_V current occurred in 1~2 min, and reached a maximum and steady value in about 7 min. In order to ensure the maximum effect of Hon, K_V current was recorded after application of Hon for 10 min. I_A was estimated as the peak of the transient component, and I_K was measured at the ending point of 400 ms step depolarizations (Fig. 2e, f). As shown in Fig. 2g, h, during exposure to 30 μM Hon, the maximal peak I_A and I_K were reduced by 46.8 ± 2.8% and 48.4 ± 1.7% (n = 14), respectively. Hon inhibited the peak amplitudes of I_A and I_K in a concentration-dependent manner. The data were fitted well with the following Hill equation, $y=1/[1+(IC_{50}/C)^n]$, where IC_{50} is the drug concentration causing the half-maximum response, C is the concentration of drug, y and n are the fraction of the maximum inhibition percentage and the Hill coefficient. The IC_{50} values for block of I_A and I_K were 30.5 μM and 25.7 μM (n = 6), respectively. The maximal suppressions of Hon on I_A and I_K were 90.0 ± 2.1% and 91.5 ± 2.7% at 300 μM, respectively. The Hill coefficients were 1.04 and 1.15, respectively (Fig. 3a, b). These results suggest that the inhibition of Hon on K_V current is preferential for I_K.

Effects of Hon on the current–voltage curves of I_A and I_K

Total K^+ currents (K_V currents) were elicited by a series of 400 ms test pulses ranging from –60 mV to +80 mV in 10 mV increments, preceded by a holding potential of –100 mV (Fig. 4a, b). When the voltage was held at –40 mV, the depolarization pulses activated I_K and inactivated I_A (Fig. 4c, d). Subtraction of the I_K from the total K_V current attained I_A (Fig. 4e, f). The peak current-voltage (*I-V*) curves for I_A and I_K in control and after the exposure to 30 μM Hon are shown in Fig. 4g, h. Hon markedly reduced I_A and I_K by 43 ± 5% and 62 ± 3% at +80 mV, respectively (n = 10, $P < 0.05$).

Effects of Hon on the activation characteristics of I_A and I_K

Activation of K^+ channel was evaluated by a conductance transform of the peak current–voltage relationship. The conductance was calculated from the peak current value at each test pulse potential using the equation: $G = I/(V-V_{rev})$, where V is the membrane potential at which I was recorded, V_{rev} is reversal potential (calculated as –86 mV for the potassium concentrations used). The normalized conductance was fitted well with a Boltzmann equation: $G/G_{max} = 1/\{1+\exp[(V_{1/2}-V)/k]\}$, where G_{max} is the maximal conductance, $V_{1/2}$ is the half activation voltage and k is a slope factor. The steady-state activation curves for I_A and I_K are shown in Fig. 5a, b. The values of $V_{1/2}$ for activation of I_A in control and in the presence of 30 μM Hon were –25.9 ± 3.8 mV and –8.3 ± 4.8 mV (n = 10, $P < 0.05$), with k values of 19.7 ± 2.4 mV and 26.0 ± 0.9 mV (n = 10, $P < 0.05$), respectively. Thus Hon produced a 17.6 mV positive shift in the $V_{1/2}$ of the steady-state activation curve. For I_K, Hon markedly shifted $V_{1/2}$ from 6.9 ± 1.7 mV for control neurons to 23.6 ± 2.5 mV for 30 μM Hon treated neurons (n = 10, $P < 0.05$). In contrast, no significant difference in the slope factor k was observed between control (20.5 ± 1.9 mV) and 30 μM Hon treated neurons (22.9 ± 0.7 mV, n = 10, $P > 0.05$).

Effects of Hon on the inactivation of I_A

To determine the voltage dependence of the steady-state inactivation of I_A, two-pulse voltage protocols were employed in this experiment. The holding potential was at –100 mV, a 1 s conditional prepulse of various voltages from –120 to –30 mV in 10 mV increment was followed by a 400 ms testing pulse to +60 mV. The inactivation curve was fitted by the Boltzmann equation : $I/I_{max} = 1/\{1+\exp[(V-V_{1/2})/k]\}$, where I is the peak current evoked from the conditional potential V, I_{max} is the maximal peak current, $V_{1/2}$ is the half inactivation voltage, and k is a slope factor. The $V_{1/2}$ and k of I_A were –78.0 ± 2.3 mV and 13.7 ± 1.6 mV in control , and were –81.8 ± 3.2 mV and 15.4 ± 1.9 mV in the presence of 30 μM Hon (n = 7, P > 0.05), respectively. Hon slightly shifted the steady-state inactivation curve of I_A towards negative potential, but not statistically significant (Fig. 6). These results suggested that Hon did not obviously affect the steady-state inactivation of I_A.

Effects of Hon on the recovery of I_A from inactivation

The time course of I_A recovery from inactivation was assessed using a standard two-pulse protocol. A 400 ms prepulse depolarized to +60 mV from a holding potential of –100 mV was followed by recovery gap potential at –100 mV for variable length and then by a 400 ms test pulse to +60 mV. The ratio of test pulse currents to prepulse currents plotted against recovery time. The recovery process of I_A could be well fitted by a single exponential function, with recovery time constant of 79.9 ± 5.2 ms and 89.7 ± 7.5 ms before and after application of 30 μM Hon (n = 7, P > 0.05) , respectively. The results showed that

Hon had no significant effect on the time course of recovery of I_A from inactivation (Fig. 7).

Effects of Hon on the frequency-dependence of I_A

To study the effect of Hon on frequency-dependence, K_V currents were elicited by 20 consecutive 400 ms depolarizing pulses to +80 mV from a holding potential of –100 mV at different frequencies (1, 5 and 10 Hz). I_A was measured as the peak of the transient component, and I_K was measured at the ending point of 400 ms voltage steps. Data were normalized with respect to the first step. As shown in Fig. 8, the responses of I_A and I_K to depolarizing pulses remained unchanged at different frequencies before and after application of 30 μM Hon (n = 6). Thus, Hon did not show a frequency-dependent effect on K_V currents (I_A and I_K).

Discussion

In the present study, inhibitory effects of Hon on voltage-dependent K^+ channels were investigated in mouse dorsal root ganglion neurons. The results indicated that Hon significantly inhibited two important voltage-gated K^+ (K_V) channel currents, the transient A-type K^+ current (I_A) and the sustained delayed rectifier K^+ current (I_K) , in a concentration-dependent manner. IC_{50} were 30.5 μM and 25.7 for I_A and I_K, respectively. In addition, Hon shifted the activation curves of I_K and I_A to more positive potentials. However, Hon had no effect on inactivation, recovery from the inactivated state and frequency-dependence of I_A.

Voltage-gated K^+ channels play a critical role in the control of electrophysiological properties and excitability of neurons [29]. A-type K^+ channels have been implicated in the delay of the spike onset and

the decrease in the action potential (AP) firing frequency, while the sustained delayed rectifier K^+ channels have been involved in action potential threshold and the number of APs of neurons, implying that the two types of K^+ channels in DRG may play different roles in neuronal excitability [22, 30]. In this study, we confirmed that Hon significantly inhibited I_K and I_A in a concentration-dependent manner. It is thus likely that Hon regulates the excitability of DRG neurons by suppressing two types of K^+ channels. However, It is noteworthy that Hon produces varying effects on both types of K^+ currents, the blocking potency of Hon on I_K (IC_{50}: 25.7 μM) is stronger than on I_A (IC_{50}: 30.5 μM). This difference may be due to the possibility that there is the different sensitivity for Hon to I_K and I_A. Functionally, I_A was relatively small and inactivated fast , repolarizing phases of action potentials were usually slow and mainly formed by I_K in DRG neurons [31]. Consequently, the modification of I_K by Hon may affect membrane potential and thereby cause changes in the neuronal activity.

The state-dependent K^+ channel blockade of Hon may help to understand its kinetic mechanism. Voltage-gated K^+ channels have three distinct states: closed or resting, open, and inactivated. In present study, Hon shifted the activation curves of I_A to more positive potentials, without altering inactivation and recovery from the inactivated state and frequency-dependence of I_A. The positive shift of the activation curve of I_A indicates that Hon preferentially binds to the activated state of the A-type K^+ channel. According to Modulated receptor hypothesis for the state-dependent interaction[32], Hon displayed higher binding affinity to the open state than to the resting state or inactivated state of the A-type K^+ channel. Additionally, I_K, a slowly inactivating delayed rectifier current, was also suppressed. The activation curve of I_K was shifted to more positive potential by Hon, but frequency-dependent inhibition of I_K was not found in the presence of Hon, suggesting Hon had a higher affinity for the open state of the sustained delayed rectifier K^+ channel.

Pain arising from intense or damaging stimuli has an important physiological role, protecting the body and promoting healing from injury. It is divided into physiological pain, inflammatory pain and neuropathic pain [33]. Previous studies have shown that Hon produces the antinociceptive action in inflammatory pain models induced by formalin, substance P and prostaglandin, and do not display analgesia in physiological pain determined by the tail-flick, hot-plate paw-shaking tests [11, 33]. After peripheral inflammation or nerve injury, voltage-gated K^+ currents are changed in DRG cells. In many neuropathic pain models, the two types of voltage-gated K^+ currents were downregulated or unaltered in DRG neurons, respectively [22, 23, 25-27]. On the contrary, the increases in I_K and I_A were observed in the model of peripheral inflammatory pain induced by an inflammatory agent (zymosan) [34], this is consistent with the antinociceptive effects of Hon in inflammatory pain models. However, the reasons for these differences between these various models are not yet clear. The relationships between voltage-gated K^+ currents and different types of pains are required to be further studied. Still, we speculate that the anti-inflammatory pain of Hon may be mainly attributed to its blocking effect on K_V channels. The latest study reported that diclofenac, a nonsteroidal anti-inflammatory drug with anti-nociceptive action, inhibited I_K and increased M-type K^+ current ($I_{K(M)}$) in DRG neurons, and decreased rhythmic firing of APs in hippocampal CA1 pyramidal neurons [35]. These results powerfully support our viewpoint that anti-inflammatory pain is associated with blocking potassium currents.

Hon, including most flavonoid compounds, has strong antioxidant activity and other bioactivity because of containing phenolic hydroxyl groups in its chemical structure. In an earlier report, piceatannol, a derivative of resveratrol , possessed an additional hydroxyl group in the 3′ position of resveratrol, which made it exerting more potent than resveratrol in cardiac ion channel inhibition, including I_{Na}, I_{to} and I_{Kss}, in parallel with the antiarrhythmic activity [36]. This result suggested that increasing hydroxyl

groups were beneficial to enhance inhibitory effect of drug on ion channels. Therefore, we think that the strong potencies of Hon in anti-oxidative, anti-inflammatory, and neuroprotective activities mainly depend on phenolic hydroxyl groups in its chemical structure.

In conclusion, our data demonstrate that Hon exhibits concentration- dependent blocking effects of I_A and I_K. It preferentially interacts with the active states of the I_A and I_K channels, without apparent effect on inactivation and recovery from the inactivated state of I_A. Currently, based on the knowledge what we know about analgesic effects of Hon, we propose that the antinociceptive action of Hon may be related to its blocking effect on K^+ channels, Hon may possess the therapeutic potential in the treatment of inflammatory pain. But, the regulations of K^+ channels in different pain models still remain to be investigated in future experiments.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ho KY, Tsai CC, Chen CP, Huang JS, Lin CC (2001) Antimicrobial activity of honokiol and magnolol isolated from Magnolia officinalis. Phytother Res 15:139-141
2. Fried LE, Arbiser JL (2009) Honokiol, a multifunctional antiangiogenic and antitumor agent. Antioxid Redox Signal 11:1139-1148. doi:10.1089/ARS.2009.2440
3. Han SJ, Bae EA, Trinh HT, Yang JH, Youn UJ, Bae KH, Kim DH (2007) Magnolol and honokiol: inhibitors against mouse passive cutaneous anaphylaxis reaction and scratching behaviors. Biol

Pharm Bull 30:2201-2203

4. Ko CH, Chen HH, Lin YR, Chan MH (2003) Inhibition of smooth muscle contraction by magnolol and honokiol in porcine trachea. Planta Med 69:532-536. doi:10.1055/s-2003-40654
5. Wu SN, Chen CC, Li HF, Lo YK, Chen SA, Chiang HT (2002) Stimulation of the BK_{Ca} channel in cultured smooth muscle cells of human trachea by magnolol. Thorax 57:67-74
6. Yamazaki R, Sugatani J, Fujii I, Kuroyanagi M, Umehara K, Ueno A, Suzuki Y, Miwa M (1994) Development of a novel method for determination of acetyl-CoA:1-alkyl-sn-glycero-3-phosphocholine acetyltransferase activity and its application to screening for acetyltransferase inhibitors. Inhibition by magnolol and honokiol from Magnoliae cortex. Biochem Pharmacol 47:995-1006
7. Lo YC, Teng CM, Chen CF, Chen CC, Hong CY (1994) Magnolol and honokiol isolated from Magnolia officinalis protect rat heart mitochondria against lipid peroxidation. Biochem Pharmacol 47:549-553
8. Tsai SK, Huang CH, Huang SS, Hung LM, Hong CY (1999) Antiarrhythmic effect of magnolol and honokiol during acute phase of coronary occlusion in anesthetized rats: influence of L-NAME and aspirin. Pharmacology 59:227-233. doi:28324
9. Teng CM, Chen CC, Ko FN, Lee LG, Huang TF, Chen YP, Hsu HY (1988) Two antiplatelet agents from Magnolia officinalis. Thromb Res 50:757-765
10. Kuribara H, Kishi E, Hattori N, Okada M, Maruyama Y (2000) The anxiolytic effect of two oriental herbal drugs in Japan attributed to honokiol from magnolia bark. J Pharm Pharmacol 52:1425-1429
11. Lin YR, Chen HH, Lin YC, Ko CH, Chan MH (2009) Antinociceptive actions of honokiol and magnolol on glutamatergic and inflammatory pain. J Biomed Sci 16:94.

doi:10.1186/1423-0127-16-94

12. Lin YR, Chen HH, Ko CH, Chan MH (2006) Neuroprotective activity of honokiol and magnolol in cerebellar granule cell damage. Eur J Pharmacol 537:64-69. doi:10.1016/j.ejphar.2006.03.035

13. Matsui N, Takahashi K, Takeichi M, Kuroshita T, Noguchi K, Yamazaki K, Tagashira H, Tsutsui K, Okada H, Kido Y, Yasui Y, Fukuishi N, Fukuyama Y, Akagi M (2009) Magnolol and honokiol prevent learning and memory impairment and cholinergic deficit in SAMP8 mice. Brain Res 1305:108-117. doi:10.1016/j.brainres.2009.09.107

14. Lin YR, Chen HH, Ko CH, Chan MH (2005) Differential inhibitory effects of honokiol and magnolol on excitatory amino acid-evoked cation signals and NMDA-induced seizures. Neuropharmacology 49:542-550. doi:10.1016/j.neuropharm.2005.04.009

15. Woodbury A, Yu SP, Wei L, Garcia P (2013) Neuro-modulating effects of honokiol: a review. Front Neurol 4:130. doi:10.3389/fneur.2013.00130

16. Kajander KC, Wakisaka S, Bennett GJ (1992) Spontaneous discharge originates in the dorsal root ganglion at the onset of a painful peripheral neuropathy in the rat. Neurosci Lett 138:225-228

17. Kim DS, Choi JO, Rim HD, Cho HJ (2002) Downregulation of voltage-gated potassium channel alpha gene expression in dorsal root ganglia following chronic constriction injury of the rat sciatic nerve. Brain Res Mol Brain Res 105:146-152

18. Stewart T, Beyak MJ, Vanner S (2003) Ileitis modulates potassium and sodium currents in guinea pig dorsal root ganglia sensory neurons. J Physiol 552:797-807. doi:10.1113/jphysiol.2003.046409

19. Everill B, Rizzo MA, Kocsis JD (1998) Morphologically identified cutaneous afferent DRG neurons express three different potassium currents in varying proportions. J Neurophysiol 79:1814-1824

20. Connor JA, Stevens CF (1971) Voltage clamp studies of a transient outward membrane current in

gastropod neural somata. J Physiol 213:21-30

21. Connor JA, Stevens CF (1971) Prediction of repetitive firing behaviour from voltage clamp data on an isolated neurone soma. J Physiol 213:31-53

22. Xiao Y, Wu Y, Zhao B, Xia Z (2016) Decreased voltage-gated potassium currents in rat dorsal root ganglion neurons after chronic constriction injury. Neuroreport 27:104-109. doi:10.1097/WNR.0000000000000505

23. Fan N, Donnelly DF, LaMotte RH (2011) Chronic compression of mouse dorsal root ganglion alters voltage-gated sodium and potassium currents in medium-sized dorsal root ganglion neurons. J Neurophysiol 106:3067-3072. doi:10.1152/jn.00752.2011

24. Fan N, Sikand P, Donnelly DF, Ma C, Lamotte RH (2011) Increased Na^+ and K^+ currents in small mouse dorsal root ganglion neurons after ganglion compression. J Neurophysiol 106:211-218. doi:10.1152/jn.00065.2011

25. Hayashi Y, Takimoto K, Chancellor MB, Erickson KA, Erickson VL, Kirimoto T, Nakano K, de Groat WC, Yoshimura N (2009) Bladder hyperactivity and increased excitability of bladder afferent neurons associated with reduced expression of Kv1.4 alpha-subunit in rats with cystitis. Am J Physiol Regul Integr Comp Physiol 296:R1661-1670. doi:10.1152/ajpregu.91054.2008

26. Takahashi R, Yoshizawa T, Yunoki T, Tyagi P, Naito S, de Groat WC, Yoshimura N (2013) Hyperexcitability of bladder afferent neurons associated with reduction of Kv1.4 alpha-subunit in rats with spinal cord injury. J Urol 190:2296-2304. doi:10.1016/j.juro.2013.07.058

27. Xu GY, Winston JH, Shenoy M, Yin H, Pasricha PJ (2006) Enhanced excitability and suppression of A-type K^+ current of pancreas-specific afferent neurons in a rat model of chronic pancreatitis. Am J Physiol Gastrointest Liver Physiol 291:G424-431. doi:10.1152/ajpgi.00560.2005

28. Murakami M, Nakagawasai O, Suzuki T, Mobarakeh, II, Sakurada Y, Murata A, Yamadera F, Miyoshi I, Yanai K, Tan-No K, Sasano H, Tadano T, Iijima T (2004) Antinociceptive effect of different types of calcium channel inhibitors and the distribution of various calcium channel alpha 1 subunits in the dorsal horn of spinal cord in mice. Brain Res 1024:122-129. doi:10.1016/j.brainres.2004.07.066

29. Vydyanathan A, Wu ZZ, Chen SR, Pan HL (2005) A-type voltage-gated K^+ currents influence firing properties of isolectin B4-positive but not isolectin B4-negative primary sensory neurons. J Neurophysiol 93:3401-3409. doi:10.1152/jn.01267.2004

30. Pongs O (1999) Voltage-gated potassium channels: from hyperexcitability to excitement. FEBS Lett 452:31-35

31. Safronov BV, Bischoff U, Vogel W (1996) Single voltage-gated K^+ channels and their functions in small dorsal root ganglion neurones of rat. J Physiol 493 (Pt 2):393-408

32. Hille B (1977) Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. J Gen Physiol 69:497-515

33. Lin YR, Chen HH, Ko CH, Chan MH (2007) Effects of honokiol and magnolol on acute and inflammatory pain models in mice. Life Sci 81:1071-1078. doi:10.1016/j.lfs.2007.08.014

34. Wang JG, Strong JA, Xie W, Zhang JM (2007) Local inflammation in rat dorsal root ganglion alters excitability and ion currents in small-diameter sensory neurons. Anesthesiology 107:322-332. doi:10.1097/01.anes.0000270761.99469.a7

35. Huang CW, Hung TY, Liao YK, Hsu MC, Wu SN (2013) Underlying mechanism of regulatory actions of diclofenac, a nonsteroidal anti-inflammatory agent, on neuronal potassium channels and firing: an experimental and theoretical study. J Physiol Pharmacol 64:269-280

36. Chen WP, Hung LM, Hsueh CH, Lai LP, Su MJ (2009) Piceatannol, a derivative of resveratrol,

moderately slows I_{Na} inactivation and exerts antiarrhythmic action in ischaemia-reperfused rat hearts. Br J Pharmacol 157:381-391. doi:10.1111/j.1476-5381.2008.00106.x

Legends for Figures

Fig. 1 Structure of Hon

Fig. 2 Hon inhibited voltage-gated K^+ (K_V) currents in DRG neurons. The total K_V currents were elicited by 400 ms depolarization from holding potential –100 mV, ranging from –60 mV to +80 mV in 10 mV

increments. Representative traces of K_V currents were shown. **a** and **b** The total K_V currents and 4-aminopyridine (4-AP)-sensitive A-type K^+ currents (I_A) (n = 5). **c** and **d** The total K_V currents and tetraethylammonium chloride (TEA)-sensitive delayed rectifier K^+ currents (I_K) (n = 5). **e** and **f** The total K_V currents before and after application of 30 μM Hon. **g** and **h** Current-voltage relationships for I_A and I_K in the absence and presence of 30 μM Hon. I_A was measured as the peak of the transient component, and I_K was measured at the ending point of 400 ms voltage steps. (n = 14, *P < 0.05 *vs* control).

Fig. 3 Concentration-response curves for inhibition of I_A (**a**) and I_K (**b**) by Hon. Data were fitted with the Hill equation, $y = 1/[1+(IC_{50}/C)^n]$, where IC_{50} is the drug concentration causing the half-maximum response, C is the concentration of drug, y and n are the fraction of the maximum inhibition percentage and the Hill coefficient (n = 6).

Fig. 4 Effects of Hon on current–voltage (I-V) relationships of I_A and I_K. The current was evoked by a series of 400 ms stimulating pulses from –60 mV to +80 mV in 10 mV steps. **a** and **b** Representative current traces for total K_V currents, cell was held at –100 mV before and after the exposure to 30 μM Hon. **c** and **d** Representative current traces for I_K, cell was held at –40 mV before and after the exposure to 30 μM Hon. **e** and **f** Representative current traces for I_A were obtained by subtracting I_K in **c** and **d** from total K_V currents in **a** and **b**, respectively. **g** and **h** Current-voltage relationships for I_A and I_K (n = 10, *P < 0.05 *vs* control).

Fig. 5 Effects of Hon on steady state activation curves of I_A (**a**) and I_K (**b**). Activation curves of K^+ currents were obtained from the data of *I-V* curves and were fitted by the Boltzmann equation.

Fig. 6 Effects of Hon on steady state inactivation of I_A. Current curves were obtained by a 1 s conditional prepulse of various voltages from –120 to –30 mV in 10 mV increment was followed by a 400 ms testing pulse to +60 mV, cell was held at –100 mV. Steady state inactivation curves were fitted by the Boltzmann equation (n = 7).

Fig. 7 Effects of Hon on recovery of I_A from inactivation. Current traces were recorded using a standard two-pulse protocol. A 400 ms prepulse depolarized to +60 mV from a holding potential of –100 mV was followed by recovery gap potential at –100 mV for variable length and then by a 400 ms test pulse to +60 mV. Data were fitted with a single exponential equation (n = 7).

Fig. 8 Frequency-dependent inhibition of Hon (30 μM) on K_V currents. K_V currents were elicited by 20 consecutive 400 ms pulses depolarized from a holding potential of -100 to +80 mV at 1, 5 and 10 Hz. I_A was measured as the peak of the transient component, and I_K was measured at the ending point of 400 ms voltage steps. I_A and I_K were normalized with respect to the value obtained for the first step (n = 6).