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Tannic acid modulates excitability of sensory neurons and nociceptive behavior and the Ionic mechanism

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ABSTRACT

M/Kv7 K^+ channels, Ca^{2+}-activated Cl^- channels (CaCCs) and voltage gated Na^+ channels expressed in dorsal root ganglia (DRG) play an important role in nociception. Tannic acid has been proposed to be involved in multiple beneficial health effects; tannic acid has also been described to be analgesic. However the underlying mechanism is unknown. In this study, we investigated the effects of tannic acid on M/Kv7 K^+, Na^+ currents and CaCCs, and the effects on bradykinin-induced nociceptive behavior. A perforated patch technique was used. The bradykinin-induced rat pain model was used to assess the analgesic effect of tannic acid. We demonstrated that tannic acid enhanced M/Kv7 K^+ currents but inhibited bradykinin-induced activation of CaCC/TMEM16A currents in rat small DRG neurons. Tannic acid potentiated Kv7.2/7.3 and Kv7.2 currents expressed in HEK293B cells, with an EC_{50} of 7.38 and 5.40 μM, respectively. Tannic acid inhibited TTX-sensitive and TTX-insensitive currents of small DRG neurons with IC_{50} of 5.25 and 8.43 μM, respectively. Tannic acid also potently suppressed the excitability of small DRG neurons. Furthermore, tannic acid greatly reduced bradykinin-induced pain behavior of rats. This study thus demonstrates that tannic acid is an activator of M/Kv7 K^+ and an inhibitor of voltage-gated Na^+ channels and CaCC/TMEM16A, which may underlie its inhibitory effects on excitability of DRG neurons and its analgesic effect. Tannic acid could be a useful agent in treatment of inflammatory pain conditions such as osteoarthritis, rheumatic arthritis and burn pain.

1. Introduction

Tannic acid (Fig. S1) presents in varying concentrations in plant foods, and in relatively high concentrations in red wines and green teas (Waterhouse, 2002; Crozier et al., 2009). Beneficial effect on health has been described for tannic acid such as vasodilatation, antimutagenic, antimicrobial activities (Scalbert et al., 2005; Serrano et al., 2009). Clinical and experimental observations suggest that tannic acid is beneficial in the management of burns for improving wound healing and reducing scar tissue formation, and for relieving the burn pain (Halkes et al., 2001). It has long been known that to press a cold tea bag with the help of teeth or sore gums, at the area where the pain is intense and the tannic acid present in it relieves the pain and provides an antiseptic effect at the same time. Tannic acid in tea, is also believed, helps to relieve sunburn pain. However, the mechanism underling this analgesic effect of tannic acid is currently not known.

A possibly untested mechanism for the analgesic effect of tannic acid could be through the modulation of ion channel functions (Namkung et al., 2010). A number of different ion channel subtypes have been identified to be related to pain. Voltage-gated Na^+ (Baker and Wood, 2001; Hoeijmakers et al., 2012), voltage-gated Ca^{2+} (Cao, 2006; Zamponi et al., 2009) and TRP channels (Cortright and Szallasi, 2009; Chung et al., 2011) have all been implicated in the pain conditions. M/Kv7 K^+ channels play an important role in excitability of the sensory neurons related to the pain transduction (Delmas and Brown, 2005; Xiong et al., 2008; Liu et al., 2010; Zheng et al., 2012). Pharmacological augmentation of M/Kv7 K^+ currents by channel opener such as retigabine represents a promising therapeutics to treat pain (Passmore et al., 2003; Du et al., 2011). Previous work revealed the

Abbreviations: DRG, dorsal root ganglia; HEK, human embryonic kidney; CaCCs, Ca^{2+}-activated Cl^- channels; TA, tannic acid; BK, bradykinin

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contribution of Ca^{2+}-activated Cl^- channels (CaCCs)/TMEM16A to sensory neuron excitability and found that inhibition of CaCCs/TMEM16A decreased firing of sensory neuron and relieved pain (Liu et al., 2010). Recently, tannic acid has been found to be an inhibitor of CaCCs/TMEM16A (Namkung et al., 2010).

Bradykinin (BK) has long been known as one of the most potent endogenous pain-inducing substances. We discovered that inhibition of M/Kv7 currents and activation of CaCCs contributed to bradykinin-induced acute pain in rats (Liu et al., 2010). In the present study, we confirm the inhibitory effect on CaCCs and describe novel stimulatory effects on M/Kv7 and inhibitory effect on voltage-gated Na^+ currents for tannic acid, and suggest a potential analgesic usage of tannic acid in treating inflammatory pain such as arthritis and burn injury.

2. Materials and methods

2.1. cDNA constructs

Plasmids encoding human Kv7.2 and Kv7.3 were kindly provided by Diomedes E. Logothetis (Virginia Commonwealth University, Richmond, VA, USA) (Zhang et al., 2003) and subcloned into pcDNA3. Kv7.2(W236L) mutant was kindly provided by Min Li (Johns Hopkins University, Baltimore, MD, USA).

2.2. Rat DRG cell culture

Use of animal in this study was approved by Animal Care and Ethical Committee of Hebei Medical University (Shijiazhuang, China). DRGs were extracted from all spinal levels of 1-week-old Sprague-Dawley rats. Ganglia were placed in modified D-Hanks' solution, and digested at 37 °C with collagenase (2 mg/ml, Worthington Biochemicals, Freehold, NJ) for 25 min, followed by another 20 min digestion with trypsin (2.5 mg/ml, Sigma-Aldrich, St. Louis, MO, USA). They were subsequently suspended at least twice in Dulbecco's modified Eagle's mediums (DMEM) plus 10% fetal bovine serum (PAA Laboratories GmbH, Linz, Austria) to stop digestion. Ganglia were then dissociated into a suspension of individual cells and plated on poly-D-lysine-coated glass coverslips in 24-well tissue culture plates. Cells were incubated at 37 °C with a 5% CO_2 and 95% air atmosphere. Electrophysiological recordings were made from cells maintained in culture from day 3 up to day 7.

2.3. HEK293 cell culture and transfection

HEK293 cells were cultured in DMEM supplemented with 10% fetal bovine serum and antibiotics in a humidified incubator at 37 °C (5% CO_2). Cells were seeded on glass coverslips in a 24-multiwell plate and transfected when 60–70% confluence was reached. For transfection of 6 wells of cells, a mixture of 2 μg KCNQ2/1 μg KCNQ2 + 1 μg KCNQ3, 2 μg pERFP cDNAs and 3 μl lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) were prepared in 1.2 ml of DMEM and incubated for 20 min. The mixture was then applied to the cell culture wells and incubated for 4 h. Recordings were made 24 h after cell transfection and cells were used within 72 h.

2.4. Electrophysiology

Perforated whole-cell patch recordings were performed on DRG neurons and HEK293 cells. Recordings were made at room temperature (23–25 °C). Pipettes were pulled from borosilicate glass capillaries and had resistances of 1.5–2.5 MΩ when filled with internal solution. Currents were recorded using an Axon patch 700B amplifier and pClamp 10.0 software (Axon Instruments, Foster City, CA, USA), and were filtered at 2 kHz. For perforated patch recording, a pipette was first front-filled with the standard internal solution, then backfilled with the same internal solution containing amphotericin B (250 μg/ml). The external solution used to record action potential and M/Kv7 currents contained (in mM): 160 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 8 glucose, pH 7.4. The internal solution consisted of (in mM): 150 KCl, 5 $MgCl_2$, 10 HEPES, pH 7.4.

2.5. Behavioral studies

Sprague-Dawley rats (body weight, 180–220 g) were randomly grouped and allowed to acclimatize for at least 20 min in a transparent observation chamber before the experiment. The right hind paw of the animal received an intraplantar injection (50 μl) of BK (10 nmol/site) or saline, and the nocifensive responses (licking, biting, lifting, and flinching) were recorded using a video camera for 20 min. The videos were analyzed by an observer unaware of treatment allocations. In the experiments with saline, tannic acid and retigabine were preinjected in the same plantar of the hind paw where 5 min later BK or saline was injected. Drugs were dissolved in saline (pH 7.4) from stock solutions and applied in a volume of 50 μl at the following doses: retigabine, 5 nmol/site; tannic acid, 50 nmol/site.

2.6. Drugs

Tannic acid and bradykinin was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). RTG and XE991 were synthesized in the Department of New Drug Development, School of Pharmacy, Hebei Medical University. The stock solutions were made in DMSO and were stored at −20 °C. All solutions were freshly prepared from stock solutions before each experiment and kept from light exposure. The final concentration of DMSO was less than 0.1%.

2.7. Statistical analysis

Currents were analyzed and fitted using Clampfit 10.2 (Molecular Devices, Sunnyvale, CA) and Origin 7.5 (OriginLab Corp., Northampton, MA) software. The concentration–response curve was fitted with logistic equation: $y=A_2+(A_1-A_2)/[1+(x/x_0)^p]$, where y is the response; A_1 and A_2 are the maximum and minimum response, respectively, x is the drug concentration, x_0 is the EC_{50}, and p is the Hill coefficient. The current activation curves were generated by plotting the normalized tail current amplitudes against the step potentials and were fitted with a Boltzmann function: $y=A/\{1+\exp[(V_h - V_m)/k]\}$, where A is the maximal current amplitude, V_h is the voltage for half-maximal activation, V_m is the test potential and k is the slope factor. All data are given as means ± S.E.M. Differences between groups were assessed by Student's t-test or 1-way ANOVA. The differences were considered significant at $P \leq 0.05$.

3. Results

3.1. Tannic acid increased the M/Kv7 K^+ currents and hyperpolarized the resting membrane potential in small DRG neurons

M/Kv7 K^+ currents play a critical role in regulating neuronal excitability and stabilizing membrane potential (Brown and Yu, 2000; Jentsch, 2000). In a preliminary experiment, we found that tannic acid was able to potentiate M/Kv7 currents in small DRG neurons. We then performed a detailed investigation into this unexpected effect of tannic acid. Recordings were made