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Inhibition of acid-sensing ion channels by chlorogenic acid in rat dorsal root ganglion neurons

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HIGHLIGHTS

- We provide evidences that the activity of ASICs is modulated by chlorogenic acid.
- Chlorogenic acid, one of the most abundant polyphenols in the human diet, relieves acidosis-evoked pain.
- We revealed for the first time a cellular and molecular mechanism underlying chlorogenic acid analgesia.

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ABSTRACT

Chlorogenic acid (CGA) is one of the most abundant polyphenol compounds in the human diet. Recently, it is demonstrated to have potent antinociceptive effect. However, little is understood about the mechanism underlying CGA analgesia. Here, we have found that CGA can exert an inhibitory effect on the functional activity of native acid-sensing ion channels (ASICs) in rat dorsal root ganglion (DRG) neurons. First, CGA decreased the peak amplitude of proton-gated currents mediated by ASICs in a concentration-dependent manner. Second, CGA shifted the proton concentration–response curve downward, with a decrease of 41.76 ± 8.65% in the maximum current response to protons but with no significant change in the $pH_{0.5}$ value. Third, CGA altered acidosis-evoked membrane excitability of rat DRG neurons and caused a significant decrease in the amplitude of the depolarization and the number of action potentials induced by acid stimuli. Finally, peripheral administered CGA attenuated nociceptive response to intraplantar injection of acetic acid in rats. ASICs are distributed in peripheral sensory neurons and participate in nociception. Our findings CGA inhibition of native ASICs indicated that CGA may exert analgesic action by modulating ASICs in the primary afferent neurons, which revealed a novel cellular and molecular mechanism underlying CGA analgesia

1. Introduction

Chlorogenic acid (5-caffeoylquinic acid, CGA) is formed by the esterification of caffeic and quinic acids. It is one of the most abundant polyphenols in the human diet and is found ubiquitously in plants, fruits and vegetables. CGA has shown to exert a number of important biological activities including anti-inflammatory, antioxidant, antigenotoxic and neuroprotective properties [5,15,24,26]. In addition, CGA is also demonstrated to have potent antinociceptive effect. In many countries, medicinal plants *Urtica urens, Urtica circularis* and *Cheilanthes farinosa* are used to relieve pain which were attributed to the CGA content [8,16,25]. As an analgesic, CGA has been found to alleviate pains in several animal models, such as inflammatory, neuropathic and visceral pain [2,5,8,10]. The mechanisms underlying CGA analgesia are thought to be related to its anti-inflammatory activity, because CGA has an inhibitory effect on peripheral synthesis or release of some inflammatory mediators such as tumor necrosis factor-α, nitric oxide and interleukins [3,5,13]. So far, it is unclear whether CGA can directly influence ion channels in nociceptive sensory terminals.

Acid-sensing ion channels (ASICs) are members of proton-gated cation channels. To date, at least four genes encoding seven ASIC subunits have been identified in mammals [4]. Among the ASIC subunits, ASIC3 have emerged as critical pH sensors predominantly expressed in nociceptors, where they have been suggested to be important for nociception [1,4]. Proton is a canonical ligand for ASICs and releases under multiple pathological conditions. It is

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well known that the local extracellular pH levels drop to 5.4 in acute inflammation [14]. The accumulating protons are sufficient to stimulate ASICs and activate nociceptors [7]. ASICs have been shown to play an important role in various pain conditions such as inflammatory and neuropathic pain [11,23].

The present study investigates whether CGA can modulate the functional activity of ASICs in rat dorsal root ganglion (DRG) neurons, aiming to further reveal mechanisms underlying the CGA-induced analgesia.

2. Materials and methods

2.1. Isolation of the DRG neurons

The experimental protocol was approved by the animal research ethics committee of Hubei University of Science and Technology (approval no. 2068). Ten–twelve-week old Sprague–Dawley male rats were anesthetized with ethyl ether. The DRGs were taken out and transferred immediately into DMEM at pH 7.4. After the removal of the surrounding connective tissues, the DRGs were minced with fine spring scissors and the ganglion fragments were placed in a flask containing 5 mL of DMEM containing trypsin (type II-S) 0.5 mg/mL, collagenase (type I-A) 1.0 mg/mL and DNase (type IV) 0.1 mg/mL, and incubated at 35 °C in a shaking water bath for 25–30 min. Soybean trypsin inhibitor (type II-S) 1.25 mg/mL was then added to stop trypsin digestion. Dissociated neurons were placed into a 35 mm Petri dish and kept for at least another 60 min before electrophysiological recordings. The neurons selected for electrophysiological experiment were 15–35 μm in diameter.

2.2. Electrophysiological recordings

Whole-cell patch clamp and voltage-clamp recordings were carried out at room temperature (22–25 °C) using a MultiClamp-700B amplifier and Digidata-1440A A/D converter (Axon Instruments, Foster City. CA, USA). Recording micropipettes were filled with internal solution containing KCl 140 mM, $MgCl_2$ 2.5 mM, HEPES 10 mM, EGTA 11 mM and ATP 5 mM and the pH was adjusted to 7.2 with KOH. Cells were bathed in an external solution containing NaCl 150 mM, KCl 5 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 2 mM, HEPES 10 mM and D-glucose 10 mM and the osmolarity was adjusted to 330 mOsm/L with sucrose and pH to 7.4. The resistance of the recording pipette was in the range of 3–6 MΩ. A small patch of membrane underneath the tip of the pipette was aspirated to form a gigaseal and then negative pressure was applied to rupture it. The adjustment of capacitance compensation and series resistance compensation was done before recording the membrane currents. Current-clamp recordings were obtained by switching to current-clamp mode after a stable whole-cell configuration was formed in voltage-clamp mode. Only cells with a stable resting membrane potential (more negative than −50 mV) were used in the study. Signals were sampled at 10–50 kHz and filtered at 2–10 kHz, and the data were stored in compatible PC computer for off-online analysis using the pCLAMP 10 acquisition software (Axon Instruments).

2.3. Drug application

CGA, hydrochloric acid, amiloride, capsazepine and tetrodotoxin (TTX) were bought from Sigma–Aldrich (St. Louis, MO, US). Stocks of drugs were made up and stored at −20 °C. They were diluted daily in the external solution at a minimum of 1:1000 to a final working concentration. Except for hydrochloric acid, the pH for all working solutions was adjusted daily to 7.4 ± 0.1 with HCl or NaOH.

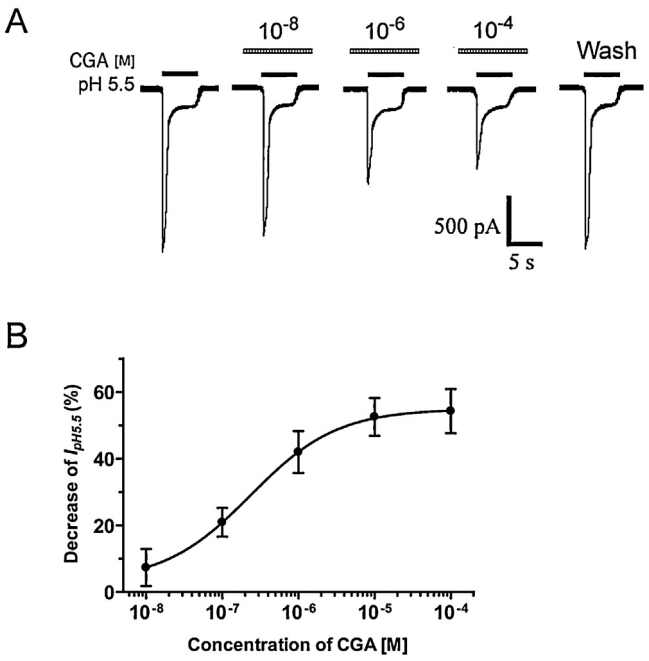

Fig. 1. CGA concentration-dependently inhibited proton-gated currents in rat DRG neurons. A and B. Representative current traces and summary data show CGA inhibited the peak amplitude of proton-gated currents in a concentration-dependent manner. The current was evoked by a rapid drop extracellular pH from 7.4 to 5.5 for 5 s in the presence of capsazepine (10^{-5} M) to block TRPV1. DRG neurons with membrane potential clamped at −60 mV. Each point represents the mean SEM of 7–9 neurons.

The application of each drug was driven by gravity and controlled by the corresponding valve.

2.4. Nociceptive behavior induced by acetic acid in rats

Rats were placed in a 30 cm × 30 cm × 30 cm Plexiglas chamber and allowed to habituate for at least 30 min before nociceptive behavior experiments. After pretreatment with 10 μL capsazepine (100 μM), a double-blind experiment was carried out. Twenty microliters of acetic acid solution (0.6%) together with 20 μL vehicle (external solution) and different doses of CGA (0.01, 0.1, 1, 10 μM) were coded, and the other experimenters subcutaneously administered them into the dorsal face of the hind paw. Nociceptive behavior (that is, number of flinches) was counted over a 5 min period starting immediately after the injection [4].

2.5. Data analysis

Data were statistically compared using the Student's *t*-test or analysis of variance (ANOVA), followed by Bonferroni's post hoc test. Statistical analysis of concentration–response data was performed using nonlinear curve-fitting program ALLFIT. Data are expressed as mean ±standard error of the mean (SEM).

3. Results

3.1. CGA inhibited ASIC currents in rat DRG neurons

To functionally characterize ASIC currents, we measured proton-gated currents in the presence of capsazepine (10 μM) to block proton-induced TRPV1 activation in the whole-cell patch-clamp configuration [17]. As previous observation [18,19], a rapid drop of extracellular pH from 7.4 to 5.5 for 5 s evoked a large inward current ($I_{pH5.5}$) in most native DRG neurons (71.77%, 89/124).