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Local subcutaneous injection of chlorogenic acid inhibits the nociceptive trigeminal spinal nucleus caudalis neurons in rats

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ABSTRACT

Acute administration of chlorogenic acid (CGA) *in vitro* was recently shown to modulate potassium channel conductance and acid-sensing ion channels (ASICs) in the primary sensory neurons; however, *in vivo* peripheral effects of CGA on the nociceptive mechanical stimulation of trigeminal neuronal activity remains to be determined. The present study investigated whether local administration of CGA *in vivo* attenuates mechanical stimulation-induced excitability of trigeminal spinal nucleus caudalis neuronal (SpVc) activity in rats. Extracellular single-unit recordings were made of SpVc wide-dynamic range (WDR) neuronal activity elicited by non-noxious and noxious orofacial mechanical stimulation in pentobarbital anesthetized rats. The mean number of SpVc WDR neuronal firings responding to both non-noxious and noxious mechanical stimuli were significantly and dose-dependently inhibited by local subcutaneous administration of CGA (0.1–10 mM), with the maximal inhibition of discharge frequency revealed within 10 min and reversed after approximately 30 min. The mean frequency of SpVc neuronal discharge inhibition by CGA was comparable to that by a local anesthetic, the sodium channel blocker, 1% lidocaine. These results suggest that local CGA injection into the peripheral receptive field suppresses the excitability of SpVc neurons, possibly *via* the activation of voltage-gated potassium channels and modulation of ASICs in the nociceptive nerve terminal of trigeminal ganglion neurons. Therefore, local injection of CGA could contribute to local anesthetic agents for the treatment of trigeminal nociceptive pain.

1. Introduction

The trigeminal spinal nucleus is an important relay station in transmitting orofacial sensory information. This nucleus is functionally subdivided into three nuclei from rostral to caudal: oralis, interpolaris, and caudalis (Sessle, 2000). Neurons of the spinal trigeminal nucleus caudalis (SpVc) and upper cervical dorsal horn also act as important relay stations for trigeminal nociceptive inputs from inflamed and injured tissue (Sessle, 2000; Takeda et al., 2012). Recently, Takehana et al. (2016) reported that in the absence of inflammatory and neuropathic pain, acute intravenous administration of resveratrol, the polyphenol in red wine, suppresses the SpVc wide-dynamic range (WDR) neurons via both peripheral and central mechanisms. We also found that subcutaneous local injection of resveratrol into the peripheral receptive field suppresses the excitability of SpVc neurons, possibly by inhibiting voltage-dependent Na^+ channels in the nociceptive nerve terminals of trigeminal ganglion (Shimazu et al., 2016). Therefore, local injection of this dietary constituent could elicit a similar effect to that observed with injection of a local anesthetic agent, providing relief from trigeminal nociceptive pain.

Chlorogenic acid (5-caffeoylquinic acid, CGA) is a natural polyphenolic compound found in many plant species consumed by humans, including fruits and vegetables. CGA exhibits various biological effects with well-known therapeutic potential, including antioxidant, neuroprotective, anticancer, and anti-inflammatory (Bagdas et al., 2013; Kang et al., 2013; Cinklic et al., 2013; Shen et al., 2012). Recently, Zhang et al. (2014) showed that CGA modulates the neuronal excitability of trigeminal ganglion (TG) neurons via voltage-gated potassium ion (Kv) channels. In addition, a rat formalin test showed that resveratrol induced peripheral antinociception *in vivo* via the opening of several Kv channels (Grannados-Soto et al., 2002). Since the opening of K^+ channels leads to hyperpolarization of the resting membrane potential, and in turn, decreased neuronal excitability, several types of Kv channels have been proposed as potential target candidates for pain therapy (Takeda et al., 2011b).

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It has also been suggested that acid-sensing ion channels (ASICs) might be mammalian cutaneous mechanoreceptor (Price et al., 2001; Borzan et al., 2010; Kang et al., 2012). Although it is unlikely that ASICs directly transduce mechanical stimuli, Kang et al. (2012) indicate that ASICs subunits influence cutaneous mechanosensitivity. Qu et al. (2014) recently demonstrated that CGA inhibit neuronal firing at the dorsal root ganglion (DRG) via blockade of ASICs and modulating the neuronal excitability of DRG neurons. Taken together, these observations suggest that local subcutaneous application of CGA into the receptive field of SpVc WDR neurons could suppress nociceptive pain transmission at the peripheral level, although the acute effect of CGA on nociceptive and non-nociceptive mechanical stimulation-induced SpVc WDR neuronal activity *in vivo* remains unknown. The present study therefore further investigated the *in vivo* effects of local subcutaneous CGA injections on SpVc WDR neuronal activity. We also compared the potency of suppressing trigeminal nociception between CGA and a clinically used local anesthetic agent.

2. Materials and methods

The experiments were approved by the Animal Use and Care Committee of Azabu University and were consistent with the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983). Every effort was made to minimize the number of animals used and their suffering.

2.1. Extracellular single-unit recording from WDR neuronal activity in the SpVc

Electrophysiological recordings were conducted on 20 adult male Wistar rats (230–280 g, body weight). Each animal was first anesthetized with pentobarbital sodium (45 mg/kg, i.p.) and maintained with additional doses of 2–3 mg/kg/h through a cannula into the jugular vein, as required. The level of anesthesia was confirmed by absence of the corneal reflex and no response to paw pinching. The rectal temperature was maintained at 37 ± 0.5 °C with a homeothermic blanket during recording. The animals were then placed in a stereotaxic apparatus, and the activity of a single neuron from the SpV_C region was recorded extracellularly using a glass micropipette filled with 2% pontamine sky blue and 0.5 M sodium acetate according to the stereotaxic coordinates of Paxinos and Watson (1986). Neuronal activity was amplified (DAM80: World Precision Instruments), filtered (0.3–10 kHz), monitored with an oscilloscope (Iwatsu, SS-7672, Tokyo), and recorded on a polygraph (NEC-Sanei 8M14). Off-line analysis used Power Lab and Chart 5 software (ADI Instruments, Oxford, UK).

2.2. Experimental protocols

Recordings of the extracellular SpVc WDR unit activity were carried out as follows and as previously described (Takehana et al., 2016; Sekiguchi et al., 2016; Shimazu et al., 2016). Mechanical stimulation was used as a search stimulus to identify the repetitive field quickly and to avoid sensitizing the peripheral receptors. A single unit that responded to stimulating the left orofacial skin (whisker pad) with a brush and a set of von Frey hairs (Semmes-Weinstein Monofilaments, North Coast Medical, Gilroy, CA, USA) was identified. Noxious pinch stimulation was applied to the orofacial area with forceps that evoke a pain sensation when applied to a human subject. After identifying WDR SpVc neurons responding to the whisker pad, we determined whether there was a spontaneous discharge. The threshold for mechanical stimulation was then determined using non-noxious and noxious mechanical stimulation (5s) of von Frey hairs (2, 4, 6, 10, 15, 26, 60 g) at 5-s intervals. The mechanical receptive fields were mapped by probing the facial skin von Frey hairs, and then outlined on life sized drawings of a rat on tracing paper. The WDR neuronal discharges induced by mechanical stimulation were quantified by subtracting the background activity from the evoked activity. Spontaneous discharge frequencies were determined over 2–5 min. If no discharge was recorded, the cell was deemed a silent neuron. Mean firing rates of SpVc WDR neurons evoked by mechanical stimulation were compared before and after drug applications, based on previous findings that SpVc WDR neurons are important in the mechanism underlying hyperalgesia and referred pain associated with orofacial pain (Takeda et al., 2000, 2005, 2012; Nishikawa et al., 2004). Post-stimulus histograms (bin = 100 ms) were generated in response to each stimulus. The effect of subcutaneous administration of CGA (0.05 ml, 0.1, 1, and 10 mM) and lidocaine (1% Xylocaine equal to 37 mM) through a Hamilton microsyringe was evaluated 5, 10, 20, 30, and 40 min after administration because the peak effect and recovery was observed in this period. CGA was dissolved in dimethyl sulfoxide (DMSO) and stock solution was stored at −20 °C. Mean spontaneous, mechanical stimulation-discharges rates, mechanical threshold before and after local administration of CGA were analyzed in this study.

2.3. Identification of recording site

The recording sites of SpVc WDR neuronal activity were identified as described previously (Takeda et al., 2000, 2012; Takehana et al., 2016). In brief, following the recordings, rats were deeply anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and anodal DC currents (30 μA, 5 min) were passed through a recording micropipette. The animals were transcardially perfused with saline and 10% formalin. Frozen coronal sections were cut into 30-μm sections and stained with hematoxylin-eosin. Recording sites were identified as blue spots, and the electrode tracks were constructed using a combination with micromanipulator readings.

2.4. Data analysis

Values are expressed as means ± SEM. Statistical analysis was performed using two-way repeated measure analysis of variances (ANOVAs) followed by the Tukey-Kramer/Dunnett's tests (post hoc test) for electrophysiological data. $P < 0.05$ was considered statistically significant.

3. Results

3.1. SpVc WDR neuronal activity innervating facial skin

Extracellular single-unit activity was recorded for 20 neurons in the SpVc to determine post-induction neuronal excitability, following subcutaneous CGA administration (16 neurons) or subcutaneous lidocaine administration (4 neurons). These SpVc neurons exhibited a somatic receptive field in the orofacial area (mainly whisker pad) in response to non-noxious and noxious mechanical stimulation (Fig. 1A), as described in previous studies (Takeda et al., 2000, 2012); they also responded to mechanical stimulation of the receptive field innervated by ophthalmic and maxillary branches. Three units showed spontaneous discharges. Histological examination revealed recording sites in layers I- III (n = 12, 60%) and IV–V (n = 8, 40%) (depth, 130–780 μm) of the SpVc (obex, −0.25 to −2.0 mm), and there was no obvious difference in location of the recording sites between the CGA and lidocaine effects on neuronal discharges. Typical examples of the SpVc WDR neuronal unit responses are shown in Fig. 1A. Graded mechanical stimulation was applied to the most sensitive area of the receptive field, which

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