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Systemic administration of the dietary constituent resveratrol inhibits the nociceptive jaw-opening reflex in rats *via* the endogenous opioid system

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ABSTRACT

The aim of the present study was to investigate whether, under *in vivo* conditions, systemic administration of resveratrol could attenuate the rat nociceptive jaw-opening reflex (JOR) *via* the endogenous opioid system. The JOR evoked by electrical stimulation of the tongue was recorded as digastric muscle electromyograms (dEMG) in pentobarbital-anesthetized rats. The amplitude of the dEMG increased significantly in proportion to the intensity of electrical stimulation (from 1× to 5 × threshold for the JOR). dEMG amplitude in response to 3× threshold electrical stimulation of the tongue was dose-dependently inhibited by intravenous administration of resveratrol (0.5–2 mg/kg). Maximum inhibition of dEMG amplitude was seen within approximately 10 min. These inhibitory effects were reversible, with dEMG responses returning to control levels after approximately 20 min. Pretreatment of rats with naloxone resulted in significant, dose-dependent attenuation of the inhibitory effects of resveratrol on dEMG amplitude compared with control. These findings suggest that resveratrol inhibits the nociceptive JOR *via* the endogenous opioid system. Further, the findings of the present study strongly support the idea that resveratrol, which is not known to have any toxic side effects, combined with an opioid could be a potential therapeutic agent for the prevention of acute trigeminal nociception.

1. Introduction

Resveratrol is plant polyphenol found in red wine and various food products (Fremont, 2000; Pervaiz, 2003). Resveratrol has been reported to have several beneficial biological actions, including anti-oxidative, anti-inflammatory, neuroprotective, anticancer, and cardioprotective effects (Leiro et al., 2005; Bermudez-Ocasna et al., 2006; Perez-Severiano et al., 2008; Pervaiz, 2003). Recent reports have described the use of complementary and alternative medicines (CAM), such as herbal medicines and acupuncture, for the treatment of persistent clinical chronic pain (Rao et al., 1999; Konvicka et al., 2008; Rosenberg et al., 2008), and considerable research has focused on the potential effects of diet and dietary supplementation on conditions associated with pain (Shir et al., 2001; Ernest, 2003; Tall and Raja, 2004). Because resveratrol has no known toxic side effects (Russo, 2007), it could be a candidate CAM for the therapeutic treatment of pain (Takeda et al., 2016). Takehana et al. (2016) reported that acute intravenous administration of resveratrol suppresses nociceptive trigeminal spinal nucleus caudalis (SpVc) wide-dynamic range (WDR) neurons *via* both peripheral and central mechanisms. Although local injection of resveratrol into the peripheral receptive field suppresses the excitability of SpVc neurons, possibly *via* inhibition of Na^+ channels in the nociceptive nerve terminals of trigeminal ganglion neurons (Shimazu et al., 2016), the central mechanisms involved in the inhibitory effects of resveratrol on nociceptive transmission remain to be determined.

Because the jaw-opening reflex (JOR) induced by electrical stimulation of the orofacial tissues, such as, tooth pulp (TP) is a valid index of reflex responses to noxious stimuli (trigeminal nociceptive reflex; Mahan and Anderson, 1970; Mason et al., 1985; Takeda et al., 1998), the JOR threshold is used as an indicator of the intensity of stimulus applied to the TP. It has been demonstrated previously that vagal afferent stimulation attenuates the activity of the trigeminal spinal nucleus oralis (SpVo) and the related JOR *via* the endogenous pain control system (Takeda et al., 1998). Ellrich (2004) reported

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electrical stimulation of tongue- evoked JOR was valid model for nociception. In addition, it has been reported that systemic administration of resveratrol results in dose-dependent antinociceptive effects *via* an opioidergic mechanism (Gupta et al., 2004). In that study, pretreatment of rats with naloxone blocked the analgesic effects of resveratrol. Together, these findings led us to speculate that intravenous injection of resveratrol would inhibit the nociceptive JOR *via* the endogenous pain control system. Thus, the aim of the present study was to investigate whether, under *in vivo* conditions, intravenous administration of resveratrol attenuated the rat nociceptive JOR *via* the endogenous opioid system.

2. Materials and methods

The experiments described herein were approved by the Animal Use and Care Committee of Azabu University and were performed in accordance with the 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. Animal preparation

Electrophysiological recordings were made in 22 adult male Wistar rats weighing 230–280 g. Rats were anesthetized with pentobarbital sodium (45 mg/kg, i.p.) and anesthesia was maintained with additional doses of 2–3 mg/kg per h pentobarbital sodium administered through a cannula into the jugular vein, as required. The level of anesthesia was confirmed by the absence of the corneal reflex and a lack of response to paw pinching. Rectal temperature was maintained at 37.0 ± 0.5 °C with a homeothermic blanket during recording.

2.2. Recording of digastric muscle electromyograms in response to electrical stimulation of the tongue

Bipolar stimulating electrodes made from stainless steel wire (150 μm diameter, enamel insulated except for 0.5–1.0 mm at the tip) were inserted longitudinally into the tongue, as described previously (Ellrich, 2004). Electrical stimulation with constant current single pulses (0.1–3.8 mA, 0.1 ms, 1 Hz) was delivered through the bipolar stimulating electrodes using PowerLab and Chart 5 software (AD Instruments, Oxford, UK). Using stainless steel electrodes (interpolar distance 2 mm, insulated except for the tip), digastric electromyograms (dEMG) were recorded from the ipsilateral anterior belly of the digastric muscle as an indicator of the JOR. Electromyogram activity was recorded using PowerLab and Chart 5 software (ADInstruments). Recordings of dEMG activity in response to electrical stimulation of the tongue and data analyses were performed as reported previously (Takeda et al., 1998, 2002). To determine the threshold for the JOR from the dEMG, electrical stimulation was applied with a pulse duration of 0.1 ms at a stimulation frequency of 1 Hz, and the pulse intensity was increased until three responses to TP stimulation were obtained from five consecutive trials. The peak-to-peak amplitudes from five stimulus trials were averaged.

2.3. Experimental protocol

The effects of resveratrol (0.5, 1, and 2 mg/kg, i.v.; equivalent to 1, 5, and 10 mM, respectively), injected through a cannula into the jugular vein, were evaluated 5, 10, 20, and 30 min after administration because peak effect and recovery were thought to occur during this period. Resveratrol was dissolved in dimethyl sulfoxide (DMSO). The stock solution was stored at −20 °C in small aliquots until use and diluted in saline to the desired concentrations. Because the nociceptive JOR is considered a valid model of pain if it is evoked by adequate nociceptive stimulation (*e.g.* using a stimulus that is between 3× and 5× the threshold [3T and 5T, respectively] of the JOR, a threshold very close to the sensory threshold in human volunteers; Mason et al., 1985; Sotigiu and Bellinzona, 1991; Takeda et al., 1998, 2002), the mean dEMG amplitude, averaged across five stimulus trials, in response to electrical stimulation (3T) of the tongue was evaluated before and after intravenous administration of resveratrol. In addition, the effects of the opioid antagonist naloxone (0.5–1 mg/kg, i.v.) were evaluated using procedures reported in previous studies, which found that the peak effect of naloxone was observed approximately 10 min after its administration (Gupta et al., 2004; Takeda et al., 1998).

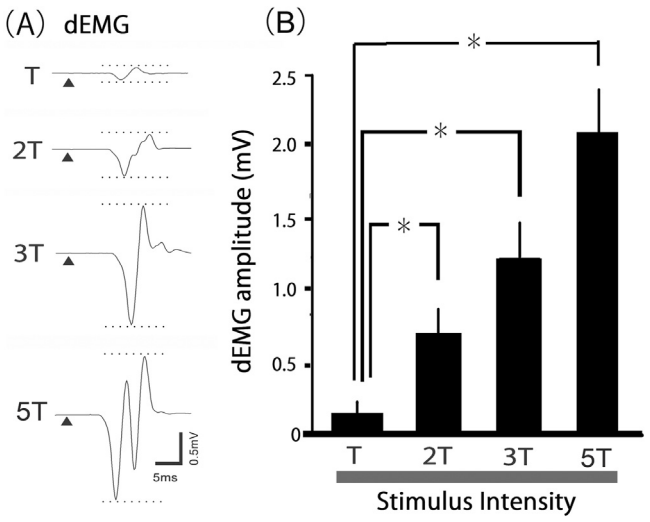

Fig. 1. Changes in digastric muscle electromyograms (dEMG) in response to electrical stimulation of tongue. (A) Changes on the dEMG in response to electrical stimulation of the tongue (1×–5× the threshold for the jaw-opening reflex [JOR]). (B) Summary of changes in the dEMG amplitude following electrical stimulation of the tongue at stimulus intensities between 1× and 5× the JOR threshold. The mean dEMG amplitude increased with increasing stimulus intensity. *$P < 0.05$, n = 9. Filled triangles indicate electrical stimulation of the tongue. T (threshold) *vs.* 2T, 3T and 5T.

2.4. Data analysis

Values are expressed as the mean ± SEM. Statistical analyses were performed using two-way repeated-measures analysis of variance (ANOVA) followed by Tukey–Kramer or Dunnett's post hoc tests for electrophysiological data. $P < 0.05$ was considered significant.

3. Results

3.1. Changes in dEMG activity in response to electrical stimulation of the tongue

Electrical stimulation of the tongue induced reflex responses of the digastric muscle with a latency of 5.4 ± 0.2 ms (n = 22). The mean threshold intensity was 0.2 ± 0.1 mA (n = 22). As shown in Fig. 1A, the peak-to-peak amplitude on the dEMG increased in proportion with increasing stimulation intensity (from 1T to 5T). These results are summarized in Fig. 1B. There was a significant increase in the mean dEMG amplitude with increasing stimulation intensity (from 1T to 5T; n = 5, F = 39.0, $P < 0.05$).

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