Brain Research Bulletin 120 (2016) 117–122

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Systemic administration of resveratrol suppress the nociceptive neuronal activity of spinal trigeminal nucleus caudalis in rats

Shiori Takehana[a], Kenta Sekiguchi[a], Maki Inoue[b], Yoshiko Kubota[c], Yukihiko Ito[c], Kei Yui[c], Yoshihito Shimazu[a], Mamoru Takeda[a,*]

[a] Laboratory of Food and Physiological Sciences, Department of Life and Food Sciences, School of Life and Environmental Sciences, Azabu University, 1-17-71, Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan
[b] Laboratory of Physiology II, Department of Veterinary Science, School of Veterinary Medicine, Azabu University, 1-17-71, Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan
[c] Fancl Health Science Research Center, Research Institute, FANCL Corporation, 12-13, Kamishinano, Totsuka-ku, Yokohama, Kanagawa 244-0806, Japan

ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form
12 November 2015
Accepted 16 November 2015

Keywords:
Nociception
Polyphenol
Resveratrol
Spinal trigeminal nucleus caudalis
Single unit recording
Alternative medicine

ABSTRACT

Although a modulatory role has been reported for the red wine polyphenol resveratrol on several types of ion channels and excitatory synaptic transmission in the nervous system, the acute effects of resveratrol in vivo, particularly on nociceptive transmission of the trigeminal system, remain to be determined. The aim of the present study was to investigate whether acute intravenous resveratrol administration to rats attenuates the excitability of wide dynamic range (WDR) spinal trigeminal nucleus caudalis (SpVc) neurons in response to nociceptive and non-nociceptive mechanical stimulation in vivo. Extracellular single unit recordings were made from 18 SpVc neurons in response to orofacial mechanical stimulation of pentobarbital-anesthetized rats. Responses to both non-noxious and noxious mechanical stimuli were analyzed in the present study. The mean firing frequency of SpVc WDR neurons in response to both non-noxious and noxious mechanical stimuli was inhibited by resveratrol (0.5–2 mg/kg, i.v.) and maximum inhibition of the discharge frequency of both non-noxious and noxious mechanical stimuli was seen within 10 min. These inhibitory effects were reversed after approximately 20 min. The relative magnitude of inhibition by resveratrol of SpVc WDR neuronal discharge frequency was significantly greater for noxious than non-noxious stimulation. These results suggest that, in the absence of inflammatory or neuropathic pain, acute intravenous resveratrol administration suppresses trigeminal sensory transmission, including nociception, and so resveratrol may be used as a complementary and alternative medicine therapeutic agent for the treatment of trigeminal nociceptive pain, including hyperalgesia.

1. Introduction

Resveratrol (*trans*-3,4′,5-trihydroxystilbene), is plant polyphenol that is found in red wine and various food products (Fremont, 2000; Pervaiz, 2003). Resveratrol has 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 the potential effects of diet and dietary supplementation on conditions associated with pain have been the focus of considerable research (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.

Recent reports suggest that resveratrol modulates the neuronal excitability of the peripheral and central nervous systems via transient receptor potential (TRP) channels and voltage-dependent ion channels (Grannados-Soto et al., 2002; Kim et al., 2005; Liew et al., 2005; Gao and Hu, 2005; Yu et al., 2013). For example, TRP ankyrin 1 (TRPA1) modulates mechanotransduction in primary sensory neurons (Kwan et al., 2009), and it has been demonstrated that

Abbreviations: SpVc, trigeminal spinal nucleus caudalis; WDR, wide dynamic range; CAM, complementary and alternative medicine; TRP, transient receptor potential; TRPA1, TRP ankyrin 1; DRG, dorsal root ganglion; ANOVA, analysis of variance; PAG, periaqueductal gray; 5-HT_3 receptor, 5-hydroxytrypytamine type 3 receptor.

* Corresponding author. Fax: +81 42 769 2212.
E-mail address: m-takeda@azabu-u.ac.jp (M. Takeda).

http://dx.doi.org/10.1016/j.brainresbull.2015.11.011

resveratrol is a potent inhibitor of TRPA1 in vitro and in vivo (Yu et al., 2013), suggesting that resveratrol attenuates the generator potential via the mechanical transduction process. In addition, resveratrol modulates the sodium and potassium currents in dorsal root ganglion (DRG) neurons that are associated with the generation of action potentials (Kim et al., 2005; Grannados-Soto et al., 2002). Conversely, in the hippocampal slice preparation, resveratrol significantly suppressed the glutamate-induced currents in post-synaptic CA1 pyramidal neurons (Gao et al., 2006) that contribute to excitatory synaptic transmission. In fact, there are reports that resveratrol decreases action potential duration and L-type Ca^{2+} currents in excitable tissues (Liew et al., 2005). Together, these findings strongly suggest that systemic resveratrol administration may suppress sensory transmission, including nociception, in the both peripheral and central nervous systems.

The spinal trigeminal nucleus is an important relay station in the transmission of orofacial sensory information and this nucleus is functionally subdivided into three nuclei (from rostral to caudal): oralis, interpolarlis and caudalis (Sessle, 2000). It is well known that, in addition to the upper cervical (C1–C2) dorsal horn, the spinal trigeminal nucleus caudalis (SpVc) is an important relay station for trigeminal nociceptive inputs from inflammation and tissue injury (Takeda et al., 2012). Chronic pathological conditions, such as tissue inflammation can, change the properties of somatic sensory pathways, leading to hyperalgesia and allodynia (Scholz and Woolf, 2002). Changes in the excitability of primary afferent neurons (peripheral sensitization) alter information processing in the spinal trigeminal nucleus or higher centers (Millan, 1999). Previous studies have demonstrated that wide dynamic range (WDR) neurons in the SpVc region have an important role in the mechanism underlying hyperalgesia/allodaynia and/or referred pain associated with orofacial pain (Takeda et al., 2000, 2005, 2012; Nishikawa et al., 2004). In addition, intravenous administration of lidocaine, a sodium channel blocker, has been shown to attenuate the trigeminal nociceptive reflex and nociceptive stimulation-induced C1 WDR neuronal excitability, possibly via an inhibitory synaptic mechanism, which may contribute to trigeminal referred pain (Takeda et al., 2009). On the basis of these observations, we hypothesized that intravenous resveratrol administration would attenuate noxious stimulation-induced excitability of SpVc neuronal activity through a central mechanism, as is the case for local anesthetic agents and/or analgesic drugs. However, the acute effects of resveratrol on trigeminal neuronal activity in vivo in response to nociceptive and non-nociceptive mechanical stimulation remain to be determined.

Thus, the aim of the present study was to investigate whether acute intravenous administration of resveratrol to rats could attenuate the excitability of nociceptive WDR SpVc neuronal activity in vivo in response to mechanical stimulation.

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. Extracellular single unit recording of WDR neuronal activity in the SpVc

Electrophysiological recordings were made in 18 adult male Wistar rats weighing 250–290 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 hour pentobarbital sodium 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 $37.0 \pm 0.5\,^{\circ}C$ with a homeothermic blanket during recording. Rats were placed in a stereotaxic apparatus, and the activity of a single neuron from the SpVC region was recorded extracellularly. Single neuron activity was recorded 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 (DAM 80; World Precision Instruments), filtered (0.3–10 kHz), monitored with an oscilloscope (SS-7672; Iwatsu, Tokyo, Japan), and recorded on a polygraph (NEC-Sanei 8M14) for subsequent off-line analysis using PowerLab and Chart 5 software (ADInstruments, Oxford, UK).

2.2. Experimental protocols

Extracellular recordings of SpVc WDR unit activity were made as follows. Mechanical stimulation was used as a stimulus to identify the receptitive field quickly and to avoid sensitization of peripheral receptors. Single units that responded to stimulation of the left side of the orofacial facial skin (whisker pad) with a brush and a set of von Frey hairs (Semmes-Weinstein Monofilaments; North Coast Medical, Gilroy, CA, USA) were identified. Noxious pinch stimulation, which evoked a pain sensation when applied to a human subject, was applied to the orofacial area using forceps. After identification of WDR SpVc neurons responding to stimulation of the whisker pad, we determined whether there was spontaneous discharge. The threshold for mechanical stimulation was determined using non-noxious and noxious mechanical stimulation (5 s) with Von Frey hairs (1, 2, 4, 6, 10, 15, 26, 60 g) at the interval of 5 s. The mechanical receptive field of neurons was mapped by probing the facial skin with Von Frey hairs, and then outlined on a life-sized drawing of a rat on tracing paper. The WDR neuronal discharges induced by mechanical stimulation were quantified by subtracting background activity from evoked activity. Spontaneous discharge frequencies were determined over a period of 2–5 min. If no discharge was recorded, the cell was deemed a silent neuron. The mean firing rate of SpVc WDR neurons evoked by mechanical stimulation was compared before and after drug administration. Since previous studies have demonstrated that wide dynamic range (WDR) neurons in the SpVc region have an important role in the mechanism underlying hyperalgesia and referred pain associated with orofacial pain (Takeda et al., 2000, 2005, 2012; Nishikawa et al., 2004), the focus of the present study was on the effects of resveratrol on SpVc WDR neuronal activity, but we did not examine nociceptive-specific neurons (Ness and Randich 2006). Post-stimulus histograms (bin = 100 ms) were generated in response to each stimulus. 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. Concerning the dose-dependent experiment, six rats were used in the each dose (0.5, 1.0 and 2 mg/kg, i.v.) of resveratrol, respectively. Resveratrol was dissolved in dimethyl sulfoxide (DMSO). The stock solution was stored at $-20^{\circ}C$ in small aliquots until use and diluted in saline. The mean spontaneous and mechanical stimulation-induced discharges rates, as well as the mechanical threshold before and after intravenous administration of resveratrol, were evaluated in the present study.

2.3. Identification of recording sites

Recording sites of SpVc WDR neuronal activity were identified as described previously (Takeda et al., 2000, 2012). Briefly, at the

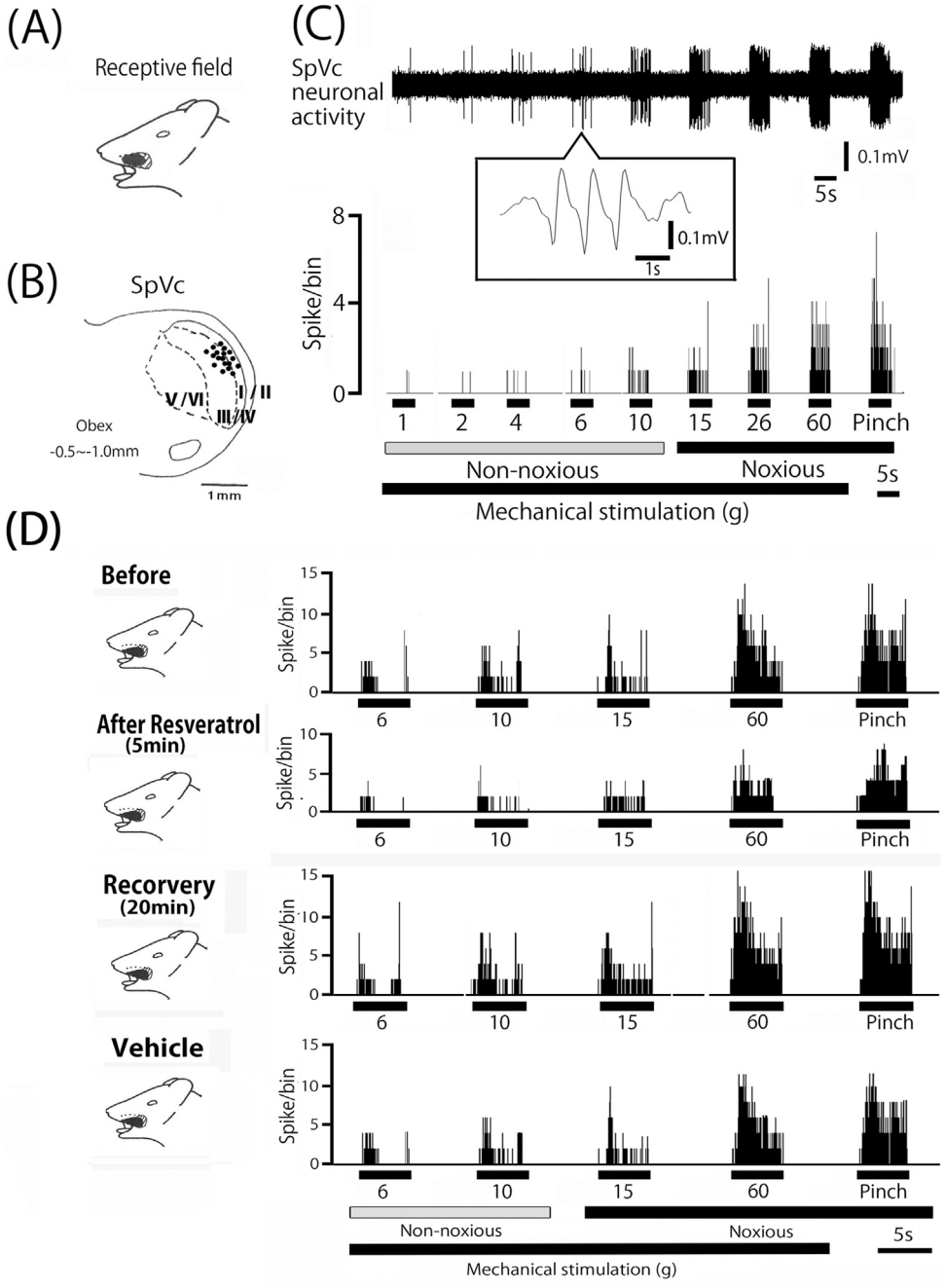

Fig. 1. Effect of intravenous injection of resveratrol on SpVc WDR neuronal activity evoked by non-noxious, noxious, and mechanical stimulation. (A) Receptive field of the whisker pad in the facial skin. Blackened area (high threshold) and hatched area (low threshold) indicate the location and size of the receptive fields. (B) Distribution of SpVc WDR neurons responding to non-noxious and noxious mechanical stimulation of the facial skin ($n = 18$). The number below the each drawing indicates the frontal plane in relation to the obex. (C) Typical example of SpVc WDR neuronal activity evoked by non-noxious (1–10 g) and noxious mechanical stimulation (15 g, 26 g, 60 g, noxious pinch) of the orofacial skin. Upper trace: SpVc WDR neuronal activity; lower trace: post-stimulus histogram. Inset: example of the waveform of an action potential evoked by mechanical stimulation. (D) Typical examples of SpVc WDR neuronal activity evoked by non-noxious (1–10 g), noxious (15–60 g) mechanical stimuli and noxious pinch mechanical stimulation: before and 5 and 20 min after administration of 2 mg/kg resveratrol. Effects of intravenous administration of vehicle on SpVc WDR neuronal activity.

end of the recording sessions, rats were deeply anesthetized and anodal DC currents (30 μA, 5 min) were passed through a recording micropipette. The rats were then perfused transcardially with saline and 10% formalin. Frozen coronal sections (30 μm) were cut and stained with hematoxylin–eosin. Recording sites were identified from the blue spots, and the path of the electrode tracks was constructed in combination with micromanipulator readings.

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.

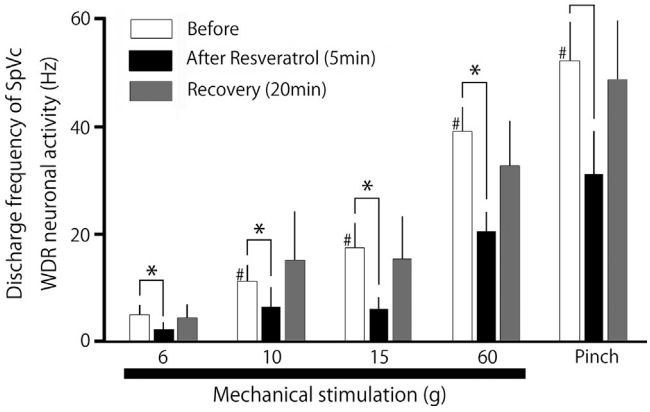

Fig. 2. Time course of intravenous resveratrol administration on the mean firing frequency of SpVc WDR neurons responding to non-noxious, noxious, and noxious pinch mechanical stimulation. $^{\#}P < 0.05$ compared with 6-g stimulus; $^{*}P < 0.05$ compared with before resveratrol administration, $n = 6$.

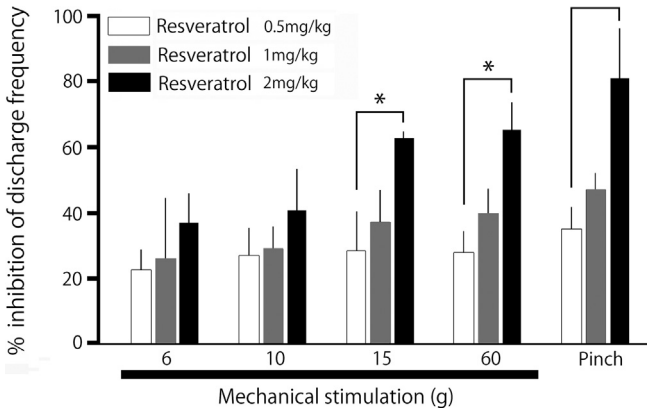

Fig. 3. Dose-dependent suppression by resveratrol of the mean firing frequency of SpVc WDR neurons responding to noxious, and noxious pinch mechanical stimulation. $^{*}P < 0.05$ compared with 0.5 mg/kg, i.v., resveratrol, $n = 6$.

3. Results

3.1. General properties of SpVc WDR neurons innervating facial skin

Extracellular single unit activity was recorded from 18 neurons in the SpVc. The SpVc neurons responding to non-noxious and noxious mechanical stimulation exhibited a somatic receptive field in the orofacial area (mainly whisker pad; Fig. 1A), as described previously (Takeda et al., 2000, 2012). In addition, all these neurons responded to mechanical stimulation of the receptive field innervated by ophthalmic and maxillary branches. Two of the 18 units exhibited spontaneous discharges. As shown in Fig. 1B, recording sites were found in Layers I–III ($n = 10$; 56%) and IV–V ($n = 8$; 44%) in the SpVc (obex—0.5 ~ −2 mm). Typical examples of SpVc WDR neuronal unit responses are shown in Fig. 1C. Graded mechanical stimulation was applied to the most sensitive area of the receptive field, which exhibited increased firing frequency of SpVc WDR neurons in proportion to stimulus intensity. The mean mechanical stimulation-induced spike threshold was 2.8 ± 1.3 g ($n = 18$). Every neuron recorded belonged to the WDR category of neurons (Takeda et al., 2012).

3.2. Effects of resveratrol on excitability of SpVc WDR neurons in response to non-noxious stimuli

A typical example of the effect of resveratrol (2 mg/kg, i.v.) on the excitability of SpVc WDR neurons in response to non-noxious mechanical stimulation is shown in Fig. 1D. Five minutes after injection of resveratrol, non-noxious (1–10 g) mechanical stimulation-evoked SpVc WDR neuronal activity was inhibited, but this inhibition disappeared, with activity returning to control levels, within approximately 20 min. There were no obvious changes in the size of the receptive field (before vs after, 19.1 ± 0.2 mm^2 vs 18.5 ± 0.1 mm^2, NS) or in the mechanical threshold after resveratrol administration. SpVc WDR neuronal activity evoked by non-noxious mechanical stimulation is summarized in Fig. 2. After resveratrol injection, there was a significant decrease in the mean firing rate of non-noxious mechanical stimulation-evoked SpVc WDR neuronal activity, which eventually returned to control levels. Mean firing rates before and after resveratrol were 4.9 ± 1.7 vs. 2.3 ± 1.0 Hz, respectively, in response to the 6-g stimulus and 10.2 ± 2.5 vs. 5.6 ± 2.9 Hz, respectively, in response to the 10-g stimulus ($n = 6$, $P < 0.05$ for both). As indicated in Fig. 3, the inhibition by resveratrol of non-noxious mechanical stimulation-evoked SpVc WDR neuronal activity had a tendency for dose-dependent, but not significant.

3.3. Effects of resveratrol on excitability of SpVc WDR neurons in response to noxious stimuli

Fig. 1 also shows typical examples of the effects of resveratrol (2 mg/kg, i.v.) on the excitability of SpVc WDR neurons in response to noxious mechanical and noxious pinch stimulation. Noxious (15–60 g) mechanical stimulation-evoked SpVc WDR neuronal activity was inhibited 5–10 min after injection of resveratrol, but this inhibition disappeared, with activity returning to control levels in approximately 20 min. Similarly, SpVc WDR neuronal activity in response to noxious pinch stimulation was inhibited 5 min after injection of resveratrol, with responses returning to control levels within 20 min.

As indicated in Fig. 2, the mean firing rates of SpVc WDR neurons evoked by noxious mechanical and pinch stimulation decreased significantly after injection of resveratrol compared with control (before drug injection): 7.7 ± 2.9 vs. 17.5 ± 2.9 Hz, respectively, for the 15-g stimulus; 22.5 ± 4.8 vs. 40.1 ± 4.3 Hz, respectively, for the 60-g stimulus; and 30.9 ± 8.1 vs 52.2 ± 7.2 Hz, respectively, for the pinch stimulus (n-6, $P < 0.05$ for all). The suppression of SpVc WDR neuronal firing in response to noxious mechanical and pinch stimulation was significantly greater following intravenous injection of 0.5 versus 2 mg/kg resveratrol ($28.1 \pm 12.2\%$ vs. $62.5 \pm 2.1\%$, respectively, for the 15-g stimulus; $27.3 \pm 5.8\%$ vs. $65.0 \pm 8.3\%$, respectively, for the 60-g stimulus; and $34.8 \pm 6.9\%$ vs. $80.9 \pm 15.1\%$, respectively, for the pinch stimulus ($n = 6$, $P < 0.05$ for all; Fig. 3). No significant change was observed in the mean receptive field size after resveratrol administration (before vs after 16.9 ± 0.3 mm^2 vs 14.5 ± 0.1 mm^2, NS). There were no changes in the spontaneous firing rate after resveratrol administration. Intravenous administration of vehicle had no significant effect on either spontaneous or evoked (non-noxious, noxious mechanical, and pinch stimulation) activity of SpVc WDR neurons ($n = 2$; Fig. 2D).

3.4. SpVc WDR neuronal activity in response to noxious vs. non-noxious stimuli after resveratrol

Finally, we compared the relative inhibitory effect of 2 mg/kg, i.v., resveratrol on responses to non-noxious and noxious stimuli. The mean magnitude of inhibition by resveratrol of SpVc WDR neuronal discharge frequency was significantly greater for noxious

than non-noxious stimuli (62.9 ± 2.3% vs. 32.7 ± 6.8%, respectively; $n = 6$, $P < 0.05$).

4. Discussion

The present study provides evidence that, in the absence of inflammatory or neuropathic pain, acute intravenous resveratrol suppresses trigeminal sensory transmission, including nociception. Therefore, resveratrol may be considered a potential CAM agent for the treatment of trigeminal nociceptive pain without side effects.

4.1. Intravenous resveratrol suppresses excitability of SpVc WDR neurons

The primary findings of the present study are as follows: (i) mean SpVc WDR neuronal firing rate in response to both non-noxious and noxious mechanical stimuli was inhibited by resveratrol (0.5–2 mg/kg, i.v.); (ii) the inhibition of the discharge frequency in response to both non-noxious and noxious mechanical stimuli was reversible and occurred within approximately 20 min; and (iii) injection of vehicle had no significant effect on SpVc WDR neuronal activity in response to either non-noxious or noxious mechanical or pinch stimuli. In a previous study, the antinociceptive effect of resveratrol against the nociceptive reflex was observed with a dose as low as 2 mg/kg, i.p. (10 mM), with no further increases in effect observed with higher doses (Gentilli et al., 2001). In the present study, following the systemic administration of 10 mM resveratrol, the drug entered the general circulation and was diluted in the bloodstream to a calculated concentration of approximately 100 μM, and this concentration still had a significant effect on the nociceptive transmission of the SpVc. It has been shown that 100 μM resveratrol significantly inhibits glutaminergic excitatory synaptic transmission in hippocampal slices (Gao et al., 2006). Together, these findings suggest that under in vivo conditions, acute intravenous resveratrol administration suppresses trigeminal nociceptive transmission in the SpVc, at the level of secondary neurons.

4.2. Suppression of SpVc WDR neuron excitability by resveratrol

4.2.1. Peripheral mechanisms

The mechanism of nociceptive sensory signaling depends on the following four general processes: (i) transduction from peripheral terminals that transduce external stimuli; (ii) generation of action potentials; (iii) propagation of action potentials along axons; and (iv) transmission to central terminals, which form the presynaptic elements of the first synapses in the sensory pathways in the central nervous system (for reviews, see Harriot and Gold 2009; Takeda et al., 2011). With regard to the effect of resveratrol on excitable tissue in the nervous system, previous reports suggest that resveratrol modulates the excitability of neurons in the peripheral nervous system via activation of voltage-dependent and TRP channels (Grannados-Soto et al., 2002; Kim et al., 2005; Liew et al., 2005; Gao and Hu, 2005; Yu et al., 2013). Recently, Meng et al. (2015) reported that, in vitro, mechanical stimuli induce mechanosensitive currents via mechanosensitive channels such as TRPA1 to trigger mechanotransduction in trigeminal neurons innervating the inner walls of the anterior eye chamber. In addition, TRPA1 has been reported to modulate mechanotransduction in primary sensory neurons (Kwan et al., 2009) and resveratrol has been shown to be a potent inhibitor of TRPA1 in vitro and in vivo (Yu et al., 2013), suggesting that resveratrol attenuates the generator potential and inhibits action potential firing via the mechanical transduction process. Moreover, resveratrol modulates the sodium and potassium currents in DRG neurons that are associated with the generation of action potentials (Kim et al., 2005; Grannados-Soto et al., 2002). Kim et al. (2005) demonstrated that resveratrol predominantly inhibits Na^+ currents in acutely dissociated DRG neurons, indicating that resveratrol inhibits the generation of action potentials. On the basis of these results, it can be speculated that resveratrol inhibits the excitability of peripheral terminals of the trigeminal nerve by modulating both the transduction (generator potential) and generation (initiation of action potentials) processes. However, further studies are needed to elucidate the precise mechanisms of action of resveratrol.

4.2.2. Central mechanisms

Gao et al. (2006) reported that in hippocampal slices resveratrol significantly suppressed glutamate-induced currents in post-synaptic CA1 pyramidal neurons without having any presynaptic effects. In addition, Gao et al. (2006) indicated that *N*-methyl-D-aspartate (NMDA) receptors were more sensitive to resveratrol than α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptors. There are also reports that resveratrol decreases action potential duration and L-type Ca^{2+} currents in ventricular myocytes (Liew et al., 2005; Zhang et al., 2006). Thus, it is possible that resveratrol suppresses the glutaminergic excitatory synaptic transmission of SpVc by inhibiting post-synaptic glutamate receptors and presynaptic Ca^{2+} channels.

Alternatively, a previous study indicated that systemic resveratrol exhibits dose-dependent antinociceptive effects via an opioidergic mechanism, because pretreatment of rats with naloxone, an opioid antagonist, completely blocked the analgesic effect of resveratrol (Gupta et al., 2004). Opiates acting via the μ-opioid receptor partially inhibit evoked inhibitory GABAergic pre- and post-synaptic potentials in the periaquadutal gray (PAG; Chieng and Christie, 1994). Blockade of μ-opioid receptors increases neuronal activity in the PAG via GABAergic disinhibition, which can then activate serotonergic (5-hydroxytrypytamine [5-HT]) neurons in the nucleus raphe magnus (the PAG–nucleus raphe magnus–trigeminal pathway; Gebahrts and Randich, 1990; Takeda et al., 2002). In addition, resveratrol facilitates 5-HT_3 receptor-mediated ion currents (Lee et al., 2011). Previous studies have reported that nociceptive stimulation-evoked SpVc/C1 neuron activity is suppressed by conditioning peripheral nerve stimulation via 5-HT_3 receptor-mediated GABAergic inhibition (Tanimoto et al., 2004; Oshima et al., 2005). Collectively, these observations suggest that resveratrol suppresses excitatory synaptic transmission of the SpVc via activation of 5-HT_3 receptor-mediated GABAergic inhibition and/or via activation of endogenous opioidergic mechanisms. However, further studies are needed to clarify these possibilities.

4.3. Functional significance of resveratrol suppression of nociceptive stimulation-induced excitability of SpVc neurons

In a previous study we found that intravenous administration of lidocaine, a sodium channel blocker, attenuated the trigeminal nociceptive reflex and nociceptive stimulation-induced C1 WDR neuronal excitability possibly via inhibitory synaptic mechanisms acting through strychnine-sensitive glycine receptors, which may contribute to trigeminal referred pain (Takeda et al., 2009). This evidence suggests that resveratrol can attenuate central trigeminal nociceptive pain transmission. In the present study, we found that resveratrol decreases the mechanical stimulation-induced discharge frequency of SpVc neurons after both non-noxious and noxious stimuli, but that nociceptive input to SpVc WDR neurons is more sensitive to resveratrol than non-nociceptive input. Although the precise reason why there is a difference in resveratrol sensitivity between nociceptive and non-nociceptive inputs remains unclear, it can be postulated that resveratrol predominantly inhibits central mechanisms through both excitatory synaptic transmission in the

SpVc and descending inhibitory (including endogenous opioidergic) mechanisms.

Finally, CAM, including herbal medicines and acupuncture, have been used to treat persistent clinical chronic pain (Rao et al., 1999; Konvicka et al., 2008; Rosenberg et al., 2008). In addition, studies have investigated the potential effects of diet and dietary supplementation on conditions associated with pain (Shir et al., 2001; Ernest, 2003; Tall and Raja, 2004). Interestingly, resveratrol has no known toxic side effects (Russo, 2007), so the possibility exists that resveratrol may be a candidate CAM, specifically a therapeutic analgesic agent. Because incisions associated with surgery cause acute pain and surgery has been identified as a potential major cause of chronic pain (Perkins and Kehlet, 2000; Kehlet et al., 2006), it is possible that resveratrol could effectively reduce clinical pain, including postoperative pain (Locher-Claus et al., 2005; Tillu et al., 2012). The results of the present study indicate that acute intravenous resveratrol administration suppresses trigeminal nociceptive transmission without inflammatory or neuropathic pain. As such, resveratrol may be a potential therapeutic agent for the treatment of trigeminal nociceptive pain, including clinical pain.

References

Bermudez-Ocasna, D.Y., Ambriz-Tiututi, M., Peres-Severiano, F., Granados-Soto, V., 2006. Pharmacological evidence for participation of NO-cyclic GMP-PKG-K^+ channel pathway in antiallodynic action of resveratrol. Pharmacol. Biochem. Behav. 84, 535–542.

Chieng, B., Christie, M.J., 1994. Hyperpolization by opioids acting on μ-receptors of a subpopulation of rat periaqueductal grey neurons in vitro. Br. J. Pharmacol. 113, 121–128.

Ernest, E., 2003. Complementary medicine. Curr. Opin. Rheumatol. 15, 151–155.

Fremont, L., 2000. Biological effects of resveratrol. Life Sci. 66, 663–673.

Gao, Z.-B., Hu, G.-Y., 2005. Trans-resveratrol, a red wine ingredient, inhibits voltage-activated potassium currents in rat hippocampal neurons. Brain Res. 1056, 68–75.

Gao, Z.-B., Chen, X.-Q., Hu, G.-Y., 2006. Inhibition of excitatory synaptic transmission by trans-resveratrol in rat hippocampus. Brain Res. 1111, 41–47.

Gebahrts, G.F., Randich, A., 1990. Brain stem modulation of nociception. In: Klem, W.R., Vertes, R.P. (Eds.), Brain Stem Mechanisms of Behavior. Wiley-Intersciences Publication, New York, pp. 315–352.

Gentilli, M., Mazoit, J.X., Bouaziz, H., Fletcher, D., Casper, R.F., Benhamou, D., Savouret, J.-F., 2001. Resveratrol decreases hyperalgesia induced by carrageenan in the rat hind paw. Life Sci. 68, 1317–1321.

Grannados-Soto, V., Argulles, C.F., Ortiz, M.I., 2002. The peripheral antinociceptive effect of resveratrol is associated with activation of potassium channels. Neuropharmacology 43, 917–923.

Gupta, Y.K., Sharma, M., Briyal, S., 2004. Antinociceptive effect of trans-resveratrol in rats: insolvent of an opioidergic mechanism. Method Find. Exp. Clin. Pharmacol. 26, 667–672.

Harriot, A.M., Gold, M.S., 2009. Contribution of primary afferent channels to neuropathic pain. Curr. Pain Headache Rep. 13, 197–207.

Kehlet, H., Jensen, T.S., Woolf, C.J., 2006. Persistent postoperative pain: risk factors and prevention. Lancet 367, 1618–12625.

Konvicka, J.J., Meyer, T.A., McDavid, A.J., Roberson, C.R., 2008. Complementary/alternative medicine use among chronic pain clinic patients. J. Periaesth. Nurs. 23, 17–23.

Kwan, K.Y., Glazer, J.M., Corey, D.P., Rice, F.L., Stucky, C.L., 2009. TRPA1 modulates mechanotransduction in cutaneous sensory neurons. J. Neurosci. 29, 4808–4819.

Kim, H.I., Kim, T.H., Song, J.H., 2005. Resveratrol inhibits Na^+ currents in rat dorsal root ganglion neurons. Brain Res. 1045, 134–141.

Lee, B.-H., Hwan g, S.-H., Choi, S.-H., Shin, T.-J., Kang, J., Lee, S.-M., Nah, S.-Y., 2011. Resveratrol enhances 5-hydroxytryptamine type 3A receptor-mediated ion currents: role of arginine 222 residue in pre-transmembrane domain I. Biol. Pharm. Bull. 34, 523–527.

Leiro, J., Arranz, J.A., Sanmmartin, M.L., Quezada, E., Orallo, F., 2005. Effect of cis-resveratrol on genes involved in nuclear factor Kappa B signaling. Int. Immunopharmacol. 5, 393–406.

Liew, R., Stagg, M.A., MacLeod, K.T., Collins, P., 2005. The red wine, resveratrol, exerts acute direct action on gunia-pig ventricular myocytes. Eur. J. Pharmacol. 519, 1–8.

Locher-Claus, M.T., Erickson, T.E., Law, A.S., Johnson, W.T., Gebaharts, G.F., 2005. Effect of pre-emptive morphine, ibuprofen of local anesthetic on fos-expression in the spinal trigeminal nucleus following tooth pulp exposure in rat. J. Endod. 31, 578–583.

Meng, Q., Fang, P., Hu, Z., Ling, Y., Liu, H., 2015. Mechanotransduction of trigeminal ganglion neurons innervating inner walls of rat anterior eye chamber. Am. J. Physiol. Cell Physiol. 309, C1–C10.

Millan, M.J., 1999. The induction of pain: an integerative review. Prog. Neurobiol. 57, 1–164.

Nishikawa, T., Takeda, M., Tanimoto, T., Matsumoto, S., 2004. Convergence of nociceptive information from temporomandibular joint and tooth-pulp afferents on C1 neurons in the rat. Life Sci. 75, 1465–1478.

Oshima, K., Takeda, M., Tanimoto, T., Katsuumi, I., Matsumoto, S., 2005. Tooth-pulp-evoked rostral spinal trigeminal nucleus neuron activity is suppressed by conditioning sciatic nerve stimulation in the rats: possible role of $5HT_3$ receptor mediated GABA ergic inhibition. Brain Res. Bull. 65, 31–40.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd edn. Academic Press, New York.

Perez-Severiano, F., Bermudes-Ocana, D.Y., Lopez-Sanchez, P., Rios, C., Granados-Soto, V., 2008. Spinal nerve ligation reduces nitric oxide synthase activity and expression: effect of resveratrol. Pharmacol. Bichem. Behav. 90, 742–747.

Perkins, F.M., Kehlet, H., 2000. Chronic pain as an outcome of surgery. A review of predictive factors. Anesthesiology 93, 1123–1133.

Pervaiz, S., 2003. Resveratrol: from grape wines to mamanlian biology. FASEB J. 17, 1975–1985.

Rao, J.K., Mihaliak, K., Kroenke, K., Bradley, J., Tierney, W.M., Weinberger, M., 1999. Use of complementary therapies for arthritis among patients of rhematologist. Ann. Intern. Med. 131, 409–416.

Rosenberg, E.I., Genao, I., Chen, I., Mechaber, A.J., Wood, J.A., Faselius, C.J., 2008. Complementary and alternative medicine use by primary care patients with chronic pain. Pain Med. 9, 1065–1072.

Russo, G.L., 2007. Ins and Outs of dietary phytochemical in cancer chemoprevention. Biochem. Pharmacol. 74, 533–544.

Sessle, B.J., 2000. Acute and chronic craniofacial pain: brainstem mechanisms of nociceptive transmission and neuroplasticity and their clinical correlates. Crit. Rev. Oral Biol. Med. 11, 57–91.

Scholz, J., Woolf, C.J., 2002. Can we conquer pain? Nat. Neurosci. 5 (Suppl), 1062–1067.

Shir, Y., Raja, S.N., Weissman, C.S., Campbell, J.N., Seltzer, Z., 2001. Consumption of soy diet before nerve injury preempts the development of neuropathic pain in rats. Anesthesiology 95, 1238–1244.

Takeda, M., Tanimoto, T., Matsumoto, S., 2000. Change in mechanical receptive field properties induced by $GABA_A$ receptor activation in the trigeminal spinal nucleus caudalis neurons in rats. Exp. Brain Res. 134, 409–416.

Takeda, M., Tanimoto, T., Matsumoto, S., 2002. Volume expansion suppress the tooth-pulp evoked jaw-opening reflex related activity of trigeminal neurons in rats. Brain Res. Bull. 58, 83–89.

Takeda, M., Tanimoto, T., Ito, M., Nasu, M., Matsumoto, S., 2005. Role of capsaicin-sensitive afferent inputs from the masseter muscle in the C1 spinal neurons responding to tooth-pulp stimulation in rats. Exp. Brain Res. 16, 107–117.

Takeda, M., Oshima, M., Takahashi, M., Matsumoto, S., 2009. Systemic administration of lidocaine suppress the excitability of rat cervical dorsal horn neurons and tooth-pulp-evoked jaw-opening reflex. Eur. J. Pain 13, 929–934.

Takeda, M., Matsumoto, S., Sessle, B.J., Shinoda, M., Iwata, K., 2011. Peripheral and central mechnaisms of trigeminal neuropathic pain and inflammatory pain. J. Oral. Biosci. 53, 318–329.

Takeda, M., Takahashi, M., Mastumoto, S., 2012. Suppression of neurokinin-1 receptor in trigeminal ganglia attenuates central sensitization following inflammation. J. Peripher. Nerv. Syst. 17, 169–181.

Tall, J.M., Raja, S.N., 2004. Dietary constituents as novel therapeutics for pain. Clin. J. Pain. 20, 19–26.

Tanimoto, T., Takeda, M., Nishikawa, T., Matsumoto, S., 2004. The role of $5\text{-}HT_3$ receptors in the vagal afferent activation-induced of C1 spinal neurons projected from tooth-pulp in the rat. J. Pharmacol. Exp. Ther. 311, 803–810.

Tillu, D.V., Melemedjian, O.K., Asiedu, M.N., Qu, N., Feline, M.D., Dussor, G., Price, T.J., 2012. Resveratrol engages AMPK to attenuates ERK and mTOR siganaling in sensory neurons and inhibits incision -induced acute and chronic pain. Mol. Pain 8 (5).

Yu, L., Wang, S., Kogure, Y., Yamamoto, S., Noguchi, K., Dai, Y., 2013. Modulation of TRP channels by resveratrol and other stilbenoids. Mol. Pain 9, 3.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.