Brain Research Bulletin 124 (2016) 262–268

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

Local administration of resveratrol inhibits excitability of nociceptive wide-dynamic range neurons in rat trigeminal spinal nucleus caudalis

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ARTICLE INFO

Article history:
Received 4 March 2016
Received in revised form 28 May 2016
Accepted 6 June 2016
Available online 7 June 2016

Keywords:
Nociception
Resveratrol
Trigeminal spinal nucleus caudalis
Extracellular single-unit recording
Lidocaine
Local anesthetic agents
Complementary and alternative medicine

ABSTRACT

Although we recently reported that intravenous administration of resveratrol suppresses trigeminal nociception, the precise peripheral effect of resveratrol on nociceptive and non-nociceptive mechanical stimulation-induced trigeminal neuron activity *in vivo* remains to be determined. The aim of the present study was to investigate whether local subcutaneous administration of resveratrol attenuates mechanical stimulation-induced excitability of trigeminal spinal nucleus caudalis (SpVc) neuron activity in rats, *in vivo*. Extracellular single-unit recordings were made of SpVc wide-dynamic range (WDR) neuron activity in response to orofacial mechanical stimulation in pentobarbital-anesthetized rats. Neurons responded to non-noxious and noxious mechanical stimulation applied to the orofacial skin. Local subcutaneous administration of resveratrol (1–10 mM) into the orofacial skin dose dependently and significantly reduced the mean number of SpVc WDR neurons firing in response to both non-noxious and noxious mechanical stimuli, with the maximal inhibition of discharge frequency in response to both stimuli being seen within 5 min. These inhibitory effects were no longer evident after approximately 20 min. The mean magnitude of inhibition by resveratrol (10 mM) of SpVc neuron discharge frequency was almost equal to that of the local anesthetic 1% lidocaine (37 mM). These results suggest that 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. Therefore, local subcutaneous administration of resveratrol may provide relief of trigeminal nociceptive pain, without side effects, thus contributing to the suite of complementary and alternative medicines used as local anesthetic agents.

1. Introduction

Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is a natural polyphenolic compound found in large number of plants that are part of the human diet, including peanuts, mulberries, grapes and red wine. It is well known that resveratrol has a variety of biological actions, including cardiovascular protection, neuroprotection, and anticancer and anti-inflammatory effects (Fremont 2000; Pervaiz 2003). Because resveratrol has no known toxic side effects (Russo, 2007) and complementary and alternative medicines (CAM), such as herbal medicines and acupuncture, have been used for the treatment of persistent clinical chronic pain (Rao et al., 1999; Konvicka et al., 2008; Rosenberg et al., 2008), resveratrol may be a candidate therapeutic CAM analgesic agent.

The trigeminal spinal nucleus is an important relay station in the transmission of orofacial sensory information and it is functionally subdivided into three nuclei (from rostral to caudal): oralis, interpolaris and caudalis (Sessle, 2000). It is well known that the spinal trigeminal nucleus caudalis (SpVc) is most important relay stations for trigeminal nociceptive inputs from inflammation and tissue injury (Sessle, 2000; Takeda et al., 2012). Recently, Takehana et al.

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; TTX-S, tetrodotoxin sensitive; TTX-R, tetrodotoxin resistant.

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http://dx.doi.org/10.1016/j.brainresbull.2016.06.001
0361-9230/

(2016) reported that, in the absence of inflammatory or neuropathic pain, acute intravenous administration of resveratrol suppresses SpVc wide-dynamic range (WDR) neurons via both peripheral and central mechanisms. Therefore, resveratrol may be a potential CAM therapeutic agent for the treatment of trigeminal nociceptive pain without side effects.

Previous reports suggest that resveratrol modulates neuronal excitability in both the peripheral and central nervous systems via voltage-dependent ion channels (sodium, potassium and calcium; Kim et al., 2005; Liew et al., 2005; Gao and Hu, 2005), as well as synaptic transmission via ligand-gated channels and various voltage-dependent ion channels (Gao et al., 2006; Lee et al., 2011). Kim et al. (2005) demonstrated that resveratrol inhibits tetrodotoxin-sensitive (TTX-S) and TTX-resistant (TTX-R) Na^+ currents in acutely dissociated dorsal root ganglion (DRG) neurons. Furthermore, it has been shown, using the rat formalin test, that resveratrol induces peripheral antinociception via opening of several K^+ channels (Grannados-Soto et al., 2002). In addition, recent findings suggest that resveratrol modulates the activity of transient receptor potential (TRP) channels, including being a potent inhibitor of TRP ankyrin 1 (TRPA1) channels both *in vitro* and *in vivo* (Yu et al., 2013). In turn, TRPA1 channels have been shown to modulate mechanotransduction via the generator potential in primary sensory neurons (Kwan et al., 2009). Together, these observations strongly suggest that local administration of resveratrol into the receptive field of SpVc WDR neurons may suppress the transmission of nociceptive pain in the periphery. Therefore, we hypothesized that local subcutaneous administration of resveratrol would attenuate both non-noxious and noxious stimulation-induced excitability of SpVc WDR neurons. However, the acute effects of resveratrol *in vivo* on nociceptive and non-nociceptive mechanical stimulation-induced SpVc WDR neuron activity have not yet been elucidated.

Therefore, the aim of the present study was to investigate whether local subcutaneous injection of resveratrol into the receptive field of SpVc WDR neurons could attenuate non-noxious and noxious mechanical stimulation-induced excitability of these neurons, *in vivo*. In addition, we compared the magnitude of suppression of trigeminal nociception between resveratrol and the clinically used local anesthetic lidocaine, a sodium channel blocker.

2. Materials and methods

The experiments reported herein were approved by the Animal Use and Care Committee of Azabu University and were performed in accordance 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 recordings from SpVc WDR neurons

Electrophysiological recordings were made in 24 adult male Wistar rats (weighing 250–290 g). Each animal was first anesthetized with pentobarbital sodium (45 mg/kg, i.p.) and maintained with additional doses of 2–3 mg/kg per h pentobarbital sodium administered through a cannula inserted in 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 then 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 in regions identified according to the stereotaxic coordinates of Paxinos and Watson (1986). Neuronal activity was amplified (DAM80; World Precision Instruments, Sarasota, FL, USA), filtered (0.3–10 kHz), monitored on an oscilloscope (SS-7672; Iwatsu, Tokyo, Japan) and recorded on a polygraph (8M14; NEC-Sanei Instruments, Tokyo, Japan). Recordings were analyzed off-line using a Power Lab and Chart 5 software (ADInstruments, Oxford, UK).

2.2. Experimental protocols

Extracellular recordings of SpVc WDR unit activity were performed as described previously (Takeda et al., 2000, 2012; Takehana et al., 2016). Briefly, mechanical stimulation was used as a search stimulus to identify the receptive field quickly and to avoid sensitization of peripheral receptors. Single units that responded to stimulation of the left side orofacial 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 was applied to the orofacial area with forceps that evoked a pain sensation when applied to a human subject. After identification of WDR SpVc neurons responding to whisker pad stimulation, we determined whether there was spontaneous discharge. The threshold for mechanical stimulation was determined by using non-noxious and noxious mechanical stimulation (5 s) with von Frey hairs (2, 4, 6, 10, 15, 26 and 60 g) applied at 5-s intervals. The mechanoreceptive 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. WDR neuron discharges induced by mechanical stimulation were quantified by subtracting background activity from 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 administration. Because previous studies have demonstrated that 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) and that the discharge of these neurons is suppressed by intravenous resveratrol administration (Takehana et al., 2016), the focus of the present study was on the effects of resveratrol on SpVc WDR neuronal activity; we did not examine nociceptive-specific neurons. Post-stimulus histograms (bin = 100 ms) were generated in response to each stimulus. The effects of subcutaneous resveratrol (0.05 mL; 1, 5 and 10 mM) and lidocaine (1% and 2% Xylocaine; equivalent to 37 and 74 mM, respectively), administered through a Hamilton microsyringe, were evaluated 5, 10, 20, 30 and 40 min after administration because the peak effect and recovery were thought to occur within in this time frame. Resveratrol was dissolved in dimethyl sulfoxide to create a stock solution of 20 mM. The stock solution was stored at $-20\,^{\circ}C$ until use. The stock solution was diluted to the desired concentrations using saline immediately before use. Mean spontaneous and mechanical stimulation-induced discharges rates, and the mechanical threshold before and after subcutaneous administration of resveratrol were analyzed in the present study.

2.3. Identification of recording sites

The location of the recording sites for SpVc WDR neuron activity was identified as described previously (Takeda et al., 2000, 2012; Takehana et al., 2016). Briefly, at the end of the recording sessions, rats were deeply anesthetized and anodal direct current (DC; 30 μA, 5 min) was passed through the recording micropipette. The rats were perfused transcardially with saline and 10% formalin. Frozen coronal sections (30 μm) were cut and stained with hematoxylin–eosin. Recording sites were identified as blue spots