The dietary constituent resveratrol suppresses nociceptive neurotransmission via the NMDA receptor

Shiori Takehana[1], Yoshiko Kubota[2], Nobuo Uotsu[2], Kei Yui[2], Koichi Iwata[3], Yoshihito Shimazu[1] and Mamoru Takeda[1]

Abstract

Background: Although we have previously reported that intravenous resveratrol administration inhibits the nociceptive neuronal activity of spinal trigeminal nucleus caudalis neurons, the site of the central effect remains unclear. The aim of the present study was to examine whether acute intravenous resveratrol administration in the rat attenuates central glutamatergic transmission of spinal trigeminal nucleus caudalis neurons responding to nociceptive mechanical stimulation in vivo, using extracellular single-unit recordings and microiontophoretic techniques.

Results: Extracellular single-unit recordings using multibarrel electrodes were made from the spinal trigeminal nucleus caudalis wide dynamic range neurons responding to orofacial mechanical stimulation in pentobarbital anesthetized rats. These neurons also responded to iontophoretic application of glutamate, and the evoked neuronal discharge frequency was significantly increased in a current-dependent and reversible manner. The mean firing frequency evoked by the iontophoretic application of glutamate (30, 50, and 70 nA) was mimicked by the application of 10 g, 60 g, and noxious pinch mechanical stimulation, respectively. The mean firing frequency of spinal trigeminal nucleus caudalis wide dynamic range neurons responding to iontophoretic application of glutamate and N-methyl-D-aspartate were also significantly inhibited by intravenous administration of resveratrol (2 mg/kg) and the maximal inhibition of discharge frequency was observed within 10 min. These inhibitory effects lasted approximately 20 min. The relative magnitude of inhibition by resveratrol of the glutamate-evoked spinal trigeminal nucleus caudalis wide dynamic range neuronal discharge frequency was similar to that for N-methyl-D-aspartate iontophoretic application.

Conclusion: These results suggest that resveratrol suppresses glutamatergic neurotransmission of the spinal trigeminal nucleus caudalis neurons responding to nociceptive mechanical stimulation via the N-methyl-D-aspartate receptor in vivo, and resveratrol may be useful as a complementary or alternative therapeutic agent for the treatment of trigeminal nociceptive pain.

Background

Recent reports have described the use of complementary and alternative medicine (CAM), such as herbal medicines and acupuncture, for the treatment of persistent clinical chronic pain.[1–3] Additionally, the potential effects of diet and dietary supplementation on conditions associated with pain have been the focus of considerable research.[4–6] Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is a plant polyphenol[7,8] and has several beneficial biological actions, including anti-oxidative, anti-inflammatory,

[1]Laboratory of Food and Physiological Sciences, Department of Life and Food Sciences, School of Life and Environmental Sciences, Azabu University, Kanagawa, Japan
[2]FANCL Health Science Research Center, Research Institute, FANCL Corporation, Kanagawa, Japan
[3]Department of Physiology, School of Dentistry, Nihon University, Tokyo, Japan

Corresponding author:
Mamoru Takeda, 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.
Email: m-takeda@azabu-u.ac.jp

neuroprotective, anticancer, and cardioprotective effects.[8–11] Because resveratrol has no known toxic side effects,[12] it could be a candidate CAM for the therapeutic treatment of nociceptive and inflammatory pain.[13] For orofacial pain, 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.[14–16] Previous studies have demonstrated that wide dynamic range (WDR) neurons in the SpVc region have an important role in the mechanism underlying hyperalgesia/allodynia and/or referred pain associated with orofacial pain.[17–19] Recently, we have reported that intravenous resveratrol administration suppresses SpVc WDR neuronal excitability via both peripheral and central mechanisms. Therefore, resveratrol could be a CAM used for the treatment of trigeminal nociceptive pain;[20] however, the precise site of the central effects of resveratrol still needs to be determined.

Previous reports suggest that resveratrol modulates the neuronal excitability of the central nervous system. For example, in a hippocampal slice preparation, resveratrol significantly suppressed the glutamate-induced currents in post-synaptic CA1 pyramidal neurons[21] that contribute to excitatory synaptic transmission. There are reports that resveratrol decreases action potential duration and L-type Ca^{2+} currents in excitable tissues,[22,23] suggesting that presynaptic Ca^{2+} channels may contribute to the suppression of nociceptive transmission. In the trigeminal system, intravenous injection of both N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists block the excitatory responses of C1-C2 spinal neuronal activity to electrical stimulation of the sagittal sinus.[24] This also supports the report that iontophoretic application of NMDA and non-NMDA glutamate receptor antagonists attenuates tooth-pulp-evoked C1 neuronal excitation.[25] We previously demonstrated, using extracellular single-unit recordings and microiontophoretic techniques, that activation of post-synaptic glutamate receptor-evoked C1 neuronal excitation was modulated by α_2-adrenergic receptor agonists.[26] Since the intrathecal administration of NMDA receptor antagonists inhibits nociceptive behavior,[27,28] it can be assumed that the NMDA receptor signaling system contributes to spinal nociceptive transmission. These observations led us to hypothesize that intravenous resveratrol administration would attenuate the noxious mechanical stimulation-induced glutamatergic neurotransmission in SpVc neurons through a central mechanism, possibly a postsynaptic NMDA receptor mechanism. However, the local effects of resveratrol on trigeminal neuronal activity in vivo in response to nociceptive mechanical stimulation remain to be determined. Therefore, the aim of the present study was to investigate whether acute intravenous resveratrol administration attenuates central postsynaptic glutamatergic neuronal transmission in the SpVc neurons responding to nociceptive mechanical stimulation in vivo, by using extracellular single-unit recording and microiontophoretic application combined with multibarrel electrodes.

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.[29] Every effort was made to minimize the number of animals used and their suffering.

Extracellular single-unit recording of WDR neuronal activity in the SpVc with multibarrel electrodes

Electrophysiological recordings were made in 15 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 pentobarbital sodium (2–3 mg/kg/h) through a jugular vein cannula, 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 $37.0 \pm 0.5°C$ with a homeothermic blanket during recording. Rats were placed in a stereotaxic apparatus, and the activity of a single neuron from the SpVc region, according to the stereotaxic coordinates of Paxinos and Watson,[30] was recorded extracellularly.

Single neuronal activity was recorded by means of a three-barreled glass micropipette filled with 2% pontamine sky blue with 0.5 M sodium acetate as described in our previous studies.[17,18] Neuronal activity was amplified (DAM 80; World Precision Instruments, Sarasota, FL), filtered (0.3–10 kHz), monitored with an oscilloscope (SS-7672; Iwatsu, Tokyo, Japan), and recorded on a polygraph (8M14; NEC-Sanei, Tachikawa, Japan) for subsequent off-line analysis using Power Lab and Chart 5 software (AD Instruments, Oxford, UK). The technique of iontophoretic application was the same as described in our previous reports.[17,18,25,31] One of the two lateral barrels of the micropipette contained 160 mmol/L NaCl which was used for balancing currents to prevent the occurrence of tip polarization artifacts. The remaining lateral barrel contained NMDA (200 mmol/L in 160 mmol/L NaCl, pH 8.5) or L-glutamate (100 mmol/L in 160 mmol/L NaCl, pH 8.5; Nacalai Tesque, Kyoto, Japan), as described previously.[18,26] The currents for ejecting, retaining, and balancing were provided by a constant current unit (Dia Medical, DPI-25, Japan). The drugs were ejected with 30–70 nA cationic currents, and 10–25 nA retaining currents were used.

Experimental protocols

Extracellular recordings of SpVc WDR unit activity were made as follows. Mechanical stimulation was used as a stimulus to identify the repetitive field quickly and to avoid sensitization of peripheral receptors. Single units that responded to stimulation of the left side of the orofacial skin (whisker pad) with a brush and a set of von Frey hairs (Semmes-Weinstein Monofilaments; North Coast Medical, Gilroy, CA) 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, 4, 6, 10, 15, 26, and 60 g). 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 resveratrol administration. 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.[32] For this reason, we must stress that WDR neurons in the SpVc region contribute to the mechanism of hyperalgesia and/or referred pain associated with orofacial pain.[16,19] Post-stimulus histograms (bin = 100 ms) were generated in response to each stimulus.

The effects of resveratrol (2 mg/kg, i.v., equivalent to 10 mmol/L), 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. The stock solution was stored at $-20°C$ in small aliquots until use and diluted in saline. After the identification of SpVc WDR neurons responding to mechanical stimulation, we determined the optimum currents of iontophoretically applied glutamate and NMDA. In brief, to assess the effectiveness of iontophoretically applied glutamate and NMDA on the firing frequency of SpVc WDR neurons, we varied the current intensity (30, 50, and 70 nA; 5 s) to enable us to determine the iontophoretic conditions. Iontophoretic application of glutamate (>60 nA) showed a maximal increase in spinal neuronal activity at a minimal current, as described in our previous study.[31] The responses of SpVc WDR neuron activity to the iontophoretic application of glutamate or NMDA (50 nA; 5 s) were compared before and after intravenous resveratrol. SpVc WDR neuronal activity after the iontophoretic application of glutamate or NMDA was recorded three times at intervals of 5 s.

Identification of recording sites

Recording sites of SpVc WDR neuronal activity were identified as described previously.[16,17] Briefly, at the 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 track was constructed in combination with micromanipulator readings.

Data analysis

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

Results

Effect of iontophoretic application of glutamate on the excitability of SpVc WDR neurons responding to mechanical stimulation of the orofacial area

Figure 1(a) shows a typical example of SpVc WDR neurons responding to non-noxious and noxious mechanical stimulation of the whisker pad. Since graded mechanical stimulation applied to the most sensitive area of the receptive field showed increased firing frequency of SpVc neurons as proportional to stimulus intensity, these neurons were recorded as belonging to the category of WDR neurons, as described previously.[20,33,34] Their recording sites were found in layers I–III (n = 14, 63%) and IV–V (n = 5, 27%) in the dorsolateral region of SpVc (obex $-1.0 \sim -2.0$ mm). In this study, we found that 19 SpVc WDR neurons responding to non-noxious and noxious mechanical stimulation of the whisker pad were also activated by iontophoretic application of glutamate (Figure 1(b)). Glutamate-evoked discharge was reproducible at intervals of 5 s as illustrated in Figure 1(b). The firing frequency evoked by the iontophoretic application of glutamate was current-dependently increased (30, 50, and 70 nA; Figure 1(c)). The mean discharge frequencies of SpVc WDR neurons evoked by iontophoretic glutamate application
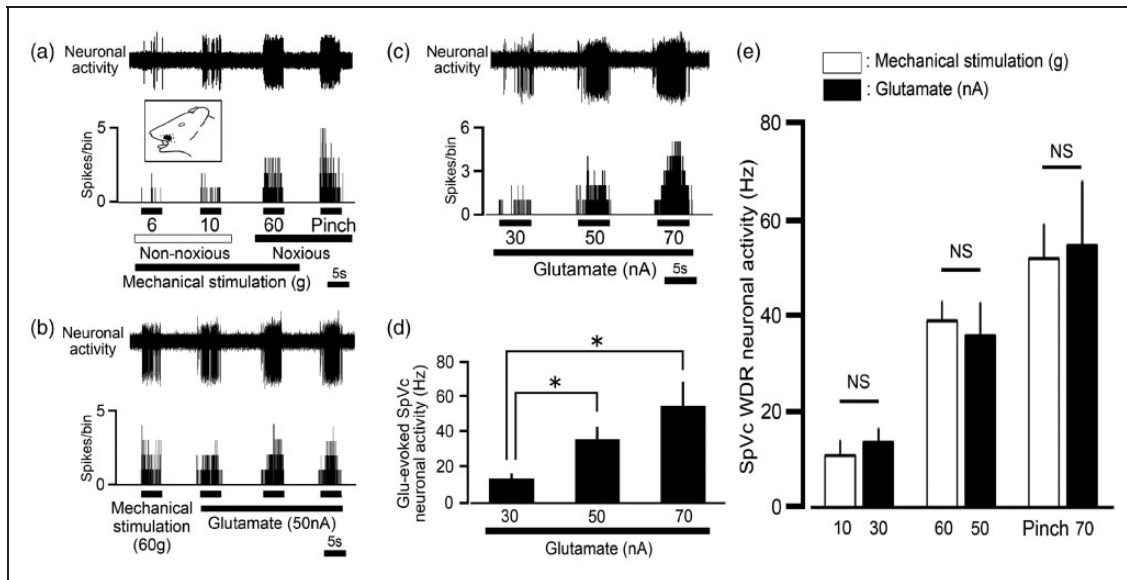
Figure 1. Glutamate iontophoretic application-evoked neuronal discharges of spinal trigeminal nucleus caudalis (SpVc) wide dynamic range (WDR) neurons responding to mechanical stimulation of the orofacial area. (a) Typical example of SpVc WDR neuronal activity evoked by non-noxious (6, 10 g) and noxious mechanical stimulation (60 g, noxious pinch) of the orofacial skin. Upper trace: SpVc WDR neuronal activity. Lower trace: poststimulus histogram. Inset: Receptive field of the whisker pad in the orofacial area. Blackened area indicates the location and size of the receptive field. (b) Iontophoretic application of glutamate (50 nA, 5 s) evoked reproducible neuronal discharges from SpVc WDR neurons (interval of 10 s) responding to noxious mechanical stimulation of the orofacial area (60 g, von Frey hair, 5 s). (c) Typical example of glutamate iontophoretic application-induced SpVc WDR neuronal activity (30, 50 and 70 nA; 5 s). (d) Summary of the effect of iontophoretic application of glutamate (30, 50, 70 nA; 5 s) on evoked neuronal discharges of SpVc WDR neurons. $P < 0.05$, 30 nA vs. 50 nA and 70 nA. (e) Comparison of neuronal discharges of SpVc WDR neurons evoked by iontophoretic application of glutamate (30, 50, 70 nA; 5 s) and mechanical stimulation. NS, not significant.

significantly increased in a current-dependent manner (Figure 1(d); $P < 0.05$, n = 4). There were no significant differences between the discharge frequencies evoked by iontophoretic glutamate (30, 50, and 70 nA) and mechanical stimulation (10 g, 60 g, and noxious pinch), respectively (Figure 1(e)). We also observed that iontophoretic application of vehicle (160 mmol/l, NaCl, pH 4.5) had no significant effect on SpVc WDR neuronal activity, as previously described (n = 4, data not shown).[16]

Effect of intravenous administration of resveratrol on the iontophoretic application of glutamate-evoked neuronal discharge

Figure 2(a) shows a typical example of the effect of intravenous administration of resveratrol on SpVc WDR neuronal excitability in response to iontophoretic application of glutamate. Glutamate-evoked discharges of SpVc WDR neurons were inhibited by resveratrol administration (2 mg/kg, i.v.) in a reversible manner. As shown in Figure 2(b), the mean firing frequency of SpVc WDR neurons in response to iontophoretic application of glutamate was significantly inhibited by intravenous administration of resveratrol (2 mg/kg) and

maximal inhibition of discharge frequency was seen within 10 min ($P < 0.05$, n = 4). These inhibitory effects were reversed after approximately 20 min (Figure 2(a) and (b)). No significant change was observed in the mean receptive field size after resveratrol administration, as described previously.[20] The relative magnitude of inhibition by resveratrol of SpVc WDR neuronal discharge frequency was significantly greater for 50 nA than 30 nA glutamate iontophoretic application (67% ± 5% vs. 40% ± 5%, respectively; $P < 0.05$). Also, the relative magnitude of inhibition by resveratrol of SpVc WDR neuronal discharge frequency was significantly greater for 70 nA than 30 nA glutamate iontophoretic application (71% ± 7% vs. 40% ± 5%, respectively; $P < 0.05$).

Effect of intravenous administration of resveratrol on the iontophoretic application of NMDA-evoked neuronal discharge frequency

Next, we tested whether intravenous administration of resveratrol affected the iontophoretic application of NMDA-evoked neuronal discharge frequencies. Figure 3(a) shows the effect of intravenous administration of resveratrol on SpVc WDR neuronal excitability
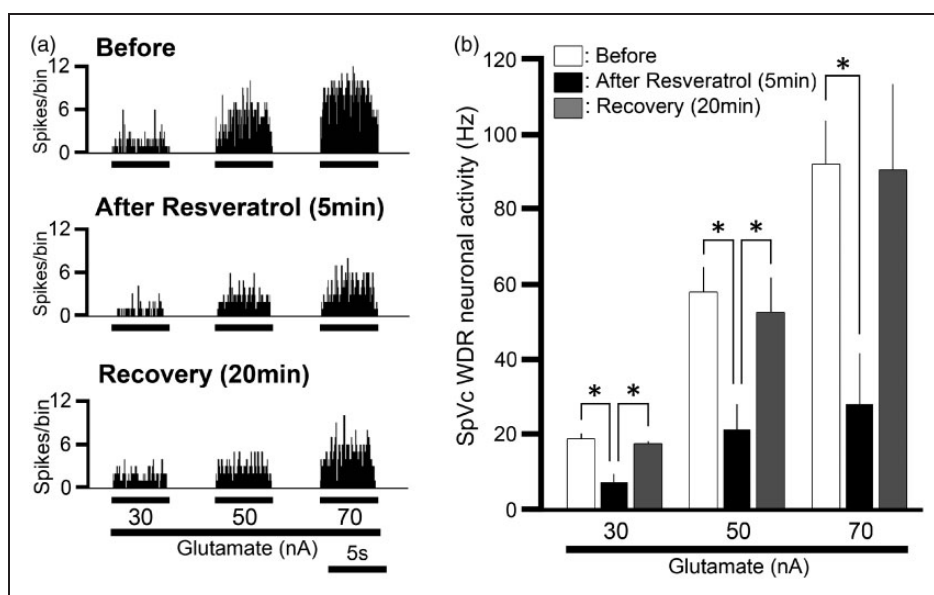
Figure 2. Effect of intravenous administration of resveratrol on glutamate iontophoretic application-evoked spinal trigeminal nucleus caudalis (SpVc) wide dynamic range (WDR) neuronal discharge frequency. (a) Typical examples of glutamate iontophoretic application-evoked (30, 50, 70 nA; 5 s) neuronal discharges of SpVc WDR neurons responding to mechanical stimulation of the orofacial area before, and 5 and 20 min after, intravenous administration of 2 mg/kg resveratrol. (b) Summary of glutamate iontophoretic application-evoked neuronal discharges of SpVc WDR neurons responding to mechanical stimulation of the orofacial area before, and 5 and 20 min after, intravenous administration of resveratrol. $*P < 0.05$.
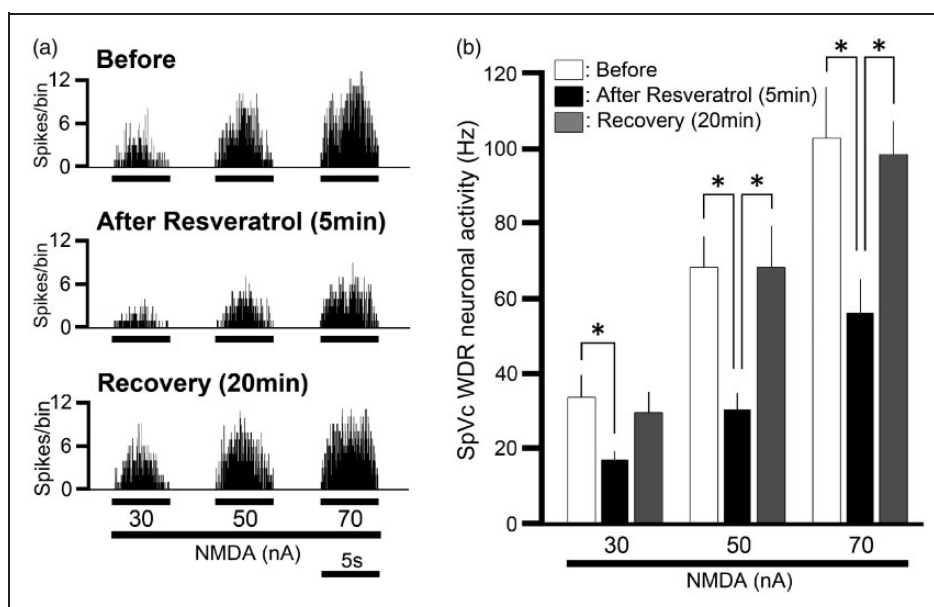

Figure 3. Effect of intravenous administration of resveratrol on N-methyl-D-aspartate (NMDA) iontophoretic application-evoked spinal trigeminal nucleus caudalis (SpVc) wide dynamic range (WDR) neuronal discharge frequency. (a) Typical examples of NMDA iontophoretic application-evoked (30, 50, 70 nA; 5 s) neuronal discharges of SpVc WDR neurons responding to mechanical stimulation of the orofacial area before, and 5 and 20 min after, intravenous administration of 2 mg/kg resveratrol. (b) Summary of NMDA iontophoretic application-evoked neuronal discharges of SpVc WDR neurons responding to mechanical stimulation of the orofacial area before, and 5 and 20 min after, intravenous administration of resveratrol. $*P < 0.05$.

in response to iontophoretic application of NMDA. The NMDA-evoked discharges of SpVc WDR neurons were inhibited by resveratrol administration (2 mg/kg, i.v.) in a reversible manner. As shown in Figure 3(b), the mean firing frequency of SpVc WDR neurons responding to iontophoretic application of NMDA were significantly inhibited by intravenous administration of resveratrol (2 mg/kg, $P < 0.05$), and maximal inhibition of discharge frequency was seen within 10 min. These inhibitory effects lasted approximately 20 min.

Comparison of the inhibition of discharge frequency of SpVc WDR neurons between noxious mechanical stimuli, iontophoretic glutamate, and NMDA

Finally, we compared the magnitude of the inhibitory effect of resveratrol on SpVc WDR neuronal activity evoked by noxious mechanical stimulation (60 g), glutamate (50 nA), and NMDA (50 nA), because the mean firing frequency evoked by 30, 50, and 70 nA iontophoretic glutamate application was mimicked by the application of 10 g, 60 g, and pinch mechanical stimulation, respectively (Figure 1(e)). In this study, the mean magnitude of inhibition by resveratrol of 60 g noxious mechanical stimulation evoked SpVc neuronal discharge frequency was $62.1 \pm 2.8\%$ (n = 4). Figure 4 shows the mean magnitude of inhibition of noxious stimulation (60 g)-, glutamate (50 nA)-, and NMDA (50 nA)-induced SpVc WDR neuronal firing by resveratrol. The mean magnitude of inhibition by resveratrol of glutamate-evoked SpVc neuronal discharge frequency was almost equal to that evoked by NMDA iontophoretic application.
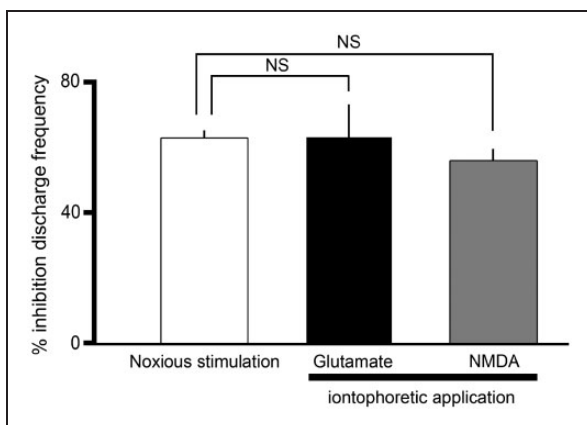

Figure 4. Comparison of the inhibition of discharge frequencies by resveratrol of spinal trigeminal nucleus caudalis (SpVc) wide dynamic range (WDR) neurons evoked by noxious mechanical stimuli, glutamate and N-methyl-D-aspartate (NMDA) iontophoretic application currents. Noxious mechanical stimulation (60 g), glutamate and NMDA iontophoretic application (50 nA each). NS, not significant.

Discussion

Methodological consideration

We have recently reported that acute intravenous resveratrol administration suppresses SpVc WDR neuronal excitability via both peripheral and central mechanisms.[20] More recently, we also demonstrated subcutaneous local injection of resveratrol into the peripheral receptive field suppresses the excitability of SpVc neurons to respond to non-noxious and noxious stimulation, possibly by inhibiting sodium channels in the nociceptive nerve terminals of primary sensory neurons (trigeminal ganglion neurons).[34] These findings suggest that acute intravenous resveratrol administration in the rat attenuates the excitability of neuronal transmission of SpVc neurons responding to nociceptive mechanical stimulation. However, the precise site of the central effects of resveratrol remains to be determined. In the present study, we aimed to test whether intravenous administration of resveratrol attenuates central postsynaptic glutamatergic transmission of SpVc neurons responding to noxious mechanical stimulation of the receptive field. In this study, we found the following findings: (i) SpVc WDR neurons responding to non-noxious and noxious mechanical stimulation of the whisker pad were also activated by iontophoretic application of glutamate; (ii) the firing frequency of iontophoretic glutamate-evoked spikes was current-dependently increased (30, 50, and 70 nA) and reversible; and (iii) the mean firing frequency evoked by 30, 50, and 70 nA iontophoretic glutamate application was mimicked by the application of 10 g, 60 g, and pinch mechanical stimulation, respectively. When taking these observations together, the iontophoretic application of glutamate is a valid method for testing the central effects of resveratrol and SpVc neuronal excitability.

Resveratrol suppresses the glutaminergic excitatory transmission of SpVc neurons via the NMDA receptor

It is known that resveratrol significantly suppresses glutamate-induced currents in post-synaptic CA1 pyramidal neurons in hippocampal slices, without having any presynaptic effects.[21] In the present study, we found that the mean firing frequency of SpVc WDR neurons responding to the iontophoretic application of glutamate was significantly inhibited by the administration of resveratrol (2 mg/kg, i.v.). Maximal inhibition of discharge frequency of SpVc WDR neurons was observed within 10 min, and these inhibitory effects were reversed in approximately 20 min. The time course of the peak effect and recovery following systemic administration of resveratrol was in agreement with our previous data.[20] Therefore, it is possible to speculate that resveratrol suppresses the glutamatergic excitatory synaptic

transmission of SpVc neurons by inhibiting post-synaptic glutamate receptors.

Additionally, Gao et al.[21] have reported that NMDA receptors are more sensitive to resveratrol than α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptors. Since it is known that intrathecal administration of selective NMDA receptor antagonists depresses nociceptive behavior (tail-flick and hot plate test),[27,28] it is more likely that NMDA receptors are involved in nociceptive spinal transmission.[35] In the present study, we found that the relative magnitude of inhibition by resveratrol of glutamate-evoked SpVc WDR neuronal discharge frequency was similar to that of iontophoretic application of NMDA. Taken together, these findings suggest that it is likely that noxious stimulation-induced NMDA receptor-activated SpVc neuronal excitability was suppressed by the intravenous administration of resveratrol. This notion is also supported by the observation that NMDA receptors are involved in mediating the enhanced responses to noxious peripheral inputs, whereas AMPA receptors are concerned with responses to non-noxious inputs in the spinal dorsal horn of rats with spinal nerve ligation neuropathy.[36]

Alternatively, it is also reported that resveratrol decreases action potential duration and L-type Ca^{2+} currents in ventricular myocytes,[22,23] suggesting the possibility that resveratrol suppresses the glutamatergic excitatory synaptic transmission of SpVc neurons by inhibiting pre-synaptic Ca^{2+} channels. In addition, using the hot plate test, Gupta et al.[37] have reported that intraperitoneal administration of resveratrol exhibits a dose-dependent antinociceptive effect via an opioidergic mechanism. It is also known that enkephalin inhibits nociceptive transmission through the inhibition of glutamate release in the superficial layer of spinal dorsal horn neurons in a slice preparation.[38] We previously reported that activation of μ-opioid receptors inhibits the excitability of rat nociceptive small-diameter trigeminal ganglion neurons projecting to the superficial layer of the upper cervical dorsal horn and this inhibition is mediated by potentiation of voltage-gated K^+ currents.[39] These results suggest that resveratrol may activate μ-opioid receptors in the presynaptic terminal causing potentiation of voltage-gated K^+ currents. These effects inhibit the excitability of neuronal transmission in the SpVc via depression of glutamate release. However, further study is needed to elucidate this possibility.

Functional significance of suppression of nociceptive transmission by the dietary constituent, resveratrol

The majority of spinal dorsal horn neurons are responsive to both NMDA and non-NMDA receptor antagonists in naïve rats, suggesting the coexistence of both types of excitatory amino acid receptors on the spinal neurons of naïve rats.[35] In the trigeminal system, intravenous injection of both NMDA and non-NMDA receptor antagonists block the excitatory responses of C1-C2 spinal neurons to electrical stimulation of the sagittal sinus.[24] In addition, local iontophoretic application of NMDA and non-NMDA glutamate receptor antagonists attenuates tooth-pulp-evoked C1 neuronal excitation.[25] Bereiter and Bereiter[40] have also reported that both NMDA and non-NMDA antagonists significantly reduce the number of neurons at the trigeminal spinal nucleus interpolaris/caudalis (SpVi/Vc) and SpVc/C1 transition regions that produce the neuronal excitation marker, *c-fos* protein, following mustard oil stimulation of the cornea. We previously provided evidence that chronic administration of resveratrol attenuates inflammation-induced mechanical hyperalgesia and that this effect is due primarily to the suppression of SpVc WDR neuron hyperexcitability via inhibition of both peripheral and central cyclooxygenase cascade signaling pathways. Up-regulation of NMDA receptors in the trigeminal spinal nociceptive neurons have been reported under inflammatory and neuropathic conditions,[41,42] suggesting that up-regulation of the NMDA receptor signaling pathway plays an important role in the hyperalgesia and allodynia of central sensitization.[39] These findings suggest that in inflammatory/neuropathic conditions, systemic resveratrol attenuates the excitability of SpVc neurons via the mechanism of NMDA receptor antagonism.

Recently, an increasing number of CAMs have been used for the treatment of chronic pain.[3,43] It is known that patients frequently turn to CAM therapies, such as herbal medicines and acupuncture, for pain control when other medical treatments are ineffective.[2,3] There has also been much research concerning the potential influence of diet and dietary supplementation on conditions associated with pain,[4,6,44] and a recent paper has reviewed the modulatory mechanism of the dietary constituent, resveratrol, on nociceptive neuronal activity.[13] Therefore, our results contribute to the development of analgesic drugs for the treatment of orofacial pathological pain. The findings from our present in vivo study support the idea that resveratrol, as well as being a candidate molecular target for NMDA receptor signaling studies, is a potential CAM for the prevention of nociceptive and inflammatory hyperalgesia.

Conclusions

The present study provides evidence that resveratrol suppresses glutamatergic neurotransmission of the SpVc neurons that respond to nociceptive mechanical stimulation in vivo via an NMDA receptor. These findings

suggest that resveratrol may be used as a CAM for the treatment of trigeminal nociceptive pain.

Authors' Contributions

ST, YK, and NU performed the electrophysiological and histological experiments. KU, YS, and KI interpreted the data and helped finalize the manuscript. MT participated in the design of the present study and wrote the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest, with respect to the research, authorship or publication of this article.

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