Biochemical and Biophysical Research Communications 459 (2015) 498–503

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Spontaneous L-glutamate release enhancement in rat substantia gelatinosa neurons by (−)-carvone and (+)-carvone which activate different types of TRP channel

Qin Kang, Chang-Yu Jiang, Tsugumi Fujita, Eiichi Kumamoto*

Department of Physiology, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan

ARTICLE INFO

Article history:
Received 17 February 2015
Available online 5 March 2015

Keywords:
(−)-Carvone
(+)-Carvone
TRPV1
TRPA1
Glutamate release
Spinal substantia gelatinosa

ABSTRACT

Transient receptor potential (TRP) channels in the spinal dorsal horn lamina II (substantia gelatinosa; SG), which are involved in the modulation of nociceptive transmission, have not yet been fully examined in property. Activation of the TRP channels by various plant-derived chemicals results in an increase in the spontaneous release of L-glutamate onto the SG neurons. We examined the effects of a monoterpene ketone (−)-carvone (contained in spearmint) and its stereoisomer (+)-carvone (in caraway) on glutamatergic spontaneous excitatory transmission in SG neurons of adult rat spinal cord slices by using the whole-cell patch-clamp technique. (−)-Carvone and (+)-carvone increased the frequency of spontaneous excitatory postsynaptic current (sEPSC) in a reversible and concentration-dependent manner with a small increase in its amplitude. Half-maximal effective concentrations of (−)-carvone and (+)-carvone in increasing sEPSC frequency were 0.70 mM and 0.72 mM, respectively. The (−)-carvone but not (+)-carvone activity was inhibited by a TRPV1 antagonist capsazepine. On the other hand, the (+)-carvone but not (−)-carvone activity was inhibited by a TRPA1 antagonist HC-030031. These results indicate that (−)-carvone and (+)-carvone activate TRPV1 and TRPA1 channels, respectively, resulting in an increase in spontaneous L-glutamate release onto SG neurons, with almost the same efficacy. Such a difference in TRP activation between the stereoisomers may serve to know the properties of TRP channels in the SG.

1. Introduction

Transient receptor potential (TRP) channels expressed in the peripheral and central terminals of primary-afferent neuron are involved in the reception of sensory stimuli and the modulation of nociceptive transmission, respectively [1,2]. Activation of the peripheral terminal TRP channels depolarizes membrane, resulting in the production of action potentials (APs). On the other hand, activating the central terminal TRP channels leads to an increase in the spontaneous release of L-glutamate onto medullary and spinal cord lamina II (substantia gelatinosa, SG) neurons. There are plant-derived chemicals among activators of the TRP channels. For instance, TRP vanilloid-1 (TRPV1) channels are activated by capsaicin, resiniferatoxin and piperine [3–7], and TRP ankyrin-1 (TRPA1) channels by allyl isothiocyanate (AITC), cinnamaldehyde, eugenol, zingerone and carvacrol ([8–13]; for review see Ref. [14]).

Many of the properties of the TRP channels have been examined in heterologous cells expressing the channels and also in the cell body of primary-afferent neuron. We have suggested that there may be a difference in property between TRP channels in the central terminal and cell body of primary-afferent neuron. For instance, eugenol, zingerone and olvanil (the synthetic oleic acid homologue of capsaicin) activated TRPV1 channels in its cell body [15–17] but not the spinal cord SG [7,8,13]. Gonçalves et al. [18] have recently reported that a monoterpene ketone (−)-carvone contained in spearmint increases intracellular Ca^{2+} concentration in rat primary-afferent neurons in a manner sensitive to a TRPV1 antagonist capsazepine, indicating an involvement of TRPV1 channels. Considering a possible difference in property between the central terminal and peripheral TRP channels, (−)-carvone may activate other types of TRP channel but not TRPV1 channels in the SG.

(−)-Carvone has various pharmacological actions including antinociception which has been attributed to an inhibition of the

* Corresponding author. Fax: +81 952 34 2013.
E-mail address: kumamote@cc.saga-u.ac.jp (E. Kumamoto).

http://dx.doi.org/10.1016/j.bbrc.2015.02.135
0006-291X/

conduction of AP in the peripheral nerve [19]. A stereoisomer of (−)-carvone, (+)-carvone, contained in caraway is reported to exhibit actions different from those of (−)-carvone in human autonomic nervous system function [20], mouse locomotive [21], anticonvulsive [22] and guinea-pig ileum relaxation activities [23].

It is likely that at least a part of the carvone actions is due to an action on the nervous system. Although carvone is known to inhibit APs [19,24], to our knowledge, it has not been examined yet how carvone affects synaptic transmission. Considering that (−)-carvone and (+)-carvone have actions distinct from each other, it is possible that they activate different types of TRP channel. In order to address these issues, we examined the effects of (−)-carvone and (+)-carvone on glutamatergic spontaneous excitatory transmission with a focus on the involvement of TRP channels by applying the whole-cell patch-clamp technique to SG neurons of adult rat spinal cord slices.

2. Materials and methods

All animal experiments were approved by the Animal Care and Use Committee of Saga University. Slice preparations from the adult rat spinal cord were prepared as described elsewhere [4,10,13]. Briefly, male Sprague–Dawley rats (6–8 weeks old) were anesthetized with urethane (1.2 g/kg, i.p.), and then a lumbosacral segment (L_1-S_3) of the spinal cord was extracted and placed in cold pre-oxygenated Krebs solution (2–4 °C) pre-equilibrated with 95% O_2 and 5% CO_2. After cutting all ventral and dorsal roots, the pia-arachnoid membrane was removed. The spinal cord was mounted on a microslicer, and then a 650 μm-thick transverse slice was cut. The slice was transferred to a recording chamber, and completely submerged and superfused at a rate of 12–15 ml/min with Krebs solution saturated with 95% O_2 and 5% CO_2 at 36 ± 1 °C. The composition of Krebs solution used was (in mM): NaCl, 117; KCl, 3.6; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11 (pH = 7.4 when saturated with the gas).

Blind whole-cell voltage-clamp recordings from SG neurons were made at a holding potential (V_H) of −70 mV with a patch-pipette, as done previously [4,10,13]. The patch-pipette solution used (in mM) was composed of K-gluconate, 135; $CaCl_2$, 0.5; $MgCl_2$, 2; KCl, 5; EGTA, 5; HEPES, 5; and Mg-ATP, 5 (pH = 7.2). Signals were acquired using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). Data were low-pass filtered at 5 kHz, and digitized at 333 kHz with an A/D converter. The data were recorded and stored with a personal computer using pCLAMP 8.1 software (Molecular Devices). Spontaneous excitatory postsynaptic currents (sEPSCs) were detected and analyzed using Mini Analysis Program ver. 6.0.3 (Synaptosoft, Decatur, GA, USA). Numerical data are given as the mean ± SEM. Statistical significance was determined as $P < 0.05$ using the paired or unpaired Student's t test. In all cases, n refers to the number of neurons studied.

The drugs used were (−)-carvone, (+)-carvone, capsazepine and HC-030031 (Sigma–Aldrich, St Louis, MO, USA). The drugs were first dissolved in dimethyl sulfoxide (DMSO) at 1000 (>100 for carvone) and then diluted to the final concentration in Krebs solution immediately before use. DMSO at 1% did not affect sEPSC frequency and amplitude. Drugs were applied by superfusing a solution containing drugs without altering the perfusion rate or temperature.

3. Results

Whole-cell patch-clamp recordings were made from a total of 176 SG neurons. All SG neurons tested had resting membrane potentials lower than −60 mV (when measured in the current-clamp mode).

3.1. The effects of (−)-carvone and (+)-carvone on spontaneous excitatory transmission in SG neurons

(−)-Carvone (1 mM; the left of Fig. 1A) superfused for 2 min enhanced spontaneous excitatory transmission in a SG neuron (Fig. 1Ba). Its stereoisomer (+)-carvone (1 mM; the right of Fig. 1A) also exhibited a similar facilitatory effect (Fig. 1Bb). Fig. 1Ca and b demonstrate the time courses of changes in sEPSC frequency and amplitude following (−)-carvone or (+)-carvone superfusion, relative to those before its superfusion. The sEPSC frequency increased gradually over time, peaking around 3 min after carvone addition; this facilitation was accompanied by a small increase in its amplitude. This increase in sEPSC frequency subsided within 10 min after carvone washout. sEPSC frequency increases produced by (−)-carvone and (+)-carvone averaged to be, respectively, 299 ± 32% [$P < 0.05$; from 8.7 ± 1.2 Hz to 21.0 ± 2.2 Hz ($n = 41$)] and 284 ± 28% [$P < 0.05$; from 6.2 ± 0.6 Hz to 15.5 ± 1.8 Hz ($n = 45$)] around 3 min after its addition. sEPSC amplitudes before and around 3 min after (−)-carvone superfusion were, respectively, 9.5 ± 0.4 pA and 11.6 ± 0.7 pA ($n = 41$); they before and after (+)-carvone superfusion were, respectively, 9.7 ± 0.4 pA and 11.6 ± 0.6 pA ($n = 45$). In each case, the latter was significantly larger than the former ($P < 0.05$). Some (about 40%) of the neurons examined produced a small inward current following (−)-carvone or (+)-carvone superfusion. Their peak amplitudes were 14.3 ± 2.3 pA ($n = 16$) and 10.9 ± 1.1 pA ($n = 18$), respectively.

The sEPSC frequency increase produced by (−)-carvone or (+)-carvone (each 1 mM) was repeated at a time interval of 20 min, as seen in Fig. 2A and B. The initial (−)-carvone treatment increased sEPSC frequency from 9.5 ± 2.1 Hz to 23.2 ± 4.7 Hz ($n = 4$) around 3 min after its treatment. A second application was similar; sEPSC frequency increased from 10.9 ± 2.4 Hz to 26.2 ± 8.0 Hz ($n = 4$). sEPSC frequency increases by the initial and second applications were not significantly different from each other (Fig. 2Ca). On the other hand, sEPSC amplitude was minimally increased by (−)-carvone. sEPSC amplitudes before and after the initial treatment were, respectively, 8.2 ± 0.3 pA and 8.8 ± 0.5 pA ($n = 4$); those before and after the second treatment were, respectively, 8.2 ± 0.6 pA and 10.3 ± 2.0 pA ($n = 4$). sEPSC frequency increase produced by (+)-carvone was also repeated at a time interval of 20 min, as seen in Fig. 2B. The initial (+)-carvone treatment increased sEPSC frequency from 5.3 ± 1.0 Hz to 31.7 ± 4.3 Hz ($n = 4$) around 3 min after its treatment. A second application was similar; sEPSC frequency increased from 4.0 ± 0.7 Hz to 24.8 ± 4.2 Hz ($n = 4$). sEPSC frequency increases produced by the initial and second applications were not significantly different from each other (Fig. 2Cb). On the other hand, sEPSC amplitude was slightly increased by (+)-carvone. sEPSC amplitudes before and after the initial treatment were, respectively, 8.4 ± 0.5 pA and 12.3 ± 1.7 pA ($n = 4$); those before and after the second treatment were, respectively, 8.0 ± 0.5 pA and 10.7 ± 0.8 pA ($n = 4$).

3.2. Concentration dependencies for sEPSC frequency and amplitude changes produced by (−)-carvone and (+)-carvone

We next examined concentration dependencies for the facilitatory actions of (−)-carvone and (+)-carvone on spontaneous excitatory transmission. The (−)-carvone activity was concentration-dependent in a range of 0.1–1 mM. As seen from Fig. 3Aa, (−)-carvone at 1 mM exhibited a facilitation larger than that at 0.5 mM, when examined in the same neuron. Fig. 3Ba demonstrates concentration-response relationships for changes in sEPSC frequency and amplitude, produced by (−)-carvone. sEPSC frequency increased with an increase in concentration with a small increase in its amplitude. Analysis based on the Hill equation

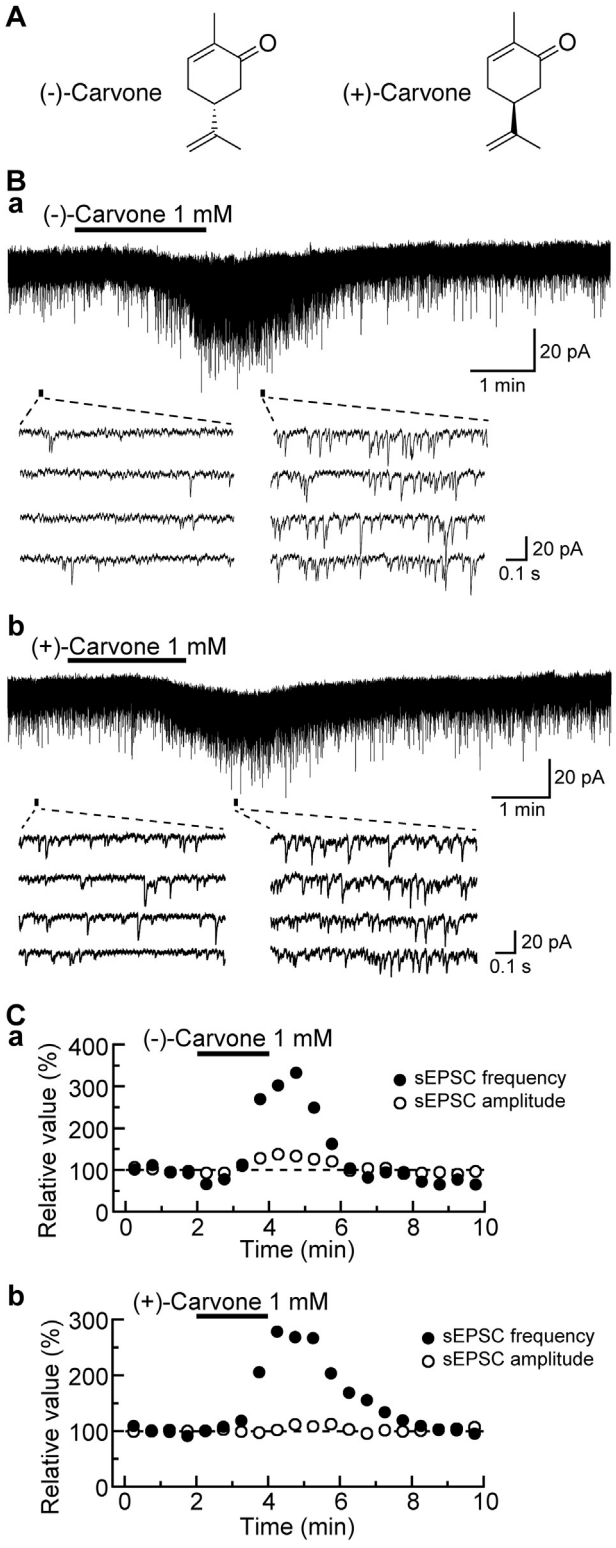

Fig. 1. (−)-Carvone and (+)-carvone reversibly increase the frequency of spontaneous excitatory postsynaptic current (sEPSC) with a small increase in its amplitude in rat substantia gelatinosa (SG) neurons. (A) The chemical structures of (−)-carvone and (+)-carvone. (Ba, b) Recordings of sEPSCs in the absence and presence of (−)-carvone (Ba) or (+)-carvone (Bb). In this and subsequent figures, duration of drug superfusion is shown by a horizontal bar above chart recording; four consecutive traces of sEPSCs for a period indicated by a short bar below the chart recording are shown in an expanded scale in time. Note that (−)-carvone and (+)-carvone produce a small inward current. (Ca, b) Time courses of changes in sEPSC frequency and amplitude during the action of (−)-carvone (Ca) or (+)-carvone (Cb), relative to those before its superfusion (control). In this and subsequent figures, control level (100%) is indicated by horizontal dotted line. Holding potential (V_H) = −70 mV.

showed that the half-maximal effective concentration (EC_{50}) of (−)-carvone in increasing sEPSC frequency is 0.70 mM.

The sEPSC frequency increase produced by (+)-carvone was also concentration-dependent; when tested in the same neuron, (+)-carvone at 1 mM increased sEPSC frequency more greatly than (+)-carvone at 0.5 mM (Fig. 3Ab). As seen in the (−)-carvone action, sEPSC frequency increase produced by (+)-carvone was concentration-dependent in a range of 0.1–1 mM, while its amplitude was slightly increased (Fig. 3Bb). Analysis based on the Hill equation showed that the EC_{50} value of (+)-carvone for sEPSC frequency increase is 0.72 mM.

3.3. The excitatory transmission enhancements produced by (−)-carvone and (+)-carvone are mediated by TRPV1 and TRPA1 channels, respectively

The excitatory transmission enhancements produced by (−)-carvone and (+)-carvone were similar to those by various plant-derived chemicals which activated TRPV1 and TRPA1 channels in the adult rat SG [4,6–11,13]. We, therefore, examined whether the facilitatory actions of (−)-carvone and (+)-carvone (each 1 mM) are mediated by TRPV1 or TRPA1 channels. The experiments were performed in single neurons, because the carvone activities were repeated.

As seen in Fig. 4Aa and b, pretreatment with a TRPV1 antagonist capsazepine (10 μM) for 4 min inhibited the facilitatory effect of (−)-carvone but not (+)-carvone on excitatory transmission. In the presence of capsazepine, (−)-carvone changed sEPSC frequency from 9.1 ± 1.9 Hz to 16.8 ± 3.6 Hz (n = 8) around 3 min after its treatment. Percentage change in sEPSC frequency was significantly smaller than that without capsazepine [change: 6.9 ± 1.1 Hz to 23.6 ± 3.8 Hz (n = 8); Fig. 4C]. Here, sEPSC amplitudes in the absence and presence of (−)-carvone in Krebs solution containing capsazepine were 10.7 ± 1.2 pA and 11.1 ± 1.1 pA (n = 8), respectively. On the other hand, in the presence of capsazepine, (+)-carvone increased sEPSC frequency from 6.7 ± 2.5 Hz to 18.9 ± 4.6 Hz (n = 6) around 3 min after its treatment. Increased sEPSC frequency percentage was not significantly different from that in the absence of capsazepine when examined in the same neuron [change: 7.9 ± 2.2 Hz to 22.4 ± 5.6 Hz (n = 6); Fig. 4D]. sEPSC amplitudes in the absence and presence of (+)-carvone in Krebs solution containing capsazepine were 9.6 ± 2.3 pA and 12.0 ± 2.5 pA (n = 6), respectively. Capsazepine itself did not affect sEPSC frequency and amplitude, as reported previously (for example, see Ref. [13]).

Unlike the TRPV1 antagonist, a TRPA1 antagonist HC-030031 (50 μM) inhibited the facilitatory effect of (+)-carvone but not (−)-carvone on excitatory transmission (Fig. 4Ba and b). Under the pretreatment with HC-030031 for 4 min, (−)-carvone changed sEPSC frequency from 7.2 ± 1.6 Hz to 23.4 ± 3.4 Hz (n = 9) around 3 min after its application. Increased sEPSC frequency percentage was not significantly different from than that in the absence of HC-030031 when examined in the same neuron [change: 9.0 ± 1.3 Hz to 27.9 ± 3.3 Hz (n = 9); Fig. 4C]. sEPSC amplitudes in the absence and presence of (−)-carvone in Krebs solution containing HC-030031 were 9.6 ± 1.2 pA and 10.9 ± 1.4 pA (n = 9), respectively. On the other hand, in neurons where (+)-carvone increased sEPSC frequency from 8.2 ± 1.5 Hz to 24.2 ± 3.9 Hz (n = 7) around 3 min after its treatment, this drug altered sEPSC frequency from 8.7 ± 1.5 Hz to 12.5 ± 2.9 Hz (n = 7) under the pretreatment with

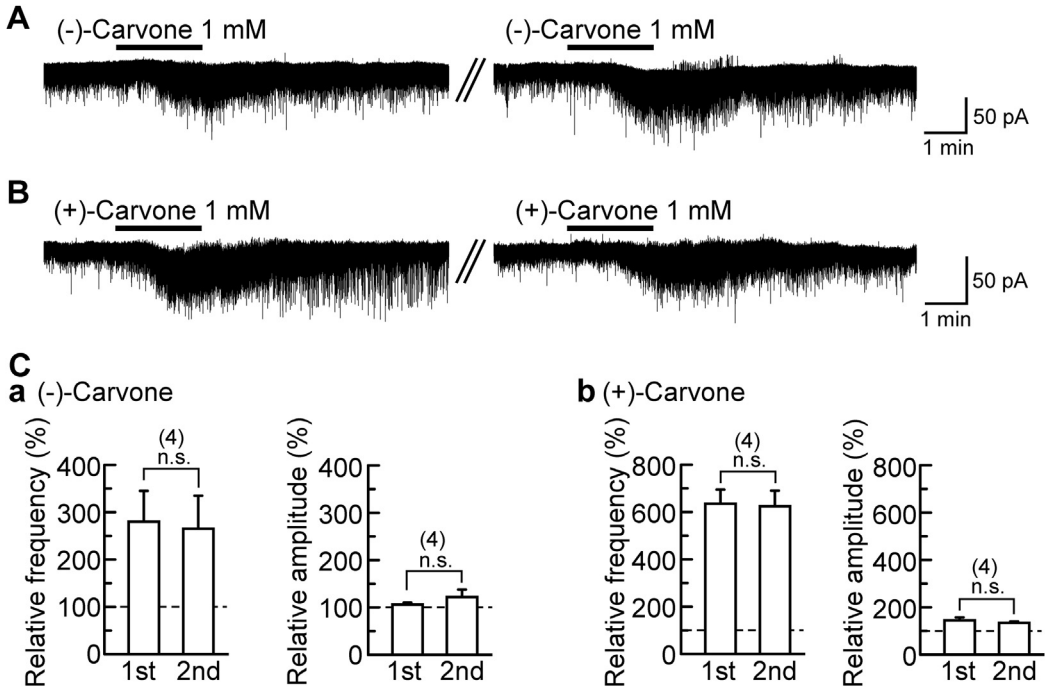

Fig. 2. The presynaptic enhancements of excitatory transmission by (−)-carvone and (+)-carvone are repeated at a time interval of 20 min. (A, B) Recordings of sEPSCs under the first application (*left*) of (−)-carvone (A) or (+)-carvone (B) and 20 min after its washout under the second application (*right*) of (−)-carvone (A) or (+)-carvone (B) in the same neuron. (Ca, b) The frequency and amplitude of sEPSC under the action of (−)-carvone (Ca) or (+)-carvone (Cb), relative to those just before its superfusion, in the first (1st) and second applications (2nd). In this and subsequent figures, values in parentheses denote the numbers of neurons examined; n.s.: not significant. $V_H = -70$ mV.

HC-030031 for 4 min. Percentage change in the latter was significantly smaller than one in the former (Fig. 4D). sEPSC amplitudes in the absence and presence of (+)-carvone in Krebs solution containing HC-030031 were 9.0 ± 0.6 pA and 11.1 ± 1.4 pA ($n = 7$), respectively. HC-030031 itself did not affect sEPSC frequency and amplitude, as reported previously (for example, see Ref. [13]).

4. Discussion

The present study demonstrated that (−)-carvone and (+)-carvone (each 1 mM) increase the frequency of sEPSC with a small increase in its amplitude in all of the SG neurons tested. In about a half of the neurons, this sEPSC frequency increase was accompanied by a small inward current, as seen in the actions of many plant-derived chemicals such as capsaicin, AITC and zingerone (for example, see Refs. [6,9,13]). The inward currents produced by (−)-carvone and (+)-carvone were not so different in peak amplitude from each other. Owing to their small amplitudes, their properties were not examined in the present study. The sEPSC amplitude increase would be due to an excess of the spontaneous release of L-glutamate from nerve terminals, as suggested in the presynaptic action of AITC in the SG [9].

The EC_{50} values of (−)-carvone and (+)-carvone for sEPSC frequency increase were 0.70 mM and 0.72 mM, respectively. The former value was similar to that (1.3 mM) of (−)-carvone in increasing intracellular Ca^{2+} concentration in HEK293 cells expressing human TRPV1 channels [18]. Moreover, their values were not so different from half-maximal inhibitory concentration values of (−)-carvone and (+)-carvone (1.4 mM and 2.0 mM, respectively) in reducing the peak amplitude of compound AP recorded from the frog sciatic nerve [24]. Thus, there was not so difference in the efficacies of sEPSC frequency increase and nerve conduction inhibition between (−)-carvone and (+)-carvone, observations different from those in human autonomic nervous system function [20], mouse locomotion [21], anticonvulsion [22] and guinea-pig ileum relaxation [23], where there was a difference in extent between (−)-carvone and (+)-carvone activities.

On the other hand, (−)-carvone and (+)-carvone activated different types of TRP channel from each other. The effect of (−)-carvone but not (+)-carvone was inhibited by the TRPV1 antagonist capsazepine. On the contrary, the effect of (+)-carvone but not (−)-carvone was inhibited by the TRPA1 antagonist HC-030031. These results indicate that (−)-carvone and (+)-carvone activate TRPV1 and TRPA1 channels, respectively, in the SG. The TRPV1 activation by (−)-carvone is consistent with the observation that (−)-carvone increases intracellular Ca^{2+} concentration in rat primary-afferent neurons and also in HEK293 cells expressing human TRPV1 channels in a manner sensitive to capsazepine [18]. With respect to TRP activation by carvone, there seem to be no reports other than those showing the activation by (−)-carvone of TRPV1 and also TRPV3 channels [18,25]. We found out that (+)-carvone has an ability to activate TRPA1 channels. The EC_{50} value (0.72 mM) for this activation was similar to that of carvacrol (0.69 mM; [10]) while being somewhat smaller than those of eugenol and zingerone (3.8 mM and 1.3 mM, respectively; [8,13]). It is well-known that there is a difference in actions on voltage-gated ion channels and neurotransmitter receptors among stereoisomers (see Refs. [26,27] for review), and there is a difference in the efficacy of TRP activation between stereoisomers such as (+)-menthol and (−)-menthol [28]. To our knowledge, the present study revealed for the first time that stereoisomers such as (−)-carvone and (+)-carvone activate different types of TRP channel.

There are TRPV1 and TRPA1 channels among proteins involved in the modulation of nociceptive transmission in the central terminal of primary-afferent neuron (see Ref. [2] for review). Although the antinociceptive activity of intraperitoneally-administrated (−)-carvone has been attributed to a peripheral nerve AP

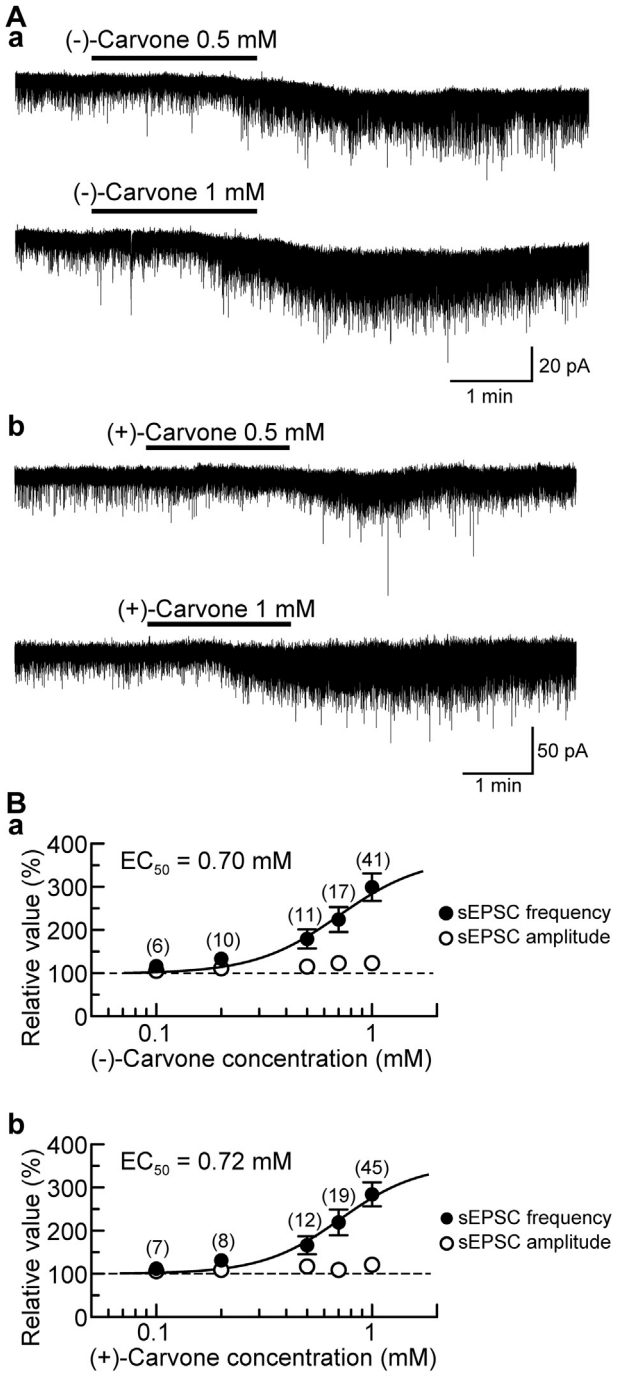

Fig. 3. The sEPSC frequency increases produced by (−)-carvone and (+)-carvone are concentration-dependent. (Aa, b) Recordings of sEPSCs in the absence and presence of (−)-carvone (Aa) or (+)-carvone (Ab) at 0.5 and 1 mM. In each of (Aa) and (Ab), upper and lower recordings were obtained from the same neuron. (Ba, b) The frequency and amplitude of sEPSC under the action of (−)-carvone or (+)-carvone, relative to those before its superfusion, which were plotted against the logarithm of its concentration. This (−)-carvone or (+)-carvone effect was measured around 3 min after the beginning of its superfusion. Each point with vertical bars represents the mean and SEM. If the SEM of the values was less than the size of symbol, the vertical bar was not shown. The concentration-response curve for sEPSC frequency was drawn according to the Hill equation [Ba, b: EC_{50} (Hill coefficient) is 0.70 mM (2.2) and 0.72 mM (2.4), respectively]. $V_H = -70$ mV.

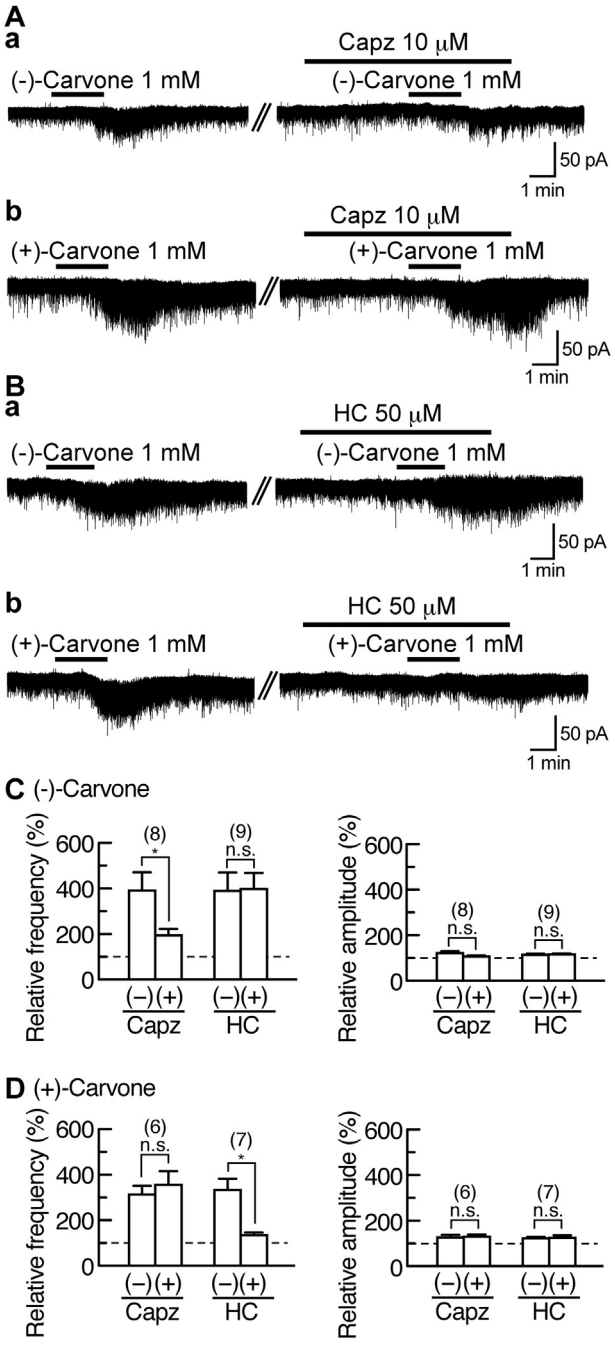

Fig. 4. The (−)-carvone and (+)-carvone activities are mediated by TRPV1 and TRPA1 channels, respectively. A TRPV1 antagonist capsazepine (Capz) inhibits the sEPSC frequency increase produced by (−)-carvone but not (+)-carvone, while a TRPA1 antagonist HC-030031 (HC) inhibits the sEPSC frequency increase produced by (+)-carvone but not (−)-carvone. (A, B) Chart recordings showing a change in sEPSCs under the action of (−)-carvone (a) or (+)-carvone (b) in the absence (*left*) and presence (*right*) of Capz (A) or HC (B). (C, D) sEPSC frequency and amplitude under the action of (−)-carvone (C) or (+)-carvone (D), relative to those before its superfusion, in the absence (−) and presence (+) of Capz or HC; *: $P < 0.05$. $V_H = -70$ mV.

conduction inhibition [19,24], TRPV1 activation produced by (−)-carvone in the SG may contribute to at least a part of the modulation of nociceptive transmission. Since TRPV1 and TRPA1 channels are expressed in not only primary-afferent neurons but also the central nervous system (see Refs. [29,30] for review), at least a part of the pharmacological actions of carvone on the nervous system may be mediated by the activation of the channels. Such a difference in TRP activation between (−)-carvone and (+)-carvone may serve to know the properties of the TRP channels.

Conflict of interest

None.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.135.

References

[1] B. Nilius, T. Voets, TRP channels: a TR(I)P through a world of multifunctional cation channels, Pflügers Archiv 451 (2005) 1–10.
[2] A. Patapoutian, S. Tate, C.J. Woolf, Transient receptor potential channels: targeting pain at the source, Nat. Rev. Drug Discov. 8 (2009) 55–68.
[3] M.L. Baccei, R. Bardoni, M. Fitzgerald, Development of nociceptive synaptic inputs to the neonatal rat dorsal horn: glutamate release by capsaicin and menthol, J. Physiol. 549 (2003) 231–242.
[4] C.-Y. Jiang, T. Fujita, H.-Y. Yue, L.-H. Piao, T. Liu, T. Nakatsuka, E. Kumamoto, Effect of resiniferatoxin on glutamatergic spontaneous excitatory synaptic transmission in substantia gelatinosa neurons of the adult rat spinal cord, Neuroscience 164 (2009) 1833–1844.
[5] V. Morisset, L. Urban, Cannabinoid-induced presynaptic inhibition of glutamatergic EPSCs in substantia gelatinosa neurons of the rat spinal cord, J. Neurophysiol. 86 (2001) 40–48.
[6] K. Yang, E. Kumamoto, H. Furue, M. Yoshimura, Capsaicin facilitates excitatory but not inhibitory synaptic transmission in substantia gelatinosa of the rat spinal cord, Neurosci. Lett. 255 (1998) 135–138.
[7] L. Yang, T. Fujita, C.-Y. Jiang, L.-H. Piao, H.-Y. Yue, K. Mizuta, E. Kumamoto, TRPV1 agonist piperine but not olvanil enhances glutamatergic spontaneous excitatory transmission in rat spinal substantia gelatinosa neurons, Biochem. Biophys. Res. Commun. 410 (2011) 841–845.
[8] M. Inoue, T. Fujita, M. Goto, E. Kumamoto, Presynaptic enhancement by eugenol of spontaneous excitatory transmission in rat spinal substantia gelatinosa neurons is mediated by transient receptor potential A1 channels, Neuroscience 210 (2012) 403–415.
[9] M. Kosugi, T. Nakatsuka, T. Fujita, Y. Kuroda, E. Kumamoto, Activation of TRPA1 channel facilitates excitatory synaptic transmission in substantia gelatinosa neurons of the adult rat spinal cord, J. Neurosci. 27 (2007) 4443–4451.
[10] Q.-T. Luo, T. Fujita, C.-Y. Jiang, E. Kumamoto, Carvacrol presynaptically enhances spontaneous excitatory transmission and produces outward current in adult rat spinal substantia gelatinosa neurons, Brain Res. 1592 (2014) 44–54.
[11] D. Uta, H. Furue, A.E. Pickering, M.H. Rashid, H. Mizuguchi-Takase, T. Katafuchi, K. Imoto, M. Yoshimura, TRPA1-expressing primary afferents synapse with a morphologically identified subclass of substantia gelatinosa neurons in the adult rat spinal cord, Eur. J. Neurosci. 31 (2010) 1960–1973.
[12] P.J. Wrigley, H.-J. Jeong, C.W. Vaughan, Primary afferents with TRPM8 and TRPA1 profiles target distinct subpopulations of rat superficial dorsal horn neurones, Br. J. Pharmacol. 157 (2009) 371–380.
[13] H.-Y. Yue, C.-Y. Jiang, T. Fujita, E. Kumamoto, Zingerone enhances glutamatergic spontaneous excitatory transmission by activating TRPA1 but not TRPV1 channels in the adult rat substantia gelatinosa, J. Neurophysiol. 110 (2013) 658–671.
[14] E. Kumamoto, T. Fujita, C.-Y. Jiang, TRP channels involved in spontaneous L-glutamate release enhancement in the adult rat spinal substantia gelatinosa, Cells 3 (2014) 331–362.
[15] L. Liu, S.A. Simon, Similarities and differences in the currents activated by capsaicin, piperine, and zingerone in rat trigeminal ganglion cells, J. Neurophysiol. 76 (1996) 1858–1869.
[16] L. Liu, Y.-C. Lo, I.-J. Chen, S.A. Simon, The responses of rat trigeminal ganglion neurons to capsaicin and two nonpungent vanilloid receptor agonists, olvanil and glyceryl nonamide, J. Neurosci. 17 (1997) 4101–4111.
[17] B.H. Yang, Z.G. Piao, Y.-B. Kim, C.-H. Lee, J.K. Lee, K. Park, J.S. Kim, S.B. Oh, Activation of vanilloid receptor 1 (VR1) by eugenol, J. Dent. Res. 82 (2003) 781–785.
[18] J.C.R. Gonçalves, A.L. Silveira, H.D.N. de Souza, A.A. Nery, V.F. Prado, M.A.M. Prado, H. Ulrich, D.A.M. Araújo, The monoterpene (-)-carvone: a novel agonist of TRPV1 channels, Cytom. Part A 83 (2013) 212–219.
[19] J.C.R. Gonçalves, F. de Sousa Oliveira, R.B. Benedito, D.P. de Sousa, R.N. de Almeida, D.A.M. de Araújo, Antinociceptive activity of (-)-carvone: evidence of association with decreased peripheral nerve excitability, Biol. Pharm. Bull. 31 (2008) 1017–1020.
[20] E. Heuberger, T. Hongratanaworakit, C. Böhm, R. Weber, G. Buchbauer, Effects of chiral fragrances on human autonomic nervous system parameters and self-evaluation, Chem. Senses 26 (2001) 281–292.
[21] G. Buchbauer, W. Jäger, A. Gruber, H. Dietrich, *R*-(+)- and *S*-(−)-carvone: influence of chirality on locomotion activity in mice, Flavour Fragrance J. 20 (2005) 686–689.
[22] D.P. de Sousa, F.F. de Farias Nóbrega, R.N. de Almeida, Influence of the chirality of (*R*)-(-)- and (*S*)-(+)-carvone in the central nervous system: a comparative study, Chirality 19 (2007) 264–268.
[23] D.P. de Sousa, G.A.S. Júnior, L.N. Andrade, F.R. Calasans, X.P. Nunes, J.M. Barbosa-Filho, J.S. Batista, Structure and spasmolytic activity relationships of monoterpene analogues found in many aromatic plants, Z. Naturforsch. C 63 (2008) 808–812.
[24] H. Kawasaki, K. Mizuta, T. Fujita, E. Kumamoto, Inhibition by menthol and its related chemicals of compound action potentials in frog sciatic nerves, Life Sci. 92 (2013) 359–367.
[25] A.K. Vogt-Eisele, K. Weber, M.A. Sherkheli, G. Vielhaber, J. Panten, G. Gisselmann, H. Hatt, Monoterpenoid agonists of TRPV3, Br. J. Pharmacol. 151 (2007) 530–540.
[26] W. Soudijn, I. van Wijngaarden, A.P. IJzerman, Stereoselectivity of drug-receptor interactions, IDrugs 6 (2003) 43–56.
[27] C. Valenzuela, C. Moreno, A. de la Cruz, Á. Macías, Á. Prieto, T. González, Stereoselective interactions between local anesthetics and ion channels, Chirality 24 (2012) 944–950.
[28] Y. Karashima, N. Damann, J. Prenen, K. Talavera, A. Segal, T. Voets, B. Nilius, Bimodal action of menthol on the transient receptor potential channel TRPA1, J. Neurosci. 27 (2007) 9874–9884.
[29] J.G. Edwards, TRPV1 in the central nervous system: synaptic plasticity, function, and pharmacological implications, Prog. Drug Res. 68 (2014) 77–104.
[30] P.M. Zygmunt, E.D. Högestätt, TRPA1, Handb. Exp. Pharmacol. 222 (2014) 583–630.