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Review

Effects of plant-derived compounds on excitatory synaptic transmission and nerve conduction in the nervous system: involvement in pain modulation

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ABSTRACT

Neuronal information is transferred by action potential (AP) conduction in nerve fibers and by chemical transmission at neuro-neuronal synapses. Inhibition of AP conduction in nociceptive nerve fibers results in antinociception. Although modulation of the chemical transmission plays an important role in various physiological functions, synapses in the neuronal pathway involved in nociceptive transmission become a target of endogenous and exogenous analgesics. Various compounds derived from plants were found to activate transient receptor potential (TRP) channels to enhance glutamatergic spontaneous excitatory transmission in rat spinal cord lamina II neurons that play a pivotal role in regulating nociceptive transmission from the periphery. This enhancement was due to an increase in the spontaneous release of L-glutamate from nerve terminals. Some of the plant-derived compounds produced a membrane hyperpolarization in rat spinal lamina II neurons without TRP channel activation. Moreover, various plant-derived compounds were found to inhibit AP conduction in the frog sciatic nerve. These activities of plant-derived compounds depended on their chemical structures. This result could serve to develop antinociceptive drugs that are related to plant-derived compounds. This article will review the effects of plant-derived compounds on excitatory transmission in rat spinal lamina II neurons and on nerve AP conduction in the frog sciatic nerve.

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1. Introduction

1.1. Excitatory transmission in rat spinal lamina II neurons and TRP channels

Information of nociceptive stimuli given to the periphery is transmitted through primary-afferent myelinated Aδ and unmyelinated C fibers contained in the dorsal root to the superficial laminae of spinal dorsal horn, especially lamina II (substantia gelatinosa; [1, 2]), and thereafter flows through the spinal cord, brain stem and then hypothalamus to the primary-sensory area of the cerebrum, producing pain sensation. Such a nociceptive transmission is due to action potential (AP) conduction in nerve fibers and chemical transmission at neuro-neuronal synapses [3].

There is much evidence showing that the spinal lamina II plays a crucial role in regulating nociceptive transmission ([1, 2]; for review, see [4, 5]). Endogenous and exogenous analgesics, which have an ability to exhibit antinociception by intrathecal administration, reduce the release of L-glutamate onto spinal lamina II neurons from nerve terminals and hyperpolarize membranes of spinal lamina II neurons, the actions of both of which lead to the reduction of the membrane excitability of the neurons [6, 7]. For instance, these effects were produced by

opioids [8-11], nociceptin [12-15], galanin [16, 17], adenosine ([18-20]; for review, see [21-23]), norepinephrine [24, 25], serotonin [26, 27], dopamine [28, 29], somatostatin [30, 31], the endocannabinoid anandamide [32, 33], a $GABA_B$-receptor agonist baclofen [34-36] and a clinically-used analgesic tramadol [37, 38].

Plant-derived compounds are well-known to activate cation-permeable transient receptor potential (TRP) channels expressed in dorsal root ganglion (DRG) neurons involved in nociceptive transmission (see [39-45] for review). For example, a red pepper component capsaicin activates TRP vanilloid-1 (TRPV1) channels ([46]; for review, see [47]), pungent chemicals contained in mustard, cinnamon and garlic activate TRP ankyrin-1 (TRPA1) channels ([48-50]; for review, see [44, 51]) and a peppermint component menthol activates TRP melastatin-8 (TRPM8) channels [52, 53].

TRP channel protein is synthesized in the cell body of DRG neuron, and then transported by axonal transport to the peripheral and central terminals of the neuron. Peripheral terminal TRP channels are opened by chemical stimuli or temperature (TRPV1, TRPA1 and TRPM8 channels: > 43 °C, < 17 °C and < 25 °C, respectively; for review, see [44, 51]), resulting in the production of AP [46, 47, 54]. On the other hand, activation of central terminal TRP channels in the spinal lamina II results in an enhancement of the spontaneous release of L-glutamate from the terminal to lamina II neurons (see [45, 55, 56] for review). Central terminal TRP channels are activated by endogenous substances: for instance, endocannabinoids and lipoxygenase metabolites for TRPV1 channels ([57, 58]; for review, see [47, 59]); and 3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597; a potent and systemically-active inhibitor of fatty acid amide hydrolase [60]), 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (a cyclopentane prostaglandin D_2 metabolite [61]), bradykinin [62] and 5,6-epoxyeicosatrienoic acid [63] for TRPA1 channels.

Various plant-derived compounds were found to enhance spontaneous excitatory transmission through TRP channel activation by applying the whole-cell patch-clamp method to neurons in the lamina II (which is easily identified as a translucent band) in transverse slice preparations dissected from the adult rat spinal cord [64, 65].

1.2. Compound action potentials recorded from the frog sciatic nerve

AP conduction in nerve fibers is mediated by voltage-gated Na^+ channels. Thus, Na^+ channels expressed in a nerve fiber are activated by a depolarizing stimulus given to a point of the fiber, allowing Na^+ entry to the cytoplasm, leading to a self-regenerative production of AP, which in turn results in an outward current (membrane depolarization) in a fiber point adjacent to the point to produce opening of other Na^+ channels, and so on. In a bundle of nerve fibers exposed to air insulator, AP conduction in each fiber produces AP current flowing through nerve bundle surface having high resistance that can be measured as a potential difference, i.e., compound action potential (CAP), by using two electrodes placed on the nerve (air-gap method). The dissected frog (*Rana nigromaculata*) sciatic nerve is a useful preparation for recording voltage-gated Na^+-channel blocker tetrodotoxin (TTX)-sensitive and fast-conducting (possibly myelinated Aα-fiber mediated) CAPs by using the air-gap method [66]. Although not only fast-conducting but also slow-conducting (C-fiber mediated) CAPs were recorded from the frog sciatic nerve, the latter had much smaller peak amplitude and conduction velocity than the former [67].

It has been reported that frog sciatic nerve fast-conducting CAPs are inhibited by clinically-used analgesics such as tramadol [68], opioids [69], an α_2-adrenoceptor agonist dexmedetomidine [70], antiepileptics [71], antidepressants [72] and non-steroidal anti-inflammatory drugs (NSAIDs [73]), many kinds of local anesthetics (lidocaine, ropivacaine, benzocaine, tetracaine and so on [74]) and a general anesthetic propofol [75]. These actions depended on the chemical structures of the drugs (for review, see [76-78]). Many plant-derived compounds were found to produce a similar CAP inhibition by applying the air-gap method to the frog sciatic nerve.

This review article will mention the effects of plant-derived compounds on both rat spinal lamina II neuron excitatory transmission and frog sciatic nerve CAPs, and then discuss how their effects are related to pain transmission and also to the chemical structures of the compounds.

2. Effects of plant-derived compounds on excitatory transmission in rat spinal lamina II neurons

2.1. Capsaicin effect

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is a natural ingredient in peppers of the *Capsicum* family [79]. Capsaicin (2 μM) superfused for 0.5 min irreversibly increased the frequency of spontaneous excitatory postsynaptic current (EPSC) recorded from rat spinal lamina II neurons by 234% with a small increase in its amplitude. This capsaicin activity was unaffected by TTX, and thus was not mediated by neurotransmistters released as a result of the production of APs. Such a capsaicin effect disappeared in the presence of a TRPV1 channel antagonist capsazepine (10 μM; [80]), indicating an involvement of TRPV1 channels [81]. Consistent with this result, TRPV1 channels are expressed in the central terminals of DRG neurons ([82-84]; also see Fig. S1 in [85]). The increase in spontaneous EPSC (sEPSC) frequency (a measure of the spontaneous release of L-glutamate from nerve terminals) would be due to a high Ca^{2+} permeability of cation-permeable TRPV1 channels in nerve terminals [46] and opened voltage-gated Ca^{2+} channels by membrane depolarization occurring as a result of TRPV1 channel activation, both of which elicit a long-lasting increase in intracellular Ca^{2+} concentration ([86]; for review, see [87]). As different from spontaneous transmission, dorsal root-evoked monosynaptic transmission in spinal lamina II neurons was depressed by capsaicin (1 μM), this inhibition occurred in primary-afferent C-fiber (EPSC peak amplitude reduction: 86%), but not Aδ-fiber-mediated transmission. This capsaicin activity was presynaptic in origin and attributed to an inhibition of voltage-gated Ca^{2+} channels located in nerve terminals (possibly due to a down-regulation of Ca^{2+} channels through a Ca^{2+}-dependent protein phosphatase calcineurin [88]) [89]. Consistent with this idea, capsaicin reduced Ca^{2+}-channel current amplitudes in cultured rat DRG neurons [90]. Similar capsaicin actions are reported in the rodent superficial spinal or medullary dorsal horn [33, 91-93]. Consistent with the sEPSC frequency increase, capsaicin produced a TTX-insensitive and capsazepine-sensitive increase of L-glutamate release in slices prepared from the dorsal horn of the rat spinal cord [94].

Such a capsaicin activity was accompanied by an inward current having an averaged peak amplitude of 32.8 pA at -70 mV (near resting membrane potential) or no change in holding currents. Although primary-afferent C-fibers have an ability to release a variety of neuroactive peptides including substance P in the dorsal horn [5, 95], the capsaicin-induced inward current was not mediated by substance P (for the idea about an involvement of this peptide in the inward current, see [80, 96, 97]). This capsaicin activity was attributed to an action of neurotransmitters other than substance P that may be released as a result of an increase in neuronal activities through the activation of primary-afferent C-fibers and non-NMDA receptors [36].

Capsaicin is similar in chemical structure to endogenous TRPV1 channel agonists such as endocannabinoids and lipoxygenase metabolites (see above). Moreover, an endogenous capsaicin-like substance, which is released from inflamed tissues, is suggested to produce nociceptive neural APs through TRPV1 channel activation in DRG neurons [98].

2.2. Resiniferatoxin effect

Resiniferatoxin is a capsaicin analog isolated from the dried latex of the cactus-like plant, *Euphorbia resinifera* [99-101]. This compound is known to ultrapotently activate TRPV1 channels [102-104]. Resiniferatoxin (0.5 μM) superfused for 1 min irreversibly increased sEPSC frequency in rat spinal lamina II neurons; this extent averaged to be 136%. This increase was accompanied by a small increase in sEPSC amplitude. Resiniferatoxin activity was concentration-dependent with a half-maximal effective concentration (EC_{50}) value of 0.21 μM [105], a value less than one (1 μM; [81]) needed for capsaicin to increase sEPSC frequency in rat spinal lamina II neurons. This result was consistent with the observation that resiniferatoxin has a higher affinity (by 3-4 orders of magnitude) for TRPV1 channels than capsaicin [101, 106, 107]. Resiniferatoxin activity was blocked by capsazepine (10 μM) and also by another TRPV1 channel antagonist SB-366791 (30 μM; [108]), indicating TRPV1 channel involvement. Consistent with this idea, there was a cross-desensitization between resiniferatoxin and capsaicin activities [105], as seen in the release of substance P or calcitonin gene-related peptide from primary-afferent central

terminals in the rat spinal cord and also in contraction involving transmitter release from primary-afferent peripheral terminals in the rat isolated urinary bladder [106]. As with capsaicin activity, resiniferatoxin-induced sEPSC frequency increase was resistant to TTX, indicating no involvement of AP production. Although resiniferatoxin (0.5 μM) produced an inward current having the averaged peak amplitude of 14.1 pA at -70 mV in some neurons, this current was resistant to TTX, as different from capsaicin's one. The other neurons did not change holding currents. The inward current produced by resiniferatoxin has been attributed to the activation of non-NMDA and NMDA receptors ([109]; also see [110]) located in postsynaptic neurons by an excess of L-glutamate released from primary-afferent C-fiber terminals owing to TRPV1 channel activation [105].

2.3. Allyl isothiocyanate effect

Allyl isothiocyanate (AITC) is the pungent major compound contained in mustard oil or wasabi [48, 49]. AITC (100 μM) superfused for 2 min increased sEPSC frequency in rat spinal lamina II neurons; this extent averaged to be 202%. This increase was accompanied by a small increase in sEPSC amplitude. AITC activity was reversible, as different from those of capsaicin and resiniferatoxin. A similar facilitatory action was produced by pungent natural compounds, cinnamaldehyde (100 μM) and allicin (100 μM; [111]; for similar observations, see [112, 113]), which are contained in cinnamon oil, ginger and garlic [48, 114]. The AITC-induced sEPSC frequency increases were unaffected by TTX, indicating that this activity was independent of AP production.

The sEPSC frequency increase produced by AITC was resistant to capsazepine (10 μM) while being inhibited by a non-selective TRP channel antagonist ruthenium red (300 μM). Moreover, there was no interaction between AITC and capsaicin activities, indicating an involvement of TRP channels other than TRPV1 channels, i.e., TRPA1 channels [111]. This idea is consistent with the observation that rat DRG neurons express TRPA1 mRNA and protein ([50, 115, 116]; also see Fig. S1 in [85]). AITC-evoked sEPSC frequency increase was not seen in a nominally Ca^{2+}-free solution, indicating that this increase is due to a high Ca^{2+} permeability of TRPA1 channels [117]. Similar AITC actions are reported in mechanically dispersed nucleus tractus solitarii neurons attached with synaptic boutons [118].

As with capsaicin and resiniferatoxin, in some neurons AITC (100 μM) produced an inward current at -70 mV; this averaged peak amplitude was 12.6 pA. The other neurons did not change holding currents. The inward current was attributed to an activation of NMDA receptors [109] located in rat spinal lamina II neurons, caused by an excess of L-glutamate released from nerve terminals as a result of TRPA1 channel activation [111]. Thus, inward currents produced by AITC and TRPV1 channel agonists (capsaicin and resiniferatoxin) appeared to be different in origin from each other.

2.4. Eugenol effect

Eugenol (2-methoxy-4-(2-propenyl) phenol), one of the vanilloid compounds, is an aromatic molecule contained in several plants including clove and bay leaves [119]. Eugenol (5 mM) superfused for 2 min reversibly increased sEPSC frequency with an averaged extent of 461% in rat spinal lamina II neurons; this activity was not affected by TTX. This increase was accompanied by a small increase in sEPSC amplitude. As with AITC, eugenol repeatedly increased sEPSC frequency, a result different from TRPV1 channel agonists (capsaicin and resiniferatoxin). The sEPSC frequency increase was concentration-dependent with an EC_{50} value of 3.8 mM [120].

The sEPSC frequency increase produced by eugenol was resistant to capsazepine (10 μM) while being reduced in extent by ruthenium red (300 μM) and a TRPA1 channel antagonist HC-030031 (50 μM; [121]), indicating an involvement of TRPA1 but not TRPV1 channels. Since a half-maximal inhibitory concentration (IC_{50}) value for HC-030031 to inhibit human TRPA1 channel activation produced by AITC is 0.7 μM [121], the HC-030031 concentration (50 μM) used may have been quite high. However, a high HC-030031 concentration such as 100 μM was necessary for inhibition of TRPA1 channel activation in the rat spinal or medullary dorsal horn [112, 122]. The involvement of TRPA1 channels was inconsistent with the observations that eugenol activated TRPV1 channels which were cloned [123] and were present in rat primary-afferent neurons ([124]; albeit less in efficacy than capsaicin). The

eugenol-elicited sEPSC frequency increase was attributed to an increase in Ca^{2+} entry from extracellular solution to the cytoplasm, because TRPA1 channels are highly permeable to Ca^{2+} ions [117] and eugenol produces Ca^{2+}-permeable currents in cultured rat DRG neurons [125].

Eugenol (5 mM) produced an outward but not inward current at -70 mV, as different from the above-mentioned TRP channel agonists. This outward current had an averaged peak amplitude of 14.2 pA, and was resistant to capsazepine (10 μM), ruthenium red (300 μM) and HC-030031 (50 μM), indicating no involvement of TRP channels [120].

2.5. Zingerone effect

Zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone), one of the vanilloid compounds, is a pungent component of ginger (rhizomes of *Zingiber officinale* Roscoe) [126, 127]. Zingerone (2 mM) superfused for 2 min reversibly increased sEPSC frequency in a manner resistant to TTX in rat spinal lamina II neurons; this extent averaged to be 260%. This increase was accompanied by a small increase in sEPSC amplitude. The sEPSC frequency increase was concentration-dependent with an EC_{50} value of 1.3 mM. sEPSC frequency increase produced by zingerone (2 mM) was sensitive to ruthenium red (300 μM) or HC-030031 (50 μM) while being resistant to capsazepine (10 μM), and AITC (100 μM) reduced the extent of zingerone (2 mM)-induced sEPSC frequency increase, indicating an involvement of TRPA1 channels [128]. This result was inconsistent with the observation that zingerone activates TRPV1 channels expressed in the cell body of the primary-afferent neuron [129, 130].

It is of interest to note that zingerone and also eugenol activate different types of TRP channels between the central terminal and cell body of primary-afferent neuron. A similar result was obtained in local anesthetic activity. Lidocaine, which activated TRPV1 channels [131] and by a less extent TRPA1 channels [132] in the cell body of DRG neuron, activated TRPA1 but not TRPV1 channels expressed in its central terminal in the spinal lamina II [133]. TRPA1 and TRPV1 channels are co-expressed in many of the DRG neurons [50, 116] and a TRPA1-TRPV1 channel complex may be formed on the plasma membrane [134]. This may contribute to a distinction in property between the peripheral and central TRP channels. This idea is supported by the observation that sEPSC frequency in rat spinal lamina II neurons was increased by piperine (1-peperoylpiperidine; a pungent component of black pepper [135]; in a manner sensitive to capsazepine (10 μM), albeit piperine does not have the vanillyl group; EC_{50} = 52.3 μM) but not olvanil (*N*-(3-methoxy-4-hydroxybenzyl) oleamide; the synthetic oleic acid homologue of capsaicin; in a range of 0.1-20 μM [136]); both compounds activated primary-afferent neuron TRPV1 channels [129, 137]. The piperine value was similar to that (35 μM) for TRPV1 activation in rat primary-afferent neurons [129] and also to that (37.9 μM) of human cloned TRPV1 channels expressed in human embryonic kidney (HEK) 293 cells [138]. Anandamide, which is thought to be an endogenous TRPV1 channel agonist ([58]; see above), did not activate TRPV1 channels located in the central terminals of DRG neurons in the spinal lamina II [32], although central terminal TRPV1 channels were activated by an anandamide-transport inhibitor AM404 [139].

With respect to evoked transmission, zingerone (2 mM) reduced the peak amplitude of dorsal root-evoked monosynaptic Aδ-fiber and C-fiber EPSCs in rat spinal lamina II neurons. These reductions were reversible and comparable in extent between Aδ-fiber and C-fiber EPSC peak amplitudes (25% and 37%, respectively; [128]). This inhibition was different from those of capsaicin and cinnamaldehyde where Aδ-fiber EPSCs were much less sensitive to these compounds than C-fiber ones [89, 112].

In some neurons, zingerone (2 mM) activity was accompanied by an inward current having an averaged peak amplitude of 14.8 pA at -70 mV; this current was resistant to TTX. The other neurons did not change holding currents. The inward current was not inhibited by a non-NMDA or NMDA-receptor antagonist, as different from capsaicin, resiniferatoxin and AITC current. Owing to a sensitivity to ruthenium red or HC-030031, zingerone current was attributed to activation of TRPA1 channels present in postsynaptic neurons [128]. This idea may be consistent with the observation that TRPA1 immunoreactivity exists in the rat superficial dorsal horn [115].

2.6. Carvacrol effect

Carvacrol (5-isopropyl-2-methylphenol) is contained in oregano and thyme essential oils [140-142]. Carvacrol (1 mM) superfused for 2 min reversibly

produced sEPSC frequency increase and/or an outward current at -70 mV in rat spinal lamina II neurons. This increase was accompanied by a small increase in sEPSC amplitude. The extent of the sEPSC frequency increase was 262% on average and the averaged peak amplitude of the outward current was 30.2 pA. These two actions were resistant to TTX, and were concentration-dependent with the EC_{50} values of 0.69 and 0.55 mM, respectively. The sEPSC frequency increase produced by carvacrol was inhibited by HC-030031 (50 μM) but not capsazepine (10 μM), indicating an involvement of TRPA1 channels existing in the central terminals of DRG neurons [143]. This result is consistent with the observation that carvacrol activates cloned TRPA1 channels [123, 144, 145].

On the other hand, the carvacrol-induced outward current was not affected by both HC-030031 (50 μM) and capsazepine (10 μM), indicating no involvement of TRPA1 and TRPV1 channels [143]. A net of carvacrol current, which was estimated from a difference between current-voltage relations in the absence and presence of carvacrol current, was reduced in amplitude with membrane hyperpolarization and then approximated to the equilibrium potential for K^+ ion, although this current did not reverse at more hyperpolarized potentials. The outward current was reduced in amplitude in 10 mM-K^+ but not K^+-channel blockers (tetraethylammonium and Ba^{2+})-containing Krebs solution and 11.0 mM-Cl^- Krebs solution. This result indicates that the carvacrol current is mediated by tetraethylammonium- and Ba^{2+}-insensitive inwardly-rectifying K^+ channels [143]. Their channel properties were similar to those of two-pore domain K^+ channels such as TASK and TREK channels (for review, see [146, 147]) located in the rat and human spinal cord (for example, see [148, 149]).

2.7. Thymol effect

As with carvacrol, thymol (5-methyl-2-isopropylphenol) is contained in oregano and thyme essential oils [140-142]. Thymol has a chemical structure where the cyclohexane ring of menthol (see below) is replaced by benzene ring, and is different only in the position of -OH bound to the benzene ring from carvacrol. Thymol (1 mM) superfused for 3 min increased sEPSC frequency in spinal lamina II neurons with an averaged extent of 326% with a slow recovery from its activity. This increase was accompanied by a small increase in sEPSC amplitude. Many of the neurons produced an outward current at -70 mV in response to thymol (1 mM); this peak amplitude averaged to be 15.6 pA. These two actions were resistant to TTX, and were concentration-dependent with the EC_{50} values of 0.18 and 0.14 mM, respectively. The sEPSC frequency increase produced by thymol was inhibited by HC-030031 (50 μM) but not capsazepine (10 μM) and a TRPM8 channel antagonist BCTC (3 μM; [150]), indicating an involvement of TRPA1 channels existing in the central terminals of DRG neurons [151]. Consistent with this result, thymol activated cloned TRPA1 channels [145] and TRPA1 channels expressed in human and rat colonic epithelial cells [152]. In spite of the facts that thymol has a chemical structure similar to menthol and both of them have an ability to activate TRPM8 channels [153], thymol did not activate TRPM8 channels in the spinal lamina II. On the other hand, the outward current produced by thymol was resistant to capsazepine (10 μM), BCTC (3 μM) and HC-030031 (50 μM), indicating no involvement of TRP channels [151].

Although like carvacrol, thymol produced both sEPSC frequency increase and outward current, thymol had efficacies about four-fold higher than carvacrol (whose EC_{50} values for their actions: 0.69 and 0.55 mM, respectively; see above).

2.8. Citral effect

Citral (3,7-dimethyl-2,6-octadienal) is a mixture of geranial (*trans*-citral) and neral (*cis*-citral), and is abundantly contained in lemongrass oil [154, 155]. Citral (1 mM) superfused for 3 min increased sEPSC frequency in a manner resistant to TTX in rat spinal lamina II neurons with a slow recovery from its activity; this extent averaged to be 256%. This increase was accompanied by a small increase in sEPSC amplitude. In some neurons, citral (1 mM) produced an inward current having an averaged peak amplitude of 13.8 pA at -70 mV; this current was sensitive to HC-030031, as seen in zingerone-induced inward current (see above), indicating an involvement of TRPA1 channels located in postsynaptic neurons. The other neurons did not change holding currents. The sEPSC frequency increase produced by citral was concentration-dependent with an EC_{50} value of 0.58 mM, which was comparable to that of carvacrol (0.69 mM).

Such an increase in sEPSC frequency was inhibited by HC-030031 (50 μM) but not capsazepine (10 μM) and BCTC (3 μM), indicating an involvement of TRPA1 channels existing in the central terminals of DRG neurons [156]. This TRPA1 activation was seen although citral was reported to activate TRPV1, TRPM8, TRPA1 and TRP vanilloid-3 (TRPV3; also activated at >33 °C) channels expressed in rat DRG neurons with a potency order of TRPM8 > TRPV1 > TRPA1 > TRPV3 channels [157].

Slow recovery from TRPA1 channel activation produced by citral as well as thymol was similar to those of capsaicin and resiniferatoxin (TRPV1 channel agonists) while being different from AITC, eugenol, carvacrol, zingerone (TRPA1 channel agonists; see above) and piperine (TRPV1 channel agonist [136]). Recovery from TRPA1 channel activation appears to depend on the chemical structures of compounds which activate TRPA1 channels, since agonist-dependent desensitization has been shown for various ligand-gated channels such as TRPV1 channels [129].

2.9. (-)-Carvone effect

(-)-Carvone ((-)-2-methyl-5-(1-methylethenyl)-2-cyclohexenone) is contained in spearmint [158, 159]. (-)-Carvone (1 mM) superfused for 2 min reversibly increased sEPSC frequency in a manner resistant to TTX in rat spinal lamina II neurons; this extent averaged to be 299%. This increase was accompanied by a small increase in sEPSC amplitude. In some neurons, (-)-carvone (1mM) produced an inward current having an averaged peak amplitude of 14.3 pA at -70 mV. The other neurons did not change holding currents. The sEPSC frequency increase produced by (-)-carvone was concentration-dependent with an EC_{50} value of 0.70 mM, which was similar to those of carvacrol (0.69 mM) and citral (0.58 mM; see above). Such an increase in sEPSC frequency was inhibited by capsazepine (10 μM) but not HC-030031 (50 μM), indicating an involvement of TRPV1 channels present in the central terminals of DRG neurons [160]. Consistent with this observation, (-)-carvone increased intracellular Ca^{2+} concentration in rat DRG neurons and also in HEK 293 cells expressing human TRPV1 channels in a manner sensitive to capsazepine [161]; EC_{50} value (1.3 mM) for the latter action was comparable to that in the spinal lamina II.

2.10. (+)-Carvone effect

(+)-Carvone, a stereoisomer of (-)-carvone, is contained in caraway [158, 162]. (+)-Carvone (1 mM) superfused for 2 min reversibly increased sEPSC frequency in a manner resistant to TTX in rat spinal lamina II neurons; this extent averaged to be 284%. This increase was accompanied by a small increase in sEPSC amplitude. In some neurons, (+)-carvone (1 mM) produced an inward current having an averaged peak amplitude of 10.9 pA at -70 mV. The other neurons did not change holding currents. The sEPSC frequency increase produced by (+)-carvone was concentration-dependent with an EC_{50} value of 0.72 mM. Their (+)-carvone activities are quantitatively similar to those of (-)-carvone. However, as different from (-)-carvone, (+)-carvone increased sEPSC frequency in a manner sensitive to HC-030031 (50 μM) but not capsazepine (10 μM), indicating an involvement of TRPA1 channels existing in the central terminals of DRG neurons [160]. Thus, (+)-carvone and (-)-carvone activated different types of TRP channel, although they are optical isomers (see [163] for review). (+)-Carvone does not necessarily appear to have the same action as that of (-)-carvone. In the mouse central nervous system, (+)-carvone exhibited a depressant action different from that of (-)-carvone [158]. It is well-known that stereoisomers affect ligand-gated or voltage-gated ion channels in a different manner [164, 165].

2.11. 1,8-Cineole effect

1,8-Cineole (1,3,3-trimethyl-2-oxabicylo[2.2.2] octane), a monoterpene, is contained in essential oils derived from many plants such as eucalyptus and rosemary, and is known as eucalyptol [166-168]. 1,8-Cineole (5 mM) superfused for 3 min reversibly increased sEPSC frequency in a manner resistant to TTX in rat spinal lamina II neurons; this extent averaged to be 159%. This increase was accompanied by a small increase in sEPSC amplitude. In some neurons, 1,8-cineole (5 mM) produced an inward current having an averaged peak amplitude of 9.7 pA at -70 mV. The other neurons did not change holding currents. The sEPSC frequency increase produced by 1,8-cineole was concentration-dependent with an EC_{50} value of 3.2 mM, which was comparable to that of eugenol (3.8 mM; see above). This 1,8-cineole's value was similar to an IC_{50} value (1.1 mM)

for 1,8-cineole to reduce K^+-induced contraction in rat isolated thoracic aorta preparations [169]. Such an increase in sEPSC frequency was inhibited by HC-030031 (50 μM) and a nicotinic acetylcholine-receptor antagonist mecamylamine (100 μM; which is reported to inhibit TRPA1 channels [170]) but not capsazepine (10 μM), SB-366791 (30 μM; see [105, 108]) and BCTC (3 μM), indicating an involvement of TRPA1 channels existing in the central terminals of DRG neurons [85]. This result was different from the observation that 1,8-cineole activated cloned TRPM8 channels, albeit with an efficacy being 50-2000 fold less than that of menthol [52, 53, 171].

With respect to evoked release, 1,8-cineole (5 mM) reduced the peak amplitude of dorsal root-evoked monosynaptic C-fiber but not Aδ-fiber EPSCs in rat spinal lamina II neurons; the extent of this reduction was 22% on average [85]. A similar inhibition was produced by capsaicin and cinnamaldehyde in spinal lamina II neurons [89, 91, 112], but the magnitude of this inhibition was smaller than those of capsaicin and cinnamaldehyde. Unlike 1,8-cineole, zingerone reduced both Aδ-fiber and C-fiber EPSC amplitudes by a small extent ([128]; see above). Reduced Aδ-fiber and C-fiber EPSC peak amplitudes appeared to vary in extent among TRPV1 and TRPA1 channel agonists.

2.12. 1,4-Cineole effect

1,4-Cineole (1-methyl-4-(1-methylethyl)-7-oxabicyclo [2.2.1]heptane) is a stereoisomer of 1,8-cineole and is contained as a minor component in plants containing 1,8-cineole [168, 172]. 1,4-Cineole (0.5 mM) superfused for 3 min reversibly increased sEPSC frequency in a manner resistant to TTX in rat spinal lamina II neurons; this extent averaged to be 226%. This increase was accompanied by a small increase in sEPSC amplitude. In some neurons, 1,4-cineole (0.5 mM) produced an inward current having an averaged peak amplitude of 8.7 pA at -70 mV. The other neurons did not change holding currents. The sEPSC frequency increase produced by 1,4-cineole was concentration-dependent with an EC_{50} value of 0.42 mM, which was about eight-fold smaller than that of 1,8-cineole. Such an increase in sEPSC frequency was inhibited by capsazepine (10 μM), SB-366791 (30 μM) but not HC-030031 (50 μM), mecamylamine (100 μM) and BCTC (3 μM), indicating an involvement of TRPV1 channels existing in the central terminals of DRG neurons [85]. This result indicates that both 1,8-cineole and 1,4-cineole activate a different type of TRP channel. This may be due to the fact that 1,8-cineole and 1,4-cineole are distinct in the placement of oxygen bridge existing in these compounds and thus there is a free dimethyl side chain in 1,4-cineole but not 1,8-cineole ([168]; see [163] for review). The lack of involvement of TRPM8 channels in 1,4-cineole activity was inconsistent with the observation that 1,4-cineole activates cloned TRPM8 channels [173].

With respect to evoked release, 1,4-cineole (0.5 mM) reduced the peak amplitude of dorsal root-evoked monosynaptic C-fiber but not Aδ-fiber EPSCs in rat spinal lamina II neurons; the extent of this reduction averaged to be 31%, a value similar to that of 1,8-cineole [85].

2.13. Menthol effect

Menthol (2-isopropyl-5-methylcyclohexanol) is a secondary alcohol, and is contained in peppermint or other mint oils [174, 175]. (-)-Menthol (the main form of menthol occurring in nature; 0.5 mM) superfused for 3 min reversibly increased sEPSC frequency by 409% with a small increase in its amplitude in a manner sensitive to BCTC (3 μM) in rat spinal lamina II neurons, indicating an involvement of TRPM8 channels ([85]; see [91, 113, 176, 177] for similar menthol activities). Consistent with this result, TRPM8 channels are located in rat DRG neurons [50, 116]. Interestingly, TRPM8 channels are known to be not co-expressed with TRPV1 and TRPA1 channels [50, 116].

2.14. Guaiacol effect

Guaiacol (2-methoxyphenol) is contained in beechwood [178]; this has a chemical structure which lacks $-CH_2CH=CH_2$ or $-(CH_2)_2COCH_3$ bound to the benzene ring of eugenol or zingerone. As different from eugenol and zingerone, guaiacol (2-5 mM) superfused for 3 min had no effect on sEPSC frequency, amplitude and holding currents in rat spinal lamina II neurons [179].

2.15. Vanillin effect

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the primary component of the extract of the vanilla bean [180]; this has a chemical structure where $-CH_2CH=CH_2$ or $-(CH_2)_2COCH_3$ bound to the benzene

ring of eugenol or zingerone is replaced by -CHO. As with guaiacol, vanillin (1-5 mM) superfused for 3 min had no effect on sEPSC frequency and amplitude in rat spinal lamina II neurons [179]. On the other hand, vanillin at a high concentration such as 5 mM produced an inward current having an averaged peak amplitude of 11.8 pA at -70 mV [179].

2.16. Vanillic acid effect

Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is contained in *Angelicae Radix* and *Ligustici Rhizoma* [181]; this has a chemical structure where -CHO of vanillin is replaced by -COOH. As with guaiacol, vanillin acid (2-5 mM) superfused for 3 min had no effect on sEPSC frequency, amplitude and holding currents in rat spinal lamina II neurons [179].

2.17. *p*-Cymene effect

p-Cymene is contained in amaranthacease in an appreciable quantity [182] and has a chemical structure which lacks -OH of carvacrol or thymol. As different from carvacrol and thymol, *p*-cymene (1-2 mM) superfused for 3 min had no effect on sEPSC frequency and amplitude in rat spinal lamina II neurons [179]. In a small number of neurons, *p*-cymene (2 mM) produced an outward current having an averaged peak amplitude of 6.8 pA at -70 mV. The other neurons did not change holding currents.

3. Effects of plant-derived compounds on compound action potentials recorded from the frog sciatic nerve

3.1. Capsaicin effect

Soaking the frog sciatic nerve into a TRPV1 channel agonist capsaicin (0.1 mM)-containing Ringer solution reduced the peak amplitude of CAP in a reversible manner; this extent averaged to be 36%. This capsaicin effect was concentration-dependent in a range of 0.005-0.2 mM. As different from sEPSC frequency increase produced by capsaicin in rat spinal lamina II neurons, this CAP inhibition was not affected by capsazepine (50 μM), indicating no involvement of TRPV1 channels [183]. CAP inhibition produced by capsaicin is due to an inhibition of voltage-gated Na^+ channels, because capsaicin at high concentrations such as 0.03-0.1 mM reduces voltage-gated Na^+-channel current amplitudes in rodents in a manner independent of TRPV1 channels [184-186], although TRPV1 channel-dependent Na^+-channel inhibition is known [187, 188]. Capsaicin is well-known to inhibit nerve AP conduction in rodents and humans [189-196].

As with capsaicin, a TRPV1 channel agonist piperine had an inhibitory effect on frog sciatic nerve CAPs (CAP peak amplitude reduction: 23% at 0.05 mM [197]) while increasing sEPSC frequency in rat spinal lamina II neurons (EC_{50} = 52.3 μM; see above).

3.2. Resiniferatoxin effect

Another TRPV1 channel agonist resiniferatoxin at 5 μM had no effect on frog sciatic nerve CAPs [183], although this concentration was much larger than the EC_{50} value (0.21 μM) for this drug to increase sEPSC frequency in rat spinal lamina II neurons (see above). This result supports the idea that TRPV1 channels are not involved in frog sciatic nerve CAP inhibition produced by capsaicin. This finding was inconsistent with the observation that voltage-gated Na^+ channels in clonal neuroendocrine cells are inhibited by resiniferatoxin at concentrations of 1-10 μM in a manner independent of TRPV1 channels [198]. Resiniferatoxin is reported to inhibit nerve conduction in rats [191]. This difference between the present and previous studies may be due to a distinction among preparations in voltage-gated Na^+ channel properties.

3.3. Allyl isothiocyanate effect

Soaking the frog sciatic nerve into a TRPA1 channel agonist AITC (2 mM)-containing Ringer solution reduced CAP peak amplitudes in a partially reversible manner; this extent averaged to be 73.4%. This AITC effect had an IC_{50} value of 1.5 mM. As different from sEPSC frequency increase produced by AITC in rat spinal lamina II neurons, this CAP inhibition was resistant to HC-030031 (50 μM), indicating no involvement of TRPA1 channels [197].

As with AITC, another TRPA1 channel agonist cinnamaldehyde reversibly reduced frog sciatic nerve CAP amplitudes with an IC_{50} value of 1.2 mM in a manner resistant to ruthenium red (300 μM), indicating no involvement of TRP channels [197].

3.4. Eugenol effect

Soaking the frog sciatic nerve into eugenol (0.5 mM)-containing Ringer solution reduced CAP peak

amplitudes in a reversible manner; this extent averaged to be 31%. This eugenol effect had an IC_{50} value of 0.81 mM [183]. This value was somewhat larger than that (0.31 mM) for reducing the peak amplitudes of intracellularly-recorded APs in rat superior cervical ganglion neurons [199] and also than those (0.3-0.6 mM) for reducing peak Na^+-channel and K^+-channel current amplitudes in rat primary-afferent neurons [200-202]. On the other hand, the frog sciatic nerve IC_{50} value was smaller than EC_{50} value (3.8 mM) for eugenol to increase sEPSC frequency in rat spinal lamina II neurons (see above). Frog sciatic nerve CAP inhibition produced by eugenol was previously reported, although this was not quantitatively examined [203]. Such an inhibitory effect of eugenol may explain at least a part of its wide usage as an analgesic to treat toothache [119].

3.5. Zingerone effect

Soaking the frog sciatic nerve into zingerone (10 mM)-containing Ringer solution reduced CAP peak amplitudes in a reversible manner; this extent averaged to be 69%. This zingerone effect had an IC_{50} value of 8.3 mM [183]. This value was larger than the EC_{50} value (1.3 mM) for zingerone to increase sEPSC frequency in rat spinal lamina II neurons (see above).

3.6. Carvacrol effect

Soaking the frog sciatic nerve into carvacrol (1 mM)-containing Ringer solution reduced CAP peak amplitudes in an irreversible manner; this extent averaged to be 97%. This carvacrol effect had an IC_{50} value of 0.34 mM [204]. This value was somewhat smaller than the EC_{50} value (0.69 mM) for carvacrol to increase sEPSC frequency in rat spinal lamina II neurons (see above). The frog sciatic nerve IC_{50} value was similar to that (0.37 mM) for inhibition by carvacrol of voltage-gated Na^+ channels in rat DRG neurons [205].

3.7. Thymol effect

Soaking the frog sciatic nerve into thymol (0.5 mM)-containing Ringer solution reduced CAP peak amplitudes in an irreversible manner; this extent averaged to be 70%. This thymol effect had an IC_{50} value of 0.34 mM [204]. This value was the same as that (0.34 mM) of carvacrol in the frog sciatic nerve. This could be due to the fact that thymol and carvacrol are different only in the position of -OH bound to the benzene ring. The frog sciatic nerve IC_{50} value was somewhat larger than EC_{50} value (0.18 mM) for thymol to increase sEPSC frequency in rat spinal lamina II neurons (see above). These values were not so different from that (0.149 mM) for inhibition by thymol of neuronal voltage-gated Na^+ channels [206].

3.8. Citral effect

Soaking the frog sciatic nerve into citral (0.5 mM)-containing Ringer solution reduced CAP peak amplitudes in an irreversible manner; this extent averaged to be 55%. This citral effect had an IC_{50} value of 0.46 mM [207]. This value was comparable to the EC_{50} value (0.58 mM) for citral to increase sEPSC frequency in rat spinal lamina II neurons (see above). Such values were about 3-fold larger than one (0.163 mM) for citral to inhibit high K^+-induced rabbit ileum contraction [208]. Although citral has an ability to activate TRPV1, TRPA1, TRPM8 and TRPV3 channels ([157]; see above), citral activity was resistant to ruthenium red (300 μM), indicating no involvement of TRP channels [207].

3.9. (-)-Carvone effect

Soaking the frog sciatic nerve into (-)-carvone (2 mM)-containing Ringer solution reduced CAP peak amplitudes in a nearly reversible manner; this extent averaged to be 79%. This (-)-carvone effect had an IC_{50} value of 1.4 mM [204]. This value was somewhat larger than the EC_{50} value (0.70 mM) for (-)-carvone to increase sEPSC frequency in rat spinal lamina II neurons (see above).

3.10. (+)-Carvone effect

Soaking the frog sciatic nerve into (+)-carvone (2 mM)-containing Ringer solution reduced CAP peak amplitudes in a reversible manner; this extent averaged to be 47%. This (+)-carvone effect had an IC_{50} value of 2.0 mM [204]. This value was somewhat larger than the EC_{50} value (0.72 mM) for (+)-carvone to increase sEPSC frequency in rat spinal lamina II neurons (see above). There was not a large difference between (+)-carvone and (-)-carvone in inhibiting frog sciatic nerve CAPs (for a smilar result, see [209]).

3.11. 1,8-Cineole effect

Soaking the frog sciatic nerve into 1,8-cineole (10 mM)-containing Ringer solution reduced CAP peak amplitudes in an irreversible manner; this extent averaged to be 98%. This 1,8-cineole effect had an IC_{50} value of 5.7 mM [204]. This value was comparable to the EC_{50} value (3.2 mM) for 1,8-cineole to increase sEPSC frequency in rat spinal lamina II neurons (see above).

3.12. 1,4-Cineole effect

Soaking the frog sciatic nerve into 1,4-cineole (10 mM)-containing Ringer solution reduced CAP peak amplitudes in an irreversible manner; this extent averaged to be 73%. This 1,4-cineole effect had an IC_{50} value of 7.2 mM [204]. This value was much larger than the EC_{50} value (0.42 mM) for 1,4-cineole to increase sEPSC frequency in rat spinal lamina II neurons (see above). On the other hand, the IC_{50} value of 1,4-cineole in the frog sciatic nerve was not so different from that (5.7 mM) of 1,8-cineole, although their chemical structures are distinct in terms of the presence of a free dimethyl side chain in 1,4-cineole but not 1,8-cineole (see above).

3.13. Menthol effect

Soaking the frog sciatic nerve into a TRPM8 channel agonist (-)-menthol (2 mM)-containing Ringer solution reduced CAP peak amplitudes in an irreversible manner; this extent averaged to be 93%. This (-)-menthol effect had an IC_{50} value of 1.1 mM. As different from sEPSC frequency increase produced by (-)-menthol in rat spinal lamina II neurons, this CAP inhibition was resistant to ruthenium red (300 μM), indicating no involvement of TRPM8 channels [204]. This idea was supported by the observation that a powerful TRPM8 channel agonist icilin (0.2 mM) [171, 210] had no effect on frog sciatic nerve CAPs [204].

As with (-)-menthol, (+)-menthol (2 mM) reduced frog sciatic nerve peak CAP amplitudes in an irreversible manner; this extent averaged to be 97%. This (+)-menthol effect had an IC_{50} of 0.93 mM, a value comparable to that of (-)-menthol [204]. These values were similar to that (0.571 mM) for inhibition of neuronal voltage-gated Na^+ channels by menthol [206] and that (0.807 mM; estimated at a holding potential of -100 mV) for inhibition of TTX-sensitive Na^+ channels by menthol in immortalized DRG neuron-derived F11 cells [211]. (+)-Menthol and (-)-menthol are reported to inhibit electrically-evoked contractions of the rat phrenic hemidiaphragm in a similar manner [212]. On the contrary, (+)-menthol and (-)-menthol activated TRPA1 channels expressed in Chinese hamster ovary cells with an efficacy different from each other [213]. (+)-Menthol and (-)-menthol did not necessarily appear to exhibit a similar effect.

Tetrahydrolavandulol (where the six-membered ring of menthol is opened) inhibited frog sciatic nerve CAPs with an IC_{50} of 0.38 mM, a value smaller than that of menthol, indicating that the six-membered ring of menthol is not necessary for its CAP inhibitiory action [197]. Consistent with this idea, many aroma-oil compounds having no cyclic structure, such as citral, citronellol, geraniol, (±)-linalool, (-)-linalool and linalyl acetate, have an ability to inhibit frog sciatic nerve CAPs (see below).

3.14. Guaiacol effect

Soaking the frog sciatic nerve into guaiacol (7 mM)-containing Ringer solution reduced CAP peak amplitudes in a reversible manner; this extent averaged to be 50%. This guaiacol effect had an IC_{50} value of 7.7 mM [204]. As different from such an activity, guaiacol (5 mM) had no effect on glutamatergic transmission in rat spinal lamina II neurons (see above).

3.15. Vanillin effect

Soaking the frog sciatic nerve into vanillin (10 mM)-containing Ringer solution reduced CAP peak amplitudes in a reversible manner; this extent averaged to be 61%. This vanillin effect had an IC_{50} value of 9.0 mM [204]. As different from such an activity, vanillin (5 mM) had no effect on glutamatergic transmission in rat spinal lamina II neurons, albeit an inward current was produced (see above).

3.16. Vanillic acid effect

Vanillic acid (7 mM) did not affect frog sciatic nerve CAPs. This was confirmed in the frog sciatic nerve whose CAPs were inhibited by vanillin (7 mM). The lack of CAP inhibition by vanillic acid compared to guaiacol was attributed to the presence of a polar group -COOH in vanillic acid but not guaiacol; this idea also could explain a difference

in CAP inhibition between vanillic acid and vanillin [183]. As with CAPs, glutamaergic transmission in rat spinal lamina II neurons was not affected by vanillin acid (5 mM; see above).

3.17. *p*-Cymene effect

Soaking the frog sciatic nerve into *p*-cymene (2 mM)-containing Ringer solution reduced CAP peak amplitudes in an irreversible manner; this extent averaged to be 22%. Such an inhibitory action of *p*-cymene was concentration-dependent in a range of 0.5-2 mM [207]. As with such a small effect, a small outward current was produced by *p*-cymene (2 mM) in a small number of rat spinal lamina II neurons, albeit glutamatergic transmission was unaffected in all neurons tested (see above).

Table 1 summarizes the above-mentioned results in rat spinal lamina II neurons and the frog sciatic nerve.

4. Role of plant-derived compounds in modulating excitatory transmission and nerve conduction, and their structure-activity relationships

4.1. Modulation of excitatory transmission

4.1.1. Involvement of TRP channels

Many plant-derived compounds activated TRPV1 or TRPA1 channels in the central terminals of DRG neurons to increase sEPSC frequency, i.e., the spontaneous release of L-glutamate onto spinal lamina II neurons. Although this L-glutamate release increase was accompanied by a small increase in sEPSC amplitude, this amplitude increase may have been caused by a highly synchronized multivesicular release of L-glutamate, as revealed in the activation of ionotropic P2X-type ATP receptors at glutamatergic terminals in the brainstem [214].

An efficacy sequence for TRPV1 channel activation was resiniferatoxin (EC_{50} = 0.00021 mM) > capsaicin

Table 1. sEPSC frequency increase and outward current in rat spinal lamina II neurons and frog sciatic nerve CAP inhibition, produced by plant-derived compounds*[1].

Plant-derived compound	Rat spinal lamina II neuron		Frog sciatic nerve	References
	sEPSC frequency increase (EC_{50}, mM)*[2]	Outward current (EC_{50}, mM)*[3]	CAP inhibition (IC_{50}, mM)	
Capsaicin	+ (n.d.; TRPV1)	−	n.d. (36% at 0.1 mM)*[4]	[81, 183]
Resiniferatoxin	+ (0.00021; TRPV1)	−	−	[105, 183]
AITC	+ (n.d.; TRPA1)	−	1.5	[111, 197]
Eugenol	+ (3.8; TRPA1)	+ (n.d.)	0.81	[120, 183]
Zingerone	+ (1.3; TRPA1)	−	8.3	[128, 183]
Carvacrol	+ (0.69; TRPA1)	+ (0.55)	0.34	[143, 204]
Thymol	+ (0.18; TRPA1)	+ (0.14)	0.34	[151, 204]
Citral	+ (0.58; TRPA1)	−	0.46	[156, 207]
(-)-Carvone	+ (0.70; TRPV1)	−	1.4	[160, 204]
(+)-Carvone	+ (0.72; TRPA1)	−	2.0	[160, 204]
1,8-Cineole	+ (3.2; TRPA1)	−	5.7	[85, 204]
1,4-Cineole	+ (0.42; TRPV1)	−	7.2	[85, 204]
(-)-Menthol	+ (n.d.; TRPM8)	−	1.1	[85, 204]
Guaiacol	−	−	7.7	[179, 204]
Vanillin	−	−	9.0	[179, 204]
Vanillic acid	−	−	−	[179, 204]
p-Cymene	−	+ (n.d.)	n.d. (22% at 2 mM)*[4]	[179, 207]

*[1]+: yes; −: none; n.d.: not determined. *[2]TRP channel involved in sEPSC frequency increase is shown with EC_{50} value in parentheses. *[3]Compounds except for guaiacol and vanillic acid, which did not elicit any outward currents, produced an inward or no current. *[4]CAP peak amplitude reduction (%) at each concentration is shown in parentheses.

(n.d.) > piperine (0.0523 mM) > 1,4-cineole (0.42 mM) ≥ (-)-carvone (0.70 mM); that for TRPA1 channel activation was thymol (EC_{50} = 0.18 mM) > citral (0.58 mM) ≥ carvacrol (0.69 mM) ≥ (+)-carvone (0.72 mM) ≥ zingerone (1.3 mM) > 1,8-cineole (3.2 mM) ≥ eugenol (3.8 mM; see Table 1). This facilitation resulted in an increase in the excitability of the neurons, an action opposite to those of analgesics, and was thus suggested to lead to pain. Consistent with this idea, excessive or ectopical expression of TRPV1 channels in the central and peripheral nervous system resulted in neuropathic pain and hyperalgesia [215-218]. Intrathecally-administrated TRPV1 channel antagonist AS1928370 reversed mechanical allodynia in a mouse model of neuropathic pain [219]. Peripheral inflammation and nerve injury upregulated TRPA1 channels in rat DRG neurons [220]. After peripheral inflammation occurred by intraplantar injection of complete Freund's adjuvant, there was an excessive expression of TRPA1 channels in the mouse spinal cord and DRG; hyperalgesia occurring in mouse models of neuropathic pain disappeared by intrathecal administration of HC-030031 [221]. Pain hypersensitivity elicited by formalin in rats was reversed by a TRPA1 channel antagonist A-967079 [222].

It is of interest to note that all of guaiacol, vanillin, vanillic acid and *p*-cymene did not increase the spontaneous release of L-glutamate in the rat spinal lamina II, as different from the other plant-derived compounds. Thus, addition to guaiacol of -$CH_2CH{=}CH_2$ (eugenol) or -$(CH_2)_2COCH_3$ (zingerone) would be necessary for TRPA1 channels to be activated. Furthermore, replacement of the substituted groups of eugenol or zingerone with -CHO (vanillin) or -COOH (vanillic acid), or deleting -OH from carvacrol or thymol (*p*-cymene), resulted in the lack of TRPA1 channel activation [179]. Activation of TRPA1 channels by various compounds is reported to be mediated by covalent modification of cysteine residues within the cytoplasmic N terminus of the channels [223]. No effect of guaiacol, vanillin, vanillic acid and *p*-cymene may result from a less electrophilicity of modifications by guaiacol, vanillin and vanillic acid (compared with eugenol and zingerone) and by *p*-cymene (compared with carvacrol and thymol) [179]. Alternatively, there was a difference in TRP channel activation between carvacrol and thymol; (-)-carvone and (+)-carvone; 1,8-cineole and 1,4-cineole (see above; for review, see [163]). These findings may serve to reveal a chemical structure that is necessary for TRP channels to be activated in the rat spinal lamina II.

4.1.2. Outward current

As seen in Table 1, eugenol, carvacrol, thymol and *p*-cymene produced an outward current at -70 mV (membrane hyperpolarization) in rat spinal lamina II neurons. The other plant-derived compounds produced an inward current or did not change holding currents; this variation may be due to the fact that the spinal lamina II is comprised of a heterogenous cell group [224].

All of the outward currents were resistant to TRP channel antagonists, indicating no involvement of TRP channels. Since the hyperpolarization reduces the excitability of spinal lamina II neurons, this activity may contribute to at least a part of antinociception produced by the compounds. For example, intrathecally-administrated eugenol inhibited formalin-induced nociceptive responses in mice [225] and alleviated neuropathic pain in sciatic nerve ligation rat models [226]. A similar alleviation of pain was elicited by orally-administrated eugenol [227]. Oral administration of carvacrol produced antinociception in the formalin test in mice [228, 229]. Orally-administrated thymol elicited antinociception in mice when examined in the hot-plate test [230].

The observation that eugenol but not guaiacol produced an outward current suggests that the -$CH_2CH{=}CH_2$ bound to the benzene ring of eugenol but not guaiacol is necessary to produce an outward current. An outward current was not produced by either vanillin or vanillic acid, which has -CHO or -COOH but not -$CH_2CH{=}CH_2$, respectively. The -$CH_2CH{=}CH_2$ of eugenol has no electric charges, while the -CHO of vanillin has a negative charge that is biased to the oxygen atom in the carbon-oxygen bond owing to a difference in electronegativity between carbon and oxygen atoms. Thus, it is suggested that vanillin cannot produce any outward currents. This idea was supported by the observation that zingerone, which has -$(CH_2)_2COCH_3$ in place of the -CHO of vanillin, produced no outward current. The same result was obtained in the action of vanillic acid whose -COOH dissociates to -COO^-

and H^+ in Krebs solution, leading to the presence of a negative charge in vanillic acid [179].

With respect to *p*-cymene, carvacrol and thymol, *p*-cymene is distinct from carvacrol or thymol only in terms of -OH bound to the benzene ring. Although *p*-cymene produced a small outward current (6.8 pA at 2 mM) at -70 mV in a small number of neurons examined, carvacrol and thymol elicited an outward current having a large amplitude (carvacrol: 30.2 pA at 1 mM; thymol: 15.6 pA at 1 mM) at -70 mV in many of the neurons tested (see above). This observation suggests that the -OH in carvacrol and thymol plays a role in outward current production [179].

4.2. Nerve conduction inhibition

Many plant-derived compounds inhibited frog sciatic nerve CAPs, i.e., nerve conduction, without TRP channel activation. Many of these actions have been attributed to voltage-gated Na^+-channel inhibition by the compounds (for review, see [231]). This inhibition could account for at least a part of antinociception or local anesthetic effects, produced by plant-derived compounds such as capsaicin [232-234], menthol [212, 235], eugenol [225-227], carvacrol [228, 229], thymol [230], (-)-carvone [159] and *p*-cymene [182]. Many of the plant-derived compounds other than the above-mentioned ones have an ability to inhibit CAPs recorded from the frog sciatic nerve in a manner dependent on their chemical structures.

4.2.1. Capsaicin-related compounds

As with capsaicin, eugenol, zingerone and vanillin, frog sciatic nerve CAPs were inhibited by dihydrocapsaicin (a secondary compound from *Capsicum* family [236]; 59% peak amplitude reduction at 0.1 mM), capsiate (a non-pungent capsaicin analog (where -NH- group in capsaicin is replaced by -O- group), (E)-8-methyl-6-nonenoic acid (4-hydroxy-3-methoxyphenyl)methyl ester; which is contained in CH-19 sweet peppers [237]; 22% reduction at 0.1 mM), vanillylamine (4-hydroxy-3-methoxybenzylamine; which is contained in *Capsicum* family as a precursor for the biosynthesis of capsaicin [238]; 12% reduction at 10 mM), curcumin ((1E,6E)-1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; the major component of turmeric [239]; 10% reduction at 0.05 mM); and olvanil (2% reduction at 0.03 mM). An efficacy sequence of the CAP inhibitions produced by their compounds was capsaicin = dihydrocapsaicin > capsiate > eugenol > guaiacol ≥ zingerone ≥ vanillin > vanillylamine (see Table 1); curcumin appeared to be less effective than capsaicin [183]. More effectiveness of capsaicin compared to eugenol, guaiacol, zingerone and vanillin may be due to the fact that capsaicin has a long hydrophobic side chain bound to the vanillyl group. Since olvanil having a side chain longer than capsaicin is much less effective in CAP inhibition, there may be an appropriate length of hydrophobic side chain bound to the vanillyl group in inhibiting CAPs [183]. In conclusion, CAP inhibition produced by vanilloids appeared to depend on the property of the side chain bound to the vanillyl group.

4.2.2. Menthol-related compounds

As with (-)-carvone, (+)-carvone, 1,8-cineole, 1,4-cineole, (-)-menthol and (+)-menthol, frog sciatic nerve CAPs were inhibited by (-)-menthone ((2S,5R)-5-methyl-2-(propan-2-yl) cyclohexanone; which is contained in pennyroyal, peppermint or other oils [140, 175]; IC_{50} = 1.5 mM), (+)-menthone (IC_{50} = 2.2 mM), (+)-pulegone ((+)-1-methyl-4-isopropylidene-3- cyclohexanone; which is contained in oils of various plants including catnip and pennyroyal [175, 240]; IC_{50} = 1.4 mM) and (-)-carveol (2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol; which is contained in spearmint [241]; IC_{50} = 1.3 mM). In summary, an efficacy sequence of the CAP inhibitions produced by their compounds was (+)-menthol ≥ (-)-menthol ≥ (-)-carveol ≥ (+)-pulegone = (-)-carvone ≥ (-)-menthone ≥ (+)-carvone ≥ (+)-menthone > 1,8-cineole > 1,4-cineole [204] (see Table 1). On the other hand, frog sciatic nerve CAPs were minimally inhibited by *p*-menthane (a basic structure of menthol; 1-methyl-4-propan-2-yl-cyclohexane), (+)-limonene (1-methyl-4-(1-methylethenyl) -cyclohexene; which is contained in oranges [242]) and menthyl chloride at 10 mM [204].

4.2.3. Other aroma-oil compounds

Carvacrol, thymol, eugenol, (-)-menthol, (+)-menthol, (-)-carveol, (+)-pulegone, citral, (-)-carvone, (+)-carvone, (-)-menthone, (+)-menthone, 1,8-cineole, 1,4-cineole and (+)-limonene, as mentioned above, belong to aroma-oil compounds. As with these

compounds, frog sciatic nerve CAPs were inhibited by other aroma-oil compounds, an aldehyde, citronellal (contained in lemongrass [243]; IC_{50} = 0.50 mM); esters, linalyl acetate (contained in lavender [244]; IC_{50} = 0.71 mM), geranyl acetate (contained in ylang ylang [182]; IC_{50} = 0.51 mM) and bornyl acetate (contained in conifer leaf oil [245]; IC_{50} = 0.44 mM); alcohols, (+)-borneol (contained in rosemary [246]; IC_{50} = 1.5 mM; this inhibition was resistant to ruthenium red, albeit (+)-borneol is a TRPV3 channel agonist [247]; [207]), (-)-borneol (IC_{50} = 2.3 mM), citronellol (contained in rose [248]; IC_{50} = 0.35 mM), geraniol (contained in rose and geranium; IC_{50} = 0.53 mM), (±)-linalool (IC_{50} = 1.7 mM), (-)-linalool (contained in lavender [244]; IC_{50} = 2.0 mM) and α-terpineol (contained in eucalyptus [249]; IC_{50} = 2.7 mM); and an oxide, rose oxide (contained in rose by a small extent [250]; IC_{50} = 2.6 mM) [207]. On the other hand, a hydrocarbon myrcene (contained in pimenta racemota [251]; 7% peak amplitude reduction at 5 mM) and a ketone camphor (contained in camphor tree [252]; 33% reduction at 5 mM; this inhibition was resistant to ruthenium red, albeit camphor is a TRPV1 and TRPV3 channel agonist [253, 254]; [207]) exhibited a smaller inhibitory efficacy than them [207]. In conclusion, an efficacy sequence for the CAP inhibitions produced by aroma-oil compounds was phenols (carvacrol, thymol and eugenol) ≥ aldehydes (citral and citronellal) ≥ esters (linalyl acetate, geranyl acetate and bornyl acetate) ≥ alcohols (citronellol, geraniol, (±)-linalool, (-)-linalool, (+)-borneol, (-)-borneol, α-terpineol, (-)-menthol, (+)-menthol and (-)-carveol) ≥ ketones ((+)-pulegone, (-)-carbone, (+)-carbone, (-)-menthone and (+)-menthone) > oxides (rose oxide, 1,8-cineole and 1,4-cineole) >> hydrocarbons (*p*-cymene, myrcene and (+)-limonene) except for camphor that was less effective than oxides [207]. A part of this sequence has been reported by other investigators. For example, frog sciatic nerve CAPs were inhibited by linalool more effectively than 1,8-cineole and *p*-cymene [255]; an efficacy sequence for rat sciatic nerve CAP inhibition was carvacrol > (-)-carveol > carbone >> (+)-limonene [256].

It may be of interest to note that the extents of the CAP inhibitions by menthol-related aroma-oil compounds are related to their chemical structures. Replacing the six-membered ring of menthol by benzene ring (thymol) results in CAP inhibition more than menthol. Removing the -OH group of menthol (*p*-menthane) or substituting the -OH group of menthol with -Cl group (menthyl chloride) leads to CAP inhibition less than menthol. Oxygen bridge in *p*-menthane (1,8-cineole and 1,4-cineole) produces CAP inhibition more than *p*-menthane. Adding =O group to *p*-mentane (menthone), or adding -OH or =O group to limonene ((-)-carveol and carvone) produces a CAP inhibition comparable to menthol. Thus, -OH or =O group, which is bound to *p*-menthane (the basic structure of menthol) and its analogs such as limonene, appeared to play a role in determining the magnitude of CAP inhibition [204].

4.2.4. Other plant-derived compounds

A seven-membered ring compound hinokitiol (β-thujaplicin; 2-hydroxy-4- isopropylcyclohepta-2,4,6-trien-1-one), which is a natural tropolone derivative contained in a species of cypress tree (*Chamaecyparis taiwanensis* [257, 258]), inhibited frog sciatic nerve CAPs with an IC_{50} value of 0.54 mM. A similar inhibition was seen by its stereoisomer β-thujaplicin (IC_{50} = 0.48 mM). On the other hand, CAPs were unaffected by tropolone that lacks isopropyl group bound to the seven-membered ring of hinokitiol. Biosol (4-isopropyl-3-methylphenol; a stereoisomer of thymol and carvacrol) and 4-isopropylphenol, which have isopropyl and hydroxyl groups bound to their six-membered ring, reduced CAP peak amplitudes with the IC_{50} values of 0.58 and 0.85 mM, respectively; the biosol value was close to those of thymol and carvacrol (0.34 mM; see above). Such a similarity in extent of inhibition among their compounds may be due to the fact that negative charge existing in the carbonyl bond of hinokitiol is biased to the oxygen atom owing to a difference in electronegativity between oxygen and carbon atoms and as a result its seven-membered ring behaves as a benzene ring. Moreover, cumene and phenol, which lack the hydroxyl and isopropyl groups, respectively, bound to the benzene ring of 4-isopropylphenol, were found to be much less effective in CAP inhibition than 4-isopropylphenol. Thus, it was concluded that hinokitiol-induced CAP inhibition is due to an interaction involving its isopropyl, carbonyl and hydroxyl groups [259].

In support of this idea, frog sciatic nerve CAPs were inhibited by general anesthetic propofol (2,6-diisopropylphenol) having two isopropyl groups and one hydroxyl group bound to the benzene ring [75]. Hinokitiol may be clinically useful to relieve pain owing to its ability to inhibit nerve AP conduction. Indeed, the application of an oral care gel having hinokitiol to the oral mucosa alleviated oral pain in patients with oral lichen planus related to hepatitis C virus infection [260].

Traditional Japanese (Kampo) medicines that are composed of plant-derived crude drugs are used with Western medicines in Japan [261] with various aims including analgesia (for review, see [262]). Taking into consideration that a variety of plant-derived compounds having an ability to inhibit nerve conduction are contained in Kampo medicine, it is likely that this medicine depresses nerve conduction. We found out that daikenchuto, rikkosan, kikyoto, rikkunshito, shakuyakukanzoto and kakkonto inhibit frog sciatic nerve CAPs (peak amplitude reduction: 70, 30, 25, 15, 15 and 12%, respectively, at 2 mg/mL); their inhibitory actions were concentration-dependent. Among these medicines, daikenchuto was the most effective with an IC_{50} value of 1.1 mg/mL [263]. Although daikenchuto is composed of three kinds of crude medicine, Japanese pepper, processed ginger and ginseng radix, CAPs were inhibited by Japanese pepper (IC_{50} = 0.77 mg/mL) and processed ginger (CAP peak amplitude reduction at 2 mg/ml: 31%) but not ginseng radix [263]. A part of the daikenchuto action was suggested to be due to hydroxyl-α-sanshool (which is contained in xanthoxylum and has an ability to activate both TRPV1 and TRPA1 channels [264]; CAP peak amplitude reduction at 0.05 mM: 60%) that has an ability to inhibit frog sciatic nerve CAPs [197, 263] and is contained in daikenchuto, particularly in Japanese peper [265-267].

It may be of interest to note that many of the plant-derived compounds (e.g., capsaicin, AITC and menthol) having an ability to suppress nerve conduction activate TRP channels. A local anesthetic lidocaine activated TRPV1 and TRPA1 channels located in DRG neurons and TRPA1 channels in the spinal lamina II (see above). Spinal lamina II TRPA1 channels were also activated by another local anesthetic tetracaine [268]. There may be a common site on TRP channels and voltage-gated Na^+ channels that is activated by a plant-derived compound (for review, see [269]).

5. Conclusion

The present study demonstrated that many plant-derived compounds facilitate the spontaneous release of L-glutamate from primary-afferent central terminals by activating TRP channels in the rat spinal lamina II and also inhibit CAPs in the frog sciatic nerve, the actions of both of which are dependent on their chemical structures. Although these two effects were examined in different species of animal, CAP inhibition in the frog sciatic nerve appeared to be similar in extent to that of the rat sciatic nerve. Frog sciatic nerve IC_{50} values for lidocaine, ropivacaine and benzocaine (0.74, 0.34 and 0.80 mM, respectively [68, 75]) were not so different from those (0.28 mM for both lidocaine and ropivacaine [270]; 40% peak amplitude reduction by benzocaine at 1.3 mM [271]) in the rat sciatic nerve. CAP amplitude reduction produced by cocaine was also comparable in extent between the frog (30% at 0.5 mM [69]) and rat sciatic nerves (40% at 0.375 mM [272]). Indeed, IC_{50} value for carvacrol in inhibiting CAPs was comparable in extent between the frog (0.34 mM [204]) and rat sciatic nerves (0.50 mM [273]). Frog sciatic nerve IC_{50} value (5.7 mM) for 1,8-cineole was similar to that (6-8 mM) of the rat sciatic nerve [274]. IC_{50} values (1.7-2.0 mM) for linalool in the frog sciatic nerve were similar to that (1.85 mM) for inhibiting intracellularly-recorded APs in rat DRG neurons [275], although they were slightly larger than that (0.56 mM) for inhibiting voltage-gated Na^+ channels in newt olfactory receptor cells [276]. Citronellol's IC_{50} value (0.35 mM) in the frog sciatic nerve was somewhat smaller than that (2.2 mM) for inhibition by citronellol, of CAPs that were recorded by applying the sucrose-gap method to the rat sciatic nerve [277].

Although CAPs examined in the present study originate from fast-conducting, possibly Aα fibers, pain transmission is transferred by slow-conducting Aδ and C fibers [3]. In future, to more know how plant-derived compounds affect pain transmission, it would be necessary to examine their effects on slow-conducting CAPs.

Although this review article focuses on an involvement in nociceptive transmission of L-glutamate released from primary-afferent fibers onto rat spinal lamina II neurons, this transmission is modulated by a neuronal circuitry composed of excitatory and inhibitory interneurons in the lamina II [278-280]. In rat spinal lamina II neurons, capsaicin and cinnamaldehyde did not affect spontaneous inhibitory transmission [81, 89, 112], while AITC and zingerone enhanced spontaneous inhibitory transmission in a manner sensitive to TTX [111, 128]. The latter observation indicates that TRPA1 channels are located in central terminals of DRG neurons innervating onto spinal inhibitory neurons that make synapses with spinal lamina II neurons. To more reveal an involvement of plant-derived compounds in nociceptive transmission, it would be necessary to examine their actions on inhibitory transmission in spinal lamina II neurons.

TRP channels activated by plant-derived compounds are expressed in not only primary-afferent neurons but also neuronal and glial cells in the central nervous system ([281, 282]; for review, see [283-286]; for example, TRPV1 channels in the periaqueductal gray [287]). Activation of TRP channels present in cells other than primary-afferent neurons also will have to be taken into consideration to reveal cellular mechanisms for antinociception produced by the intrathecal administration of plant-derived compounds (see [288, 289]).

The structure-activity relationships of TRP channel activation, outward current and nerve conduction inhibition produced by plant-derived compounds, as revealed in this study, could serve to develop antinociceptive drugs that are related to the compounds.

CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interests regarding the publication of this paper.

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