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Research paper

Action of thymol on spontaneous excitatory transmission in adult rat spinal substantia gelatinosa neurons

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HIGHLIGHTS

- Thymol activates TRPA1 channels in the spinal dorsal horn.
- Thymol enhances the spontaneous release of glutamate onto spinal dorsal horn neurons.
- Thymol produces membrane hyperpolarization in spinal dorsal horn neurons.
- The thymol activities are different in efficacy from those of its isomer carvacrol.

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ABSTRACT

Thymol, which is contained in thyme essential oil, has various actions including antinociception and nerve conduction inhibition. Although thymol activates transient receptor potential (TRP) channels expressed in heterologous cells, it remains to be examined whether this is so in native neurons. It has not yet been examined how thymol affects synaptic transmission. In order to know how thymol modulates excitatory transmission with a focus on TRP activation, we investigated its effect on glutamatergic spontaneous excitatory transmission in lamina II (substantia gelatinosa; SG) neurons with which nerve terminals expressing TRP channels make synaptic contacts. The experiment was performed by using the blind whole-cell patch-clamp technique in adult rat spinal cord slices. Superfusing thymol (1 mM) for 3 min reversibly increased the frequency of spontaneous excitatory postsynaptic current (sEPSC) with a minimal increase in its amplitude in all neurons examined. Seventy-seven% of the neurons produced an outward current at a holding potential of −70 mV. The sEPSC frequency increase and outward current produced by thymol were concentration-dependent with almost the same half-maximal effective concentration (EC_{50}) values of 0.18 and 0.14 mM, respectively. These activities were repeated at a time interval of 30 min, although the sEPSC frequency increase but not outward current recovered with a slow time course. Voltage-gated Na^+-channel blocker tetrodotoxin did not affect the thymol activities. The sEPSC frequency increase was inhibited by TRPA1 antagonist HC-030031 but not TRPV1 and TRPM8 antagonist (capsazepine and BCTC, respectively), while these antagonists had no effect on the outward current. This was so, albeit the two thymol activities had similar EC_{50} values. It is concluded that thymol increases the spontaneous release of L-glutamate onto SG neurons by activating TRPA1 channels while producing an outward current without TRP activation. Considering that the SG plays a pivotal role in modulating nociceptive transmission from the periphery, these actions of thymol could contribute to at least a part of its antinociceptive effect.

1. Introduction

Thymol (5-methyl-2-isopropylphenol; where the cyclohexane ring of menthol is replaced by benzene ring; Fig. 1A), which is contained in thyme essential oil, has various actions including mutagenicity, genotoxicity [18], antibacterial activity [3], acetylcholinesterase inhibition [10], $GABA_A$-receptor activation [21], vasorelaxation [25], inflammatory response inhibition [4] and

Abbreviations: DRG, dorsal root ganglion; EC_{50}, half-maximal effective concentration; sEPSC, spontaneous excitatory postsynaptic current; SG, substantia gelatinosa; TRP, transient receptor potential; TTX, tetrodotoxin; V_H, holding potential.

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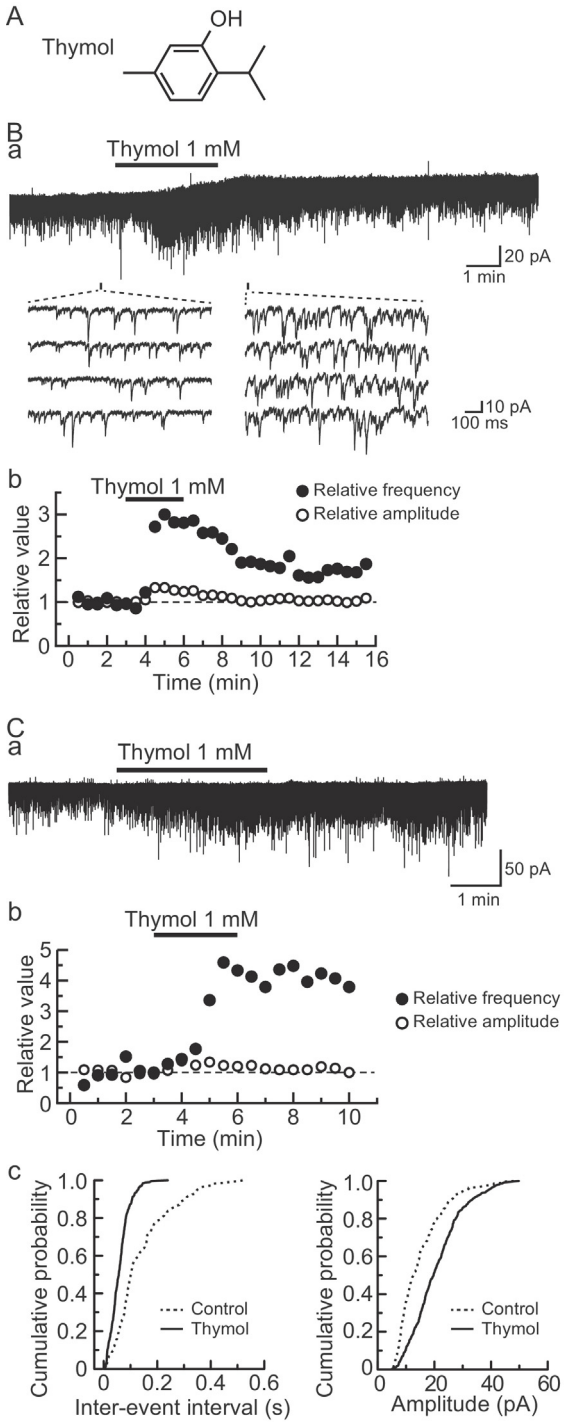

Fig. 1. Effect of thymol (1 mM) on glutamatergic spontaneous excitatory transmission and holding current in rat SG neurons. (A) The chemical structure of thymol. (Ba, Ca) Recordings showing sEPSCs and holding current in the absence and presence of thymol. In the lower of (Ba), four consecutive traces of sEPSCs for a period indicated by a short bar below the chart recording are shown in an expanded time scale. (Bb, Cb) Time courses of changes in sEPSC frequency and amplitude during the action of thymol, relative to those before its superfusion. In this and subsequent figures, the duration of drug superfusion is shown by a horizontal bar above the chart recording; control level (1) is indicated by horizontal dotted line. (Cc) Cumulative histograms of the inter-event interval (left) and amplitude (right) of sEPSC in controls and after thymol. The histograms were created during 0.5 min in the control (214 sEPSC events) and 5 min after the onset of thymol application (507 sEPSC events). Thymol shortened the inter-event interval distribution ($P < 0.01$) and increased the amplitude distribution ($P < 0.01$; Kolmogorov–Smirnov test). (Ba) and (Bb) ((Ca)–(Cc)) were obtained from the same neuron. $V_H = -70$ mV.

antinociception [2]. Some of their effects are likely related to an action of thymol on the neuronal system. Although thymol is known to have an ability to inhibit nerve conduction [12], to our knowledge, there are no reports about its effect on synaptic transmission.

Like various plant-derived chemicals, thymol opens transient receptor potential (TRP) channels that are non-selectively permeable to cations. Thymol has been reported to activate TRP vanilloid-3 (TRPV3; [30]), TRP ankyrin-1 (TRPA1; [15]) and TRP melastatin-8 (TRPM8) channels [23] expressed in heterologous cells. On the other hand, it has not yet been examined which types of TRP channel in native neurons are activated by thymol, although Kaji et al. [11] have reported TRPA1 activation by this drug in human and rat colonic epithelial cells.

There are TRP vanilloid-1 (TRPV1), TRPA1 and TRPM8 channels among protein involved in the modulation of nociceptive transmission in the spinal dorsal horn lamina II (substantia gelatinosa; SG) where the channels are mainly located in the central terminal of dorsal root ganglion (DRG) neuron (for review see [24]). Activation of the TRP channels results in an increase in the spontaneous release of L-glutamate onto the SG neurons ([8,13,22,27,29,31–33]; for review see [14]). In order to know how thymol affects synaptic transmission with a focus on TRP activation, we examined its effect on glutamatergic spontaneous excitatory transmission by applying the whole-cell patch-clamp technique to SG neurons of adult rat spinal cord slices.

2. Materials and methods

2.1. Slice preparation

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 [8,19,33]. Briefly, adult male Sprague-Dawley rats were anesthetized with urethane, 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.

2.2. Whole-cell patch-clamp recordings

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 [8,19,33]. 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. Signals were acquired using an amplifier; data were stored and analyzed with a personal computer. Spontaneous excitatory postsynaptic currents (sEPSCs) were detected and analyzed using Mini Analysis Program; when sEPSC frequency and amplitude changed >5% following superfusion of a drug, the effect of this drug on the excitatory transmission was considered to be effective, as done previously [33]. 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.