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Research Report

# Carvacrol presynaptically enhances spontaneous excitatory transmission and produces outward current in adult rat spinal substantia gelatinosa neurons

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ABSTRACT

Carvacrol, which is abundantly contained in oregano essential oils, has various pharmacological actions including antinociception. Although the oral administration of carvacrol results in antinociception, cellular mechanisms for this action have not been examined yet. We investigated the action of carvacrol on glutamatergic spontaneous excitatory transmission in substantia gelatinosa neurons which play a pivotal role in regulating nociceptive transmission from the periphery by using the patch-clamp technique in adult rat spinal cord slices. Carvacrol superfused for 2 min produced either spontaneous excitatory postsynaptic current frequency increase or outward current at −70 mV, or both of them in many of the neurons tested. The frequency increase and outward current had the $EC_{50}$ values of 0.69 mM and 0.55 mM, respectively. The former action was inhibited by a selective TRPA1 antagonist HC-030031 but not a selective TRPV1 antagonist capsazepine, while the latter action was unaffected by their antagonists. The current–voltage relationship for the outward current indicated an involvement in the current of a change in the membrane permeability of $K^+$ and its outward rectification. The outward current was inhibited in 10 mM-$K^+$ but not $K^+$-channel blockers [tetraethylammonium and $Ba^{2+}$]-containing and 11.0 mM-$Cl^-$ Krebs solution. These results indicate that carvacrol increases the spontaneous release of L-glutamate from nerve terminals by activating TRPA1 but not TRPV1 channels and produces membrane hyperpolarization, which is possibly mediated by tetraethylammonium- and $Ba^{2+}$-insensitive $K^+$ channels, in substantia gelatinosa neurons. It is suggested that the hyperpolarizing effect of carvacrol could contribute to its antinociceptive action.



Abbreviations: AChE, acetylcholinesterase; CNS, central nervous system; DMSO, dimethyl sulfoxide; DRG, dorsal root ganglion; $EC_{50}$, effective concentration for producing half-maximal response; $E_K$, equilibrium potential for $K^+$; HEK, human embryonic kidney; IPSC, inhibitory postsynaptic current; sEPSC, spontaneous excitatory postsynaptic current; SG, substantia gelatinosa; TEA, tetraethylammonium; TRP, transient receptor potential; TRPA1, TRP ankyrin-1; TRPV1, TRP vanilloid-1; TRPV3, TRP vanilloid-3; TTX, tetrodotoxin; $V_H$, holding potential

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## 1. Introduction

Carvacrol (5-isopropyl-2-methylphenol), which is abundantly contained in oregano and thyme essential oils (Baser, 2008; De Vincenzi et al., 2004), has various pharmacological actions such as acetylcholinesterase (AChE) inhibition, and anxiolytic, anticonvulsive and antinociceptive actions in the neuronal system (Baser, 2008). The anxiolytic action of carvacrol may be explained by its facilitatory effect on GABAergic transmission (Kessler et al., 2014; Melo et al., 2010). Carvacrol has been recently reported to inhibit nerve action potential conduction (Joca et al., 2012; Kawasaki et al., 2013), which is consistent with its anticonvulsive action. Although the oral administration of carvacrol results in antinociception in the formalin test in mice (Cavalcante Melo et al., 2012; Guimarães et al., 2010), cellular mechanisms for this action have not yet been examined.

Many of plant-derived chemicals are known to activate transient receptor potential (TRP) channels. For example, capsaicin opens TRP vanilloid-1 (TRPV1) channels that are activated by noxious hot temperature and protons (Caterina and Julius, 2001; Caterina et al., 1997). TRP ankyrin-1 (TRPA1) channels are activated by mustard oil, cinnamon oil and garlic as well as by noxious cold temperature (Jordt et al., 2004; Nilius and Voets, 2005; Story et al., 2003). Carvacrol has an ability to activate TRPA1 and TRP vanilloid-3 (TRPV3) but not TRPV1 channels expressed in human embryonic kidney (HEK) or *Xenopus laevis* oocyte cells (de la Roche et al., 2013; Lee et al., 2008; Vogt-Eisele et al., 2007; Xu et al., 2006). It has not yet been examined what types of TRP channel in the central nervous system (CNS) are activated by carvacrol.

The substantia gelatinosa (SG; lamina II of Rexed) of the spinal dorsal horn is thought to play an important role in modulating nociceptive transmission to the CNS from the periphery (Fürst, 1999; Willis and Coggeshall, 1991). The activation of the TRPV1 or TRPA1 channels located in the central terminals of dorsal root ganglion (DRG) neurons results in the enhancement of the spontaneous release of L-glutamate to the SG neurons (Jiang et al., 2009; Kosugi et al., 2007; Morisset and Urban, 2001; Uta et al., 2010; Wrigley et al., 2009; Yang et al., 1998; for review see Kumamoto et al., 2014). Olvanil, eugenol and zingerone activate TRPV1 channels in the cell body of the DRG neuron, whereas olvanil does not activate TRPV1 and TRPA1 channels in its central terminal (Yang et al., 2011), and zingerone and eugenol activate central terminal TRPA1 but not TRPV1 channels (Inoue et al., 2012; Yue et al., 2013). The properties of TRP channels in the spinal dorsal horn remain to be unknown. In order to know what are cellular mechanisms for the carvacrol-induced antinociception and which types of TRP channel are activated by this drug in the spinal dorsal horn, the present study examined carbacrol's action on glutamatergic spontaneous excitatory transmission in the SG neurons by using the whole-cell patch-clamp technique in adult rat spinal cord slices.

## 2. Results

Whole-cell recordings were obtained from 143 SG neurons. Stable recordings could be obtained from spinal cord slices maintained *in vitro* for more than 12 h, and recordings could be made from single SG neurons for up to 4 h. All SG neurons tested had resting membrane potentials that were more negative than −55 mV (when measured in a current-clamp mode), and exhibited glutamatergic spontaneous excitatory transmission, as shown previously (see Fujita and Kumamoto, 2006; Jiang et al., 2009). Spontaneous excitatory postsynaptic currents (sEPSCs) were not significantly affected in both frequency and amplitude by a voltage-gated $Na^+$-channel blocker tetrodotoxin (TTX; 0.5 μM), as reported previously (Fujita and Kumamoto, 2006; Jiang et al., 2009), possibly owing to deafferentiation in the slices used. This indicated that all of them occurred without the propagation of spikes from cell soma, whose neuron was presynaptic to SG neurons, to the terminals, resulting in spontaneous release.

### 2.1. *Effect of carvacrol on spontaneous excitatory transmission*

In 22% ($n=31$) of the SG neurons tested ($n=143$), carvacrol (1 mM) superfused for 2 min produced an outward current, which was not accompanied by a change in sEPSC frequency, at a holding potential ($V_H$) of −70 mV, as seen in Fig. 1A. On the other hand, 11% ($n=16$) of the SG neurons produced no change in holding currents while exhibiting an increase over 5% in sEPSC frequency (Fig. 1B). In many (63%; $n=90$) of the neurons, both of the outward current and sEPSC frequency increase were produced, as seen in Fig. 1C. The outward current had the averaged peak amplitude of 25.5±1.3 pA ($n=121$), and sEPSC frequency around 3.5 min (when the frequency increase peaked) after the beginning of carvacrol superfusion averaged to be 362±20% ($P<0.05$) of that before its superfusion (control; 10.4±0.7 Hz; $n=106$) with a minimal increase in its amplitude [119±3% ($P<0.05$) of control (12.6±0.5 pA; $n=106$)]. The time for sEPSC frequency increase to be maximal was not so different from those of eugenol (2.5 min at 5 mM; Inoue et al., 2012) and zingerone (2.5 min at 2 mM; Yue et al., 2013). Remaining neurons ($n=6$; 4% of the neurons) did not respond to carvacrol (1 mM).

We next examined whether the carvacrol (1 mM) effects are produced in a repeated manner. As noted from Fig. 2Aa, Ba, the carvacrol-induced outward current was repeated at an interval of 30 min. Fig. 2Ab demonstrates peak outward current amplitudes in the first and second applications, obtained from individual neurons. The amplitudes in the first and second applications were, respectively, 26.4±3.2 pA and 25.9±3.0 pA ($n=15$); they were not different from each other ($P>0.05$). As seen in Fig. 2Ba, carvacrol also repeatedly increased sEPSC frequency. Fig. 2Bb demonstrates sEPSC frequency around 3.5 min after carvacrol addition, relative to that (control) just before its first or second application, obtained from the same neuron. The initial and second carvacrol treatments averaged to result in sEPSC frequencies of 231±32% ($n=6$; $P<0.05$) and 255±26% ($n=6$; $P<0.05$), respectively, relative to control, around 3.5 min after the onset of its treatment; these percentage values were not different from each other ($P>0.05$). Here, sEPSC amplitudes in the initial and second applications, relative to control, were 108±8% ($n=6$; $P>0.05$) and 119±8% ($n=6$; $P>0.05$), respectively.