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Full Length Article

Dual effects of eugenol on the neuronal excitability: An *in vitro* study

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ABSTRACT

Besides its well-known actions on sensory afferents, eugenol also affects general excitability of the nervous system, but the mechanisms involved in the recent effect, especially through modulation of ion channels, have received much less attention. In this study, we studied the effects of eugenol on the excitability of central neurons of land snail *Caucasotachea atrolabiata* and tried to elucidate the underlying ionic mechanisms. The lower concentration of eugenol (0.5 mM) reversibly reduced the frequency of spontaneous action potentials that was associated with elevation of threshold, reduction of maximum slope of rising phase and prolongation of actin potentials. These effects were mimicked by riluzole, suggesting that they might be mediated by inhibition of Na^+ channels. Eugenol also prolonged the single-spike afterhyperpolarization and post stimulus inhibitory period, but these effects seemed to be consequent to action potential prolongation that indirectly augment Ca^{2+} inward currents and Ca^{2+}-activated K^+ currents. This concentration of eugenol was also able to prevent or abolish pentylenetetrazole-induced epileptiform activity. On the other hand, a higher concentration of eugenol (2 mM) reversibly increased the frequency of action potentials and then induced epileptiform activity in majority of treated neurons. Several criteria suggest that the inhibition of K^+ channels by higher concentration of eugenol and indirect augmentation of Ca^{2+} currents are central to the hyperexcitability and epileptiform activity induced by eugenol. Our findings indicate that while low concentration of eugenol could have antiepileptic properties, at higher concentration it induces epileptiform activity. It seems that does dependent inhibition of the ionic currents underlying rising and falling phases of action potential is relevant to the eugenol suppressant and excitatory actions, respectively.

1. Introduction

Eugenol, the major component of clove essential oil, is a phenylpropanoid with analgesic and anti-inflammatory effects and widely used as a toothache remedy. The analgesic action of eugenol has been mainly attributed to its modulatory actions on different ion channels that mediate nociceptive signaling in sensory neurons. Eugenol can primarily elicit irritation by activating TRPV1 channels, but it induces a long-lasting desensitization of these channels after chronic exposure that provides a basis for its analgesic action (Ohkubo and Shibata, 1997). In different neurons including dental primary afferents, it has been shown that eugenol inhibits action potentials and Na^+ and Ca^{2+} currents in a TRPV1-independent manner (Cho et al., 2008; Huang et al., 2012). Although most of the researches have focused on the mechanisms that underlie the effects of eugenol on the sensory transmission in the peripheral nervous system, a few works have sought its actions on the central nervous system. Eugenol easily penetrates into the central nervous system to alleviate neuropathic pain (Lionnet et al., 2010). It inhibits excitatory synaptic transmission in neocortical and hippocampal neurons (Ardjmand et al., 2006; Muller et al., 2006). Eugenol has shown antiepileptic action that has been mainly attributed to its inhibitory action on Na^+ current, although its actions on synaptic transmission has also been suggested as a contributor to its antiepileptic activity (Dallmeier and Carlini, 1981; Huang et al., 2012; Muller et al., 2006). Huang and colleagues (2012) reported that eugenol suppresses neuronal excitability and reduces the severity of pilocarpine-induced seizures. They found that eugenol inhibits both inactivating and non-inactivating Na^+ currents and suggested this effect as responsible for the suppressive and antiepileptic action of eugenol. On the other hand, clove oil overdose has been shown to be associated with development of generalized seizure (Hartnoll et al., 1993).

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The modulatory action of eugenol on different ion channels of the neuronal membrane can affect different functions of the brain and its general excitability. The wide range application of eugenol containing products and its availability over-the-counter emphasize the necessity of more studies to clarify the effects of eugenol on neuronal excitability and the underlying mechanisms. In addition to the technical advantages offered by snail neurons for electrophysiological studies, they have a rich repertoire of ion channels in their soma membranes that make them suitable model system to study neuronal excitability. They have the essential substrates to develop and sustain epileptiform activity that in many aspects resemble those that happen in the vertebrate neurons (Altrup et al., 2003). The structure and the function of ion channels show essential conservation and this is the basis for the assumption that the principal mechanisms underlying the epileptiform activity are constant in whatever nervous systems it appears (Altrup et al., 2003; Plummer and Meisler, 1999). In the current work we took advantage of central neurons of the land snail *Caucasotachea atrolabiata* to study the effects of eugenol on the neuronal excitability and the underlying mechanisms.

2. Materials and methods

2.1. Animals and preparations

Experiments were performed on neurons in the subesophageal ganglia of land snail *Caucasotachea atrolabiata*. Animals were treated in accordance with the European Community guidelines and the experimental protocols were reviewed and approved by the animal care committee of the Shiraz University. The ganglionic mass with its main peripheral nerves and aorta was rapidly dissected out. The subesophageal ganglia was pinned by the nerve and edges of the connective tissue into a small (~1ml) sylgard-grounded recording chamber containing normal snail Ringer and the overlying connective tissue were gently stripped off using fine forcipes.

2.2. Intracellular recording

SEC-10LX amplifier (npi electronic, Tamm, Germany) and Patchmaster 2 × 73.1 software (HEKA, Lambrecht, Germany) were used to record the membrane potentials and to inject current under current clamp conditions using low resistance (1–5 MΩ) sharp microelectrodes. Data were digitized using a LIH 8 + 8 data acquisition interface and the parameters describing action potentials waveform were analyzed using the Fitmaster software (HEKA, Lambrecht, Germany). The resting membrane potential (RMP), input resistance and action potential parameters were measured as described in our previous paper (Vatanparast and Andalib-Lari, 2015).

2.3. Solutions and drugs

The normal snail Ringer solution contained (in mM): NaCl 80, KCl 4, $CaCl_2$ 10, $MgCl_2$ 5, glucose 10, HEPES 5 and pH were adjusted to 7.4 with TRISMA-base. The Na^+-free Ringer was prepared by substituting NaCl with equimolar quantities of Tris-HCl. Eugenol (99%), riluzole, dimethyl sulfoxide (DMSO) and nifedipine were purchased from Sigma-Aldrich (Germany). Other chemicals obtained from Merck (Darmstadt, Germany). Eugenol was prepared as 2 M stock in DMSO, stored under nitrogen in fridge and diluted (0.5 and 2 mM) daily in snail Ringer. Nifedipine was prepared in a stock solution of 100 mM in 95% ethanol. The final concentrations of DMSO and ethanol in bathing solutions were always less than 0.1% and 0.05%, respectively, which had no discernible effect on firing frequency and electrical properties of snail neurons.

2.4. Statistical analysis

Data were presented as means ± S.E.M. with n being the number of neurons on which the measurements were done. The statistical differences were analyzed with either paired *t*-tests, or repeated measures ANOVA followed by Bonferroni post-hoc tests. A value of $P < 0.05$ was considered as statistically significant.

3. Results

In normal snail Ringer, the studied neurons had mean RMP of −43.27 ± 1.9 mV and showed regular spontaneous action potential with mean frequency of 2.08 ± 0.2 Hz, amplitude of 73.54 ± 2.83 mV, duration of 12.38 ± 1.54 ms, threshold of −33.4 ± 1.67, integral of 271 ± 42 and maximum rising and falling slopes of 18.7 ± 2.3 V/s and −11.25 ± 1.9 V/s, respectively. Single action potentials were followed by AHP with a mean amplitude of −8.36 ± 0.84 mV and duration of 111 ± 27 ms (n = 15). Depolarizing current injections (1–5 nA, 500 ms) greatly increased the firing rate of action potentials that were followed by a prominent slow decaying AHP, appearing as a post stimulus inhibitory period (PSIP).

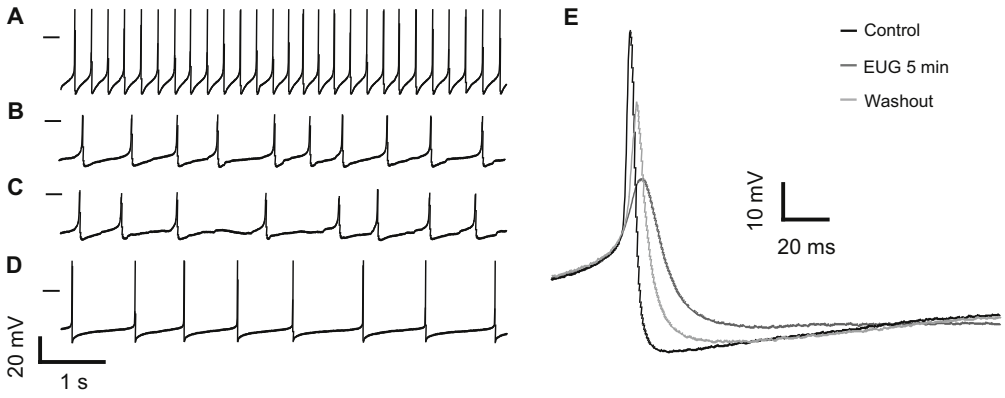

Fig 1. Eugenol (0.5 mM) reduced the frequency of spontaneous action potentials that was associated with a reduction of their amplitude and slope of rising and falling phases in a time dependent and partially reversible manner. Spontaneous action potentials recorded from a representative neuron under control condition (A), 2 min (B) and 5 min after exposure to 0.5 mM eugenol (C), and 6 min after washout with normal snail Ringer (D). The small horizontal bars right to each graph show 0 mV. E: Superimposed action potentials from a neuron under control condition, 5 min after application of eugenol and 4 min after washout. Adjustment of superimposition was based on the threshold of action potentials.