Life Sciences 92 (2013) 359–367

Contents lists available at SciVerse ScienceDirect

Life Sciences

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

Inhibition by menthol and its related chemicals of compound action potentials in frog sciatic nerves

Hiroki Kawasaki, Kotaro Mizuta, Tsugumi Fujita, Eiichi Kumamoto *

Department of Physiology, Saga Medical School, Saga, Japan

ARTICLE INFO

Article history:
Received 3 October 2012
Accepted 5 January 2013

Keywords:
TRPM8 channel
TRPV1 channel
Menthol
Local anesthetic
Compound action potential
Sciatic nerve
Frog

ABSTRACT

Aims: Transient receptor potential (TRP) vanilloid-1 (TRPV1) and melastatin-8 (TRPM8) channels play a role in transmitting sensory information in primary-afferent neurons. TRPV1 agonists at high concentrations inhibit action potential conduction in the neurons and thus have a local anesthetic effect. The purpose of the present study was to know whether TRPM8 agonist menthol at high concentrations has a similar action and if so whether there is a structure–activity relationship among menthol-related chemicals.
Main methods: Compound action potentials (CAPs) were recorded from the frog sciatic nerve by using the air-gap method.
Key findings: (−)-Menthol and (+)-menthol concentration-dependently reduced CAP peak amplitude with the IC_{50} values of 1.1 and 0.93 mM, respectively. This (−)-menthol activity was resistant to non-selective TRP antagonist ruthenium red; TRPM8 agonist icilin did not affect CAPs, indicating no involvements of TRPM8 channels. *p*-Menthane, (+)-limonene and menthyl chloride at 7–10 mM minimally affected CAPs. On the other hand, (−)-menthone, (+)-menthone, (−)-carvone, (+)-carvone and (−)-carveol (in each of which chemicals –OH or =O group was added to *p*-menthane and limonene) and (+)-pulegone inhibited CAPs with extents similar to that of menthol. 1,8-Cineole and 1,4-cineole were less effective while thymol and carvacrol were more effective than menthol in inhibiting CAPs.
Significance: Menthol-related chemicals inhibited CAPs and were thus suggested to exhibit local anesthetic effects comparable to those of lidocaine and cocaine as reported previously for frog CAPs. This result may provide information to develop local anesthetics on the basis of the chemical structure of menthol.

Introduction

Menthol (2-isopropyl-5-methylcyclohexanol), a secondary alcohol which is contained in peppermint or other mint oils (Li et al., 2011; Eccles, 1994), is well-known to activate the transient receptor potential (TRP) melastatin-8 (TRPM8) channel (McKemy et al., 2002; Peier et al., 2002) existing in the peripheral and central terminals of dorsal root ganglion (DRG) neurons (Kobayashi et al., 2005). The TRPM8 channel in the peripheral terminal is activated by not only menthol but also temperatures of <25 °C to generate a membrane depolarization, resulting in the production of action potentials (APs). On the other hand, the activation of the TRPM8 channel in the central terminal leads to a barrage of the spontaneous release of L-glutamate onto superficial dorsal horn neurons from there (Wrigley et al., 2009; Suzuki et al., 2007; Tsuzuki et al., 2004). The other type of TRP channel, TRP vanilloid-1 channel [TRPV1 channel, which is activated by capsaicin (the major pungent ingredient in hot peppers) and heat (>43 °C); Caterina et al., 1997], is also located in the peripheral and central terminals of the DRG neurons (Kobayashi et al., 2005); its activation in both of the two terminals produces actions similar to those of TRPM8 activation (Tominaga, 2007; Yang et al., 1998). These TRP activations play a role in transmitting sensory information.

Capsaicin at high concentrations inhibits voltage-gated Na^+ channels without TRPV1 activation and thus has an ability to inhibit AP conduction in nerve fibers (Cao et al., 2007; Wang et al., 2007). We have previously reported that capsaicin reduces the peak amplitude of compound AP (CAP), which is fast-conducting and sensitive to a voltage-gated Na^+-channel blocker tetrodotoxin (TTX), without TRPV1 activation in the frog sciatic nerve (Tomohiro et al., 2008). Other TRPV1 agonists derived from plants also exhibited CAP inhibition, the extent of which differed among the agonists, and this difference was thus suggested to be due to a distinction in chemical structure (Tomohiro et al., 2008). A similar CAP inhibition in a manner dependent on the structures of the chemicals tested has been shown for cocaine-related chemicals (Tokuno et al., 2004), opioids (Mizuta et al., 2008; Katsuki et al., 2006) and adrenoceptor agonists (Kosugi et al., 2010; for review see Kumamoto et al., 2011). To our knowledge, it has not yet been systematically examined how menthol and its related chemicals act on nerve AP conduction, although

* Corresponding author at: Department of Physiology, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan. Tel.: +81 952 34 2273; fax: +81 952 34 2013.
E-mail address: kumamote@cc.saga-u.ac.jp (E. Kumamoto).

0024-3205/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2013.01.012

Gaudioso et al. (2012) have very recently reported a voltage-gated Na^+ channel inhibition produced by menthol. We investigated their actions on the CAPs recorded by applying the air-gap method to the frog sciatic nerve and addressed whether there is a structure-activity relationship in the actions of menthol-related chemicals. The present study was focused on plant-derived chemicals, many of which were involved in the modulation of voltage-gated ion channels (de Araújo et al., 2011).

Materials and methods

Animals

This study was approved by the Animal Care and Use Committee of Saga University, and was conducted in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan. All efforts were made to minimize animal suffering and the number of animals used.

Preparation of frog sciatic nerves

The method used for obtaining frog sciatic nerve preparation has been described previously (Kosugi et al., 2010; Mizuta et al., 2008; Katsuki et al., 2006). In brief, either sex of frogs (*Rana nigromaculata*) was decapitated and then pithed; thereafter the sciatic nerve was dissected from the lumbar plexus to the knee in Ringer solution. The isolated sciatic nerve was carefully desheathed under a binocular microscope and then loosely placed in five platinum wires that were glued to a Lucite plate, where the two ends of the nerve were tied to the wires by using threads. The plate was put on a beaker having Ringer solution in which the sciatic nerve was soaked. The composition of Ringer solution used was (mM): NaCl, 115.5; KCl, 2.0; $CaCl_2$, 1.8; Na_2HPO_4, 1.3; and NaH_2PO_4, 0.7 (pH = 7.0).

Recordings of compound action potentials from frog sciatic nerve fibers

As performed previously (Kosugi et al., 2010; Mizuta et al., 2008; Katsuki et al., 2006), the Lucite plate having platinum wires attached with the sciatic nerve was moved from the beaker containing Ringer solution to a vacant one and then CAPs were recorded in air using a preamplifier. Here, two of the platinum wires were used to record CAPs, and other two were for stimulating the sciatic nerve. The stimulation was performed at a frequency of 1 Hz with a stimulator, where rectangular pulses having 0.1 ms duration and various strengths were used. In order not to dry the sciatic nerve in air, this procedure was quickly performed at a time interval of 2 min. When the effects of drugs on CAPs were examined, the nerve was put back into the soaking solution with drugs in between 2 measures. The data were monitored on a storage oscilloscope while being recorded on a thermal array recorder having a wave form storage module and stored on magnetic tape with a PCM tape recorder for later analyses. Stimulating the sciatic nerve produced a CAP following a stimulus artifact. The peak amplitude of the CAP was measured as a difference between baseline and CAP peak level, as done previously (Kosugi et al., 2010; Mizuta et al., 2008; Katsuki et al., 2006). The peak amplitude of the CAP depended on the strength of stimulus given to the sciatic nerve in such that the CAP peak amplitude enhanced with an increase in stimulus strength and attained a maximal value. As done previously (Kosugi et al., 2010; Mizuta et al., 2008; Katsuki et al., 2006), we analyzed the peak amplitude of the maximal CAP. A conduction velocity (CV) value was determined by using the fifth electrode as an additional stimulation site. All experiments were carried out at room temperature (22–27 °C).

Data analysis

Concentration-dependence curve for the reduction of the peak amplitude of CAP in the sciatic nerve soaked with a drug was analyzed using the following Hill equation:

$$\text{CAP amplitude (\% of control)} = 100/(1 + ([\text{Drug}]/IC_{50})^{n}_{H},$$

where [Drug] is drug concentration, IC_{50} is the concentration of drug for half-maximal inhibition and n_H is the Hill coefficient.

Data were indicated as mean ± S.E.M. and statistical significance was set at $P<0.05$ using a paired or unpaired Student's *t*-test. In all cases, *n* refers to the number of sciatic nerves studied. The peak amplitude of CAP before drug application was denoted as control.

Materials

Drugs used were (−)-menthol, (+)-menthol, thymol, carvacrol, (−)-menthone, (+)-menthone, (+)-pulegone, 1,8-cineole, 1,4-cineole, (−)-carveol, (−)-carvone, (+)-carvone, (+)-limonene, menthyl chloride, icilin, ruthenium red (Sigma-Aldrich, St. Louis, MO, USA) and *p*-menthane (Wako, Osaka, Japan). All drugs except for icilin and ruthenium red were first dissolved in dimethyl sulfoxide (DMSO) and then diluted to the final concentration in Ringer solution, where the concentration of DMSO was less than 1%. Icilin and ruthenium red were dissolved in DMSO at 20 mM and in distilled water at 10 mM, respectively, and then stored at −25 °C. These drugs were then diluted to the final concentration in Ringer solution immediately before use. DMSO at 1% did not affect CAPs. The pH of Ringer solution containing drugs was adjusted to 7.0 with NaOH. Drugs at concentrations larger than 10 mM were not tested, because a change in osmotic pressure may affect CAPs.

Results

Effects of drugs on fast-conducting CAPs were examined in a total of 271 sciatic nerves, and when measured in some of the nerves, the CAPs had the averaged CV value of 30.1 ± 0.7 m/s ($n = 213$), a value comparable to those reported previously (Kosugi et al., 2010; Mizuta et al., 2008; Katsuki et al., 2006).

Effect of (−)-menthol on frog sciatic nerve CAPs

Soaking the sciatic nerve into (−)-menthol (Fig. 1Aa; the main form of menthol occurring in nature; 2 mM)-containing Ringer solution reduced the peak amplitude of the CAP, as seen from Fig. 1Ab. Fig. 1Ac demonstrates an average of the time courses of a change in CAP peak amplitude following soaking into (−)-menthol (2 mM), relative to control, which are obtained from seven sciatic nerves. The (−)-menthol-induced reduction in CAP peak amplitude attained a maximal effect at 20 min of the soaking, where the peak amplitude of the CAP was 7 ± 4% ($P<0.05$) of control (17.8 ± 3.1 mV; $n = 7$). In nerves were treated with (−)-menthol and then returned to drug-free Ringer solution (washout) for up to 1 h, the CAP amplitude recovered to about 80% of control level. Fig. 1B shows the time courses of changes in CAP peak amplitude with an increase in time after soaking the sciatic nerve into (−)-menthol at various concentrations ranging from 0.01 to 2 mM. The rate of the CAP peak amplitude reduction produced by (−)-menthol was enhanced in extent with an increase in its concentration. CAP amplitude reduction at 20 min of the soaking increased in magnitude with an increase in (−)-menthol concentration. The concentration–response curve for the (−)-menthol-induced CAP amplitude reduction obtained from many nerve trunks is given in Fig. 1C. Table 1 shows IC_{50} and n_H values obtained from analysis based on the Hill equation.