Life Sciences 92 (2013) 368–378

Contents lists available at SciVerse ScienceDirect

Life Sciences

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

Inhibition by capsaicin and its related vanilloids of compound action potentials in frog sciatic nerves

Daisuke Tomohiro, Kotaro Mizuta, Tsugumi Fujita, Yukiko Nishikubo, Eiichi Kumamoto *

Department of Physiology, Saga Medical School, Saga, Japan

ARTICLE INFO

Article history:
Received 10 October 2012
Accepted 5 January 2013

Keywords:
Capsaicin
Vanilloid
Transient receptor potential vanilloid-1 channel
Procaine
Sciatic nerve
Compound action potential
Frog

ABSTRACT

Aims: Although capsaicin not only activates transient receptor potential vanilloid-1 (TRPV1) channels but also inhibits nerve conduction, the latter action has not yet been fully examined. The purpose of the present study was to know whether various vanilloids have an inhibitory action similar to that of capsaicin and further to compare their actions with that of local anesthetic procaine.

Main methods: Fast-conducting compound action potentials (CAPs) were recorded from frog sciatic nerve fibers by using the air-gap method.

Key findings: Capsaicin reversibly and concentration-dependently reduced the peak amplitude of the CAP. TRPV1 antagonist capsazepine did not affect the capsaicin activity, and powerful TRPV1 agonist resiniferatoxin had no effect on CAPs, indicating no involvement of TRPV1 channels. Capsaicin analogs and other various vanilloids also inhibited CAPs in a concentration-dependent manner. An efficacy sequence of these inhibitions was capsaicin = dihydrocapsaicin > capsiate > eugenol > guaiacol ≥ zingerone ≥ vanillin > vanillylamine. Vanillic acid had almost no effect on CAPs; olvanil and curcumin appeared to be effective less than capsaicin. Capsaicin and eugenol were, respectively, ten- and two-fold effective more than procaine in CAP inhibition, while each of guaiacol, zingerone and vanillin was five-fold effective less than procaine.

Significance: Various vanilloids exhibit CAP inhibition, the extent of which is determined by the property of the side chain bound to the vanillyl group, and some of them are more effective than procaine. These results may serve to unveil molecular mechanisms for capsaicin-induced conduction block and to develop antinociceptive drugs related to capsaicin.

Introduction

Transient receptor potential vanilloid-1 (TRPV1) channels expressed in the peripheral terminal of primary-afferent neuron (Kobayashi et al., 2005; Amaya et al., 2003) are activated by capsaicin (8-methyl-*N*-vanillyl-trans-6-nonenamide; Fig. 1A) to generate a membrane depolarization, resulting in the production of action potentials (APs; Caterina and Julius, 2001; Caterina et al., 1997; Szolcsányi, 1977) and thus pain sensation (Tominaga, 2007). On the other hand, an application of capsaicin at high concentrations to the skin is known to alleviate chronic pain (Malmberg et al., 2004; Nolano et al., 1999) and thus capsaicin cream is clinically used as an antinociceptive drug (Medical Letter, 1992). There are several possible mechanisms for this capsaicin action. First, an increase in intracellular Ca^{2+} concentration as a result of TRPV1 activation may desensitize TRPV1 channels by activating a dephosphorylation enzyme calcinurin or calmodulin (Numazaki et al., 2003; Docherty et al., 1996). Second, TRPV1 activation may result in a degeneration of nociceptive fibers (Yang et al., 2003b; Nolano et al., 1999) or in the depletion of substance P involved in nociceptive transmission in the central terminal of primary-afferent neuron (Kashiba et al., 1997; Maggi, 1992; Fitzgerald, 1983). Third, capsaicin may inhibit AP conduction in nociceptive fibers, resulting in antinociception (Yamanaka et al., 1984; Petsche et al., 1983; Wall and Fitzgerald, 1981).

Voltage-gated Na^+-channels are inhibited by capsaicin in a TRPV1-dependent (Binshtok et al., 2007; Cao et al., 2007; Liu et al., 2001) or -independent manner. The latter action is shown to be due to either a change in lipid bilayer elasticity (Lundbæk et al., 2005) or a direct action on Na^+ channels themselves (Cao et al., 2007; Wang et al., 2007). Like capsaicin, a powerful TRPV1 agonist resiniferatoxin (RTX; Fig. 1B; Jiang et al., 2009; Winter et al., 1990) inhibited voltage-gated Na^+ and Ca^{2+} channels (Sugimoto et al., 2008) and also AP conduction in primary-afferent fibers (Kissin, 2008). Another TRPV1 agonist eugenol [2-methoxy-4-(2-propenyl) phenol; Fig. 1C] inhibited voltage-gated Na^+ and K^+ channels without TRPV1 activation (Cho et al., 2008; Li et al., 2007; Park et al., 2006).

We have previously reported that opioids and α-adrenoceptor agonists reduce the peak amplitude of compound AP (CAP), which is fast-conducting and sensitive to a voltage-gated Na^+-channel blocker tetrodotoxin (TTX), in a manner dependent on the chemical structures

* 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.2013.01.011

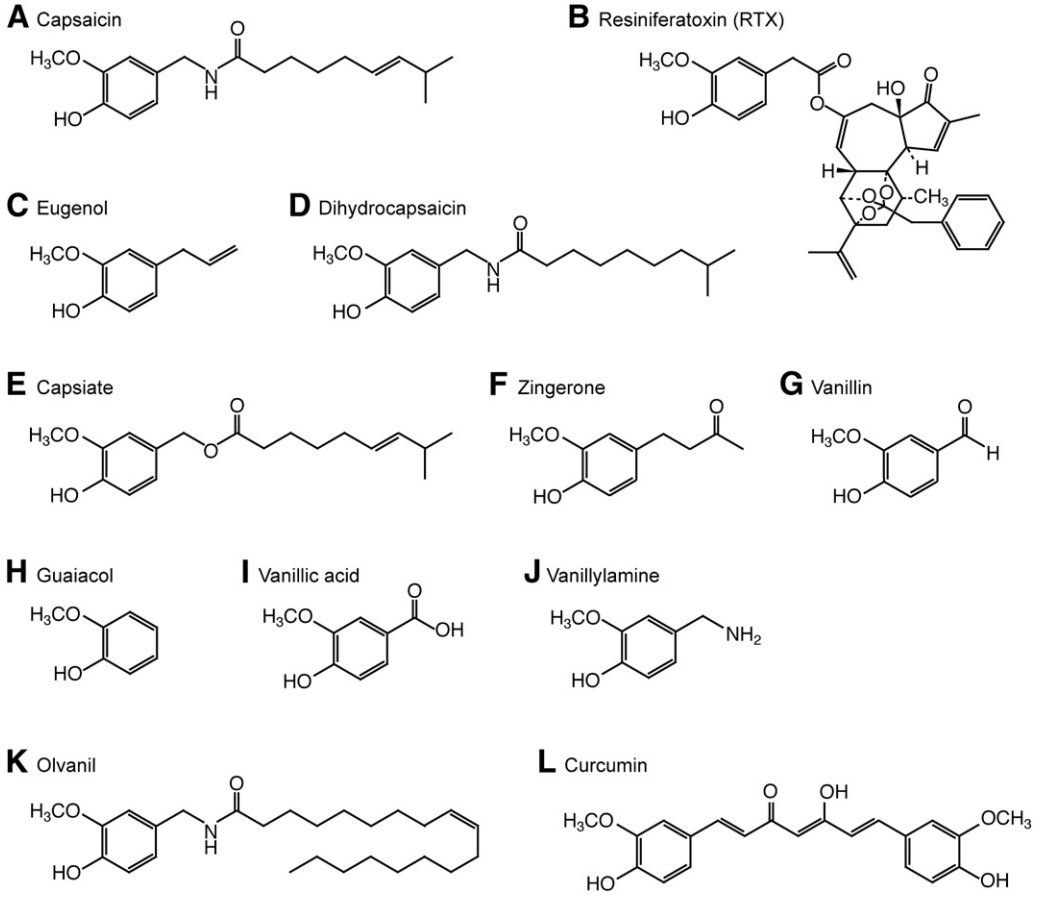

Fig. 1. The chemical structures of capsaicin (A) and its related compounds [B, resiniferatoxin (RTX); C, eugenol; D, dihydrocapsaicin; E, capsiate; F, zingerone; G, vanillin; H, guaiacol; I, vanillic acid; J, vanillylamine; K, olvanil; L, curcumin].

of the substances tested (Kumamoto et al., 2011; Kosugi et al., 2010; Mizuta et al., 2008; Katsuki et al., 2006). To our knowledge, it has not yet been examined which chemical structures of capsaicin are important in inhibiting nerve AP conduction. The present study examined the actions of capsaicin and its related various vanilloids (Fig. 1) on the CAPs recorded by applying the air-gap method to the frog sciatic nerve and addressed whether the actions are related to vanilloids' chemical structures. To know vanilloids' local anesthetic effects, their CAP inhibitions were compared with that of a local anesthetic procaine (Štolc and Mai, 1993). These results have been partly reported in abstract form (Tomohiro et al., 2008).

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 (length: 4–5 cm; diameter: 0.6–1 mm) was carefully desheathed under a binocular microscope (20–30×) and then loosely placed in five platinum wires, that were glued to a Lucite plate (8 cm×3.5 cm), 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 (100 ml) in which the sciatic nerve was soaked. Throughout experiments, the Ringer solution was continuously stirred at a rate of about 350 rpm with a Teflon-covered magnetic stirrer bar in order to maintain a uniform composition of Ringer solution around the sciatic nerve. 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). Before the start of the experiment, the sciatic nerve was preincubated for at least 15 min with Ringer solution.

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 (Model LI-75A, NF Electronics Instruments, Yokohama, Japan). 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 (SEN-3201, Nihon Kohden, Tokyo, Japan), where rectangular pulses having 0.1 ms duration and various stimulus strengths were used. In order not to dry the sciatic nerve in air, this procedure was quickly (20 s at the most) performed at a time interval of 2 min. The data were monitored on a storage oscilloscope (VC-6724, Hitachi Electronics Instruments, Tokyo, Japan) while being