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Inhibition by TRPA1 agonists of compound action potentials in the frog sciatic nerve

Akitomo Matsushita [1], Sena Ohtsubo [1], Tsugumi Fujita, Eiichi Kumamoto *

Department of Physiology, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan

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ABSTRACT

Although TRPV1 and TRPM8 agonists (vanilloid capsaicin and menthol, respectively) at high concentrations inhibit action potential conduction, it remains to be unknown whether TRPA1 agonists have a similar action. The present study examined the actions of TRPA1 agonists, cinnamaldehyde (CA) and allyl isothiocyanate (AITC), which differ in chemical structure from each other, on compound action potentials (CAPs) recorded from the frog sciatic nerve by using the air-gap method. CA and AITC concentration-dependently reduced the peak amplitude of the CAP with the IC_{50} values of 1.2 and 1.5 mM, respectively; these activities were resistant to a non-selective TRP antagonist ruthenium red or a selective TRPA1 antagonist HC-030031. The CA and AITC actions were distinct in property; the latter but not former action was delayed in onset and partially reversible, and CA but not AITC increased thresholds to elicit CAPs. A CAP inhibition was seen by hydroxy-α-sanshool (by 60% at 0.05 mM), which activates both TRPA1 and TRPV1 channels, a non-vanilloid TRPV1 agonist piperine (by 20% at 0.07 mM) and tetrahydrolavandulol (where the six-membered ring of menthol is opened; IC_{50} = 0.38 mM). It is suggested that TRPA1 agonists as well as TRPV1 and TRPM8 agonists have an ability to inhibit nerve conduction without TRP activation, although their agonists are quite different in chemical structure from each other.

1. Introduction

Plant-derived chemicals are known to activate transient receptor potential (TRP) channels such as TRP vanilloid-1 (TRPV1; [1]), TRP melastatin-8 (TRPM8; [11]) and TRP ankyrin-1 (TRPA1; [3]) channels existing in the peripheral and central terminals of primary-afferent neurons [6]. Their activations in the peripheral terminal generate a membrane depolarization, resulting in the production of action potentials (APs), while those in the central terminal lead to a barrage of the spontaneous release of L-glutamate onto superficial spinal dorsal horn or medullary neurons from there [9,17,19]. These TRP activations play a role in transmitting sensory information. On the other hand, a TRPV1 agonist capsaicin and a TRPM8 agonist menthol (which are contained in capsicum and peppermint, respectively) at high concentrations inhibit voltage-gated Na^+ channels without TRP activation and thus have an ability to inhibit AP conduction in nerve fibers [2,16]. To our knowledge, it has not yet been examined whether TRPA1 agonists have a similar action.

We have very recently reported that capsaicin and its related vanilloids [14], and also menthol and its related chemicals [5] reduce the peak amplitude of compound AP (CAP), which is fast-conducting and sensitive to a voltage-gated Na^+-channel blocker tetrodotoxin, without TRP activation in the frog sciatic nerve. In these studies, it was revealed that a difference in chemical structure among them results in a distinction in the extent of the CAP inhibition. A similar CAP inhibition in a manner dependent on the structures of the chemicals tested has been shown for opioids [4,12] and adrenoceptor agonists [8]. In order to know whether TRPA1 agonists have an ability to inhibit frog CAPs and if so this inhibition is different in extent among TRPA1 agonists having a distinct chemical structure, we examined the actions of TRPA1 agonists, cinnamaldehyde (CA, contained in cinnamon) and allyl isothiocyanate (AITC; in wasabi [15]; Fig. 1Aa, Ba), on CAPs recorded from the frog sciatic nerve by using the air-gap method. With the aim to know more about chemical structures of TRP agonists having an ability to inhibit nerve conduction, we further investigated how frog CAPs are affected by hydroxy-α-sanshool (in xanthoxylum) which activates both TRPV1 and TRPA1 channels [7], a TRPV1 agonist piperine (in black pepper) which does not have the vanillyl group [10,18], and tetrahydrolavandulol where the six-membered ring of menthol is opened.

2. Materials and methods

This study was approved by the Animal Care and Use Committee of Saga University. The method used for obtaining frog sciatic nerve preparation has been described previously [4,5,8,12,14]. In brief, either sex of frogs (*Rana nigromaculata*) was decapitated and then pithed; thereafter the sciatic nerve was dissected from

* Corresponding author. Fax: +81 952 34 2013.
E-mail address: kumamote@cc.saga-u.ac.jp (E. Kumamoto).
[1] These two authors make equal contribution to this work.

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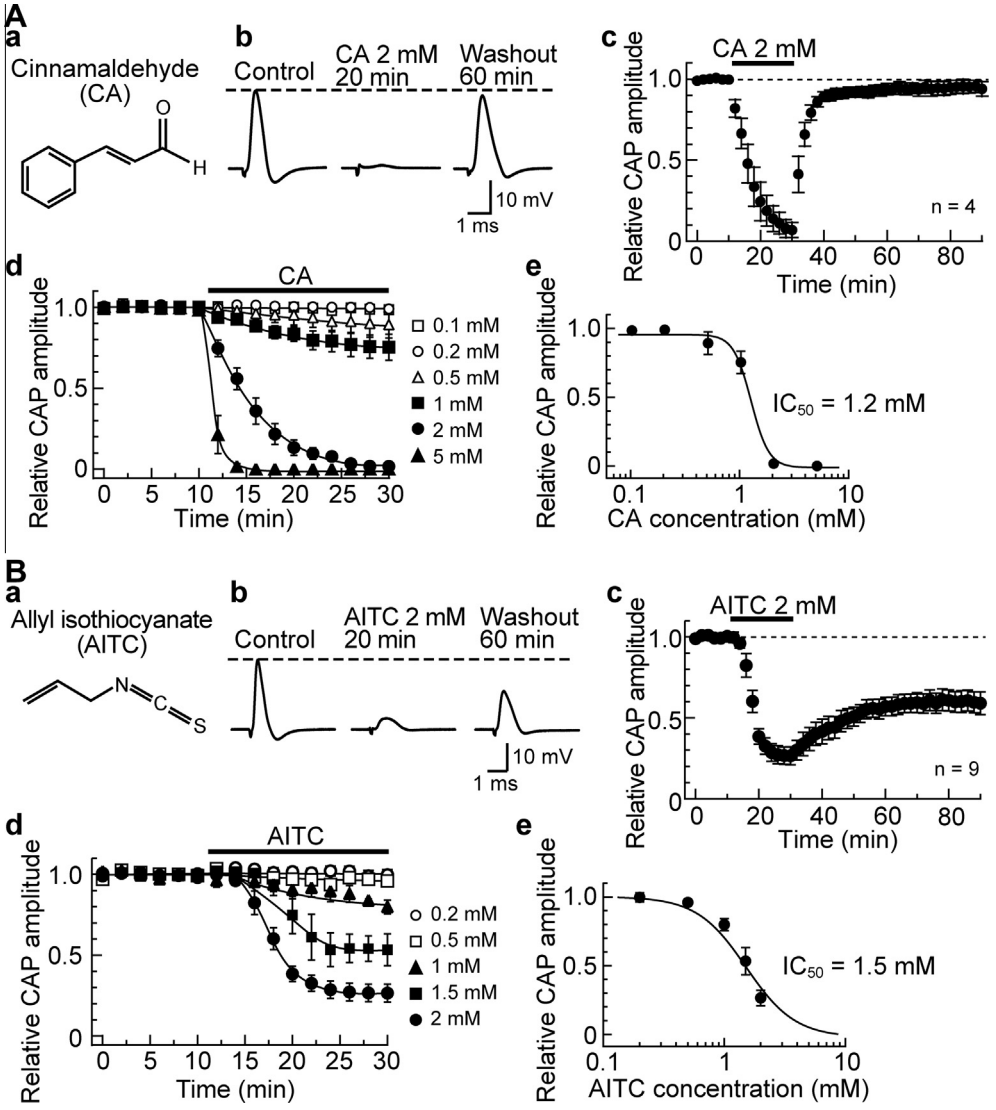

Fig. 1. TRPA1 agonists, cinnamaldehyde (CA) and allyl isothiocyanate (AITC), concentration-dependently inhibit compound action potentials (CAPs) recorded from frog sciatic nerves. (Aa, Ba) The chemical structures of CA and AITC. (Ab, Bb) Recordings of CAPs before (Control), at 20 min after exposure to CA or AITC and thereafter 60 min in the absence of CA or AITC (Washout). (Ac, Bc) Average time courses of changes in CAP peak amplitudes following exposure to CA or AITC for 20 min, relative to those before the soaking. In this and subsequent figures, *n* denotes the number of sciatic nerve examined. (Ad, Bd) Comparison in average time course among CAP peak amplitude reductions produced by CA at 0.1–5 mM (Ad; data at each concentration were obtained from 3 to 5 sciatic nerves) or by AITC at 0.2–2 mM (Bd; from 3 to 7 sciatic nerves). Here, solid lines were arbitrarily drawn. (Ae, Be) The peak amplitudes of CAPs recorded from sciatic nerve fibers treated with CA or AITC at various concentrations for 20 min, relative to control, which were plotted against its concentration. These concentration–response curves were drawn according to the Hill equation (Ae: half-maximal inhibitory concentration, IC_{50} = 1.2 mM; Hill coefficient, n_H = 5.3; Be: IC_{50} = 1.5 mM; n_H = 3.5). In this and subsequent figures, each point with vertical bars represents the mean and S.E.M.; the S.E.M. of the values without a vertical bar was within the size of symbol, and dotted line denotes the control value.

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).

As performed previously [4,5,8,12,14], 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 at 1 Hz. 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 USB flash memory with a Data logger 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 [4,5,8,12,14]. 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 [4,5,8,12,14], we analyzed the peak amplitude of the maximal CAP unless otherwise mentioned. A conduction velocity value was determined by using the fifth electrode as an additional stimulation site. All experiments were carried out at room temperature (22–27 °C).