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Traditional Japanese medicines inhibit compound action potentials in the frog sciatic nerve

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ABSTRACT

Ethnopharmacological relevance: Traditional Japanese (Kampo) medicines have a variety of clinical effects including pain alleviation, but evidence for a mechanism for their pain relief has not yet been elucidated fully. Considering that Kampo medicine contains many plant-derived chemicals having an ability to inhibit nerve action potential conduction, it is possible that this medicine inhibits nerve conduction. The purpose of the present study was to know how various Kampo medicines affect nerve conduction.

Materials and methods: We examined the effects of Kampo and crude medicines on compound action potentials (CAPs) recorded from the frog sciatic nerve by using the air-gap method.

Results: Daikenchuto, rikkosan, kikyoto, rikkunshito, shakuyakukanzoto and kakkonto concentration-dependently reduced the peak amplitude of the CAP. Among the Kampo medicines, daikenchuto was the most effective in inhibiting CAPs. Daikenchuto is composed of three kinds of crude medicine, Japanese pepper, processed ginger and ginseng radix. When the crude medicines were tested, Japanese pepper and processed ginger reduced CAP peak amplitudes, while ginseng radix hardly affected CAPs. Moreover, there was an interaction between the Japanese pepper and processed ginger activities in such that one medicine at low but not high concentrations increased the extent of the inhibition by the other one that was co-applied.

Conclusions: Kampo medicines have an ability to inhibit nerve conduction. This action of daikenchuto is due to Japanese pepper and processed ginger but not ginseng radix, probably through an interaction between Japanese pepper and processed ginger in a manner dependent on their concentrations. Nerve conduction inhibition could contribute to at least a part of Kampo medicine's clinical effects such as pain alleviation.

1. Introduction

Traditional Japanese (Kampo) medicines that are composed of crude drugs derived from plants are used alongside Western medicines in Japan (Kono et al., 2009) with various purposes such as digestive symptom treatment and pain alleviation (for review, see Hijikata, 2006; Mochiki et al., 2010; Motoo et al., 2009). This is because Kampo medicines have high quality and standardized ingredients (Kono et al., 2009). One of Kampo medicines, pharmaceutical-grade daikenchuto, has been approved as an investigational new drug to treat gastrointestinal disorders by the US Food and Drug Administration (Munekage et al., 2013). The mechanisms for the clinical effects of traditional herbal medicines such as Kampo medicines, however, have not yet been elucidated fully (Wachtel-Galor and Benzie, 2011).

It is possible that pain relief produced by Kampo medicine is due to a modulation of synaptic transmission (at the spinal cord level, see Fürst, 1999) and an action potential conduction inhibition in neural pain pathways; the latter idea is supported by the fact that local anesthetics are used for alleviating pain with an expectation of conduction inhibition (for example, see Tremont-Lukats et al., 2005). We have recently revealed that many plant-derived chemicals inhibit fast-conducting and voltage-gated Na^+-channel blocker tetrodotoxin-sensitive compound action potentials (CAPs) in the frog sciatic nerve. Among the chemicals, there are vanilloids including capsaicin (the major pungent

Abbreviations: CAP, compound action potential; IC_{50}, half-maximal inhibitory concentration; n_H, Hill coefficient; TRP, transient receptor potential; TRPA1, TRP ankyrin-1; TRPV1, TRP vanilloid-1

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ingredient in hot peppers) and zingerone (a pungent component of ginger; Tomohiro et al., 2013), menthol (a secondary alcohol contained in peppermint) and its related substances (Kawasaki et al., 2013), allyl isothiocyanate, cinnamaldehyde (which are contained in wasabi and cinnamon, respectively; Matsushita et al., 2013) and various aroma-oil compounds (Ohtsubo et al., 2015). Linalool, (−)- and (+)-carvone, which are, respectively, found in lavender, spearmint and caraway, have been reported to inhibit frog sciatic nerve CAPs (Faliagkas et al., 2015; Zalachoras et al., 2010). Similar results have been obtained in other preparations. For example, rat sciatic nerve CAPs were inhibited by plant-derived chemicals (carvacrol, carveol, carvone, 1,8-cineole and eugenol; Gonçalves et al., 2010; Lima-Accioly et al., 2006; Moreira-Lobo et al., 2010). Capsaicin inhibited rat coccygeal or saphenous nerve CAPs (Petsche et al., 1983). Lavender-oil components (linalool and linalyl acetate) and menthol inhibited electrically-evoked contractions of rat phrenic hemidiaphragm (Galeotti et al., 2001; Ghelardini et al., 1999).

It is possible that chemicals themselves contained in orally-administrated Kampo medicines and ones modified as a result of degradation by enzymes existing in alimentary tracts or of bioconversion by enteric microbiota are absorbed into the body from the epithelial cells of the tracts and then act on the nervous system through blood or lymph (see Watanabe et al., 2015). In support of this idea, Kampo medicines given with foods ameliorated age-related impairments of working memory and reversal learning in rats (Mizoguchi et al., 2011) and exhibited an antidepressant-like effect in behavioral mouse models of depression (Ito et al., 2012). To our knowledge, there are no reports examining the actions of Kampo medicines on nerve conduction. The present study examined how various Kampo medicines used to relieve pain (Table 1) affect CAPs recorded from the frog sciatic nerve by using the air-gap method.

Table 1
Kampo medicines used to examine their effects on frog sciatic nerve CAPs and examples of pain alleviated by the medicines.

Kampo medicines	Pain	References
Daikenchuto	Abdominal pain associated with intestinal obstruction	Horiuchi et al. (2010)
Rikkosan	Intractable intraoral pain	Niimi et al. (2015)
Kikyoto	Pain associated with acute upper respiratory infection	Ishimaru et al. (2013)
Rikkunshito	Abdominal pain in gastrointestinal symptoms	Kusunoki et al. (2010)
Kakkonto	Trigeminal neuralgia	Hijikata (2006)
	Chronic tension headache	Motoo et al. (2009)
Shakuyakukanzoto	Paclitaxel-induced painful peripheral neuropathy	Hidaka et al. (2009)

2. Materials and methods

2.1. 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.

2.2. Preparation of frog sciatic nerves

The method used for obtaining frog sciatic nerve preparation has been described previously (Kumamoto et al., 2011; Matsushita et al., 2013; Ohtsubo et al., 2015). 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).

2.3. Recordings of compound action potentials from frog sciatic nerve fibers

As performed previously (Kumamoto et al., 2011; Matsushita et al., 2013; Ohtsubo et al., 2015), 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 USB flash memory (ELECOM, Osaka, Japan) with a Data logger (mini LOGGER GL900, GRAPHTEC, Yokohama, Japan) 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 (Kumamoto et al., 2011; Matsushita et al., 2013; Ohtsubo et al., 2015). 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 (Kumamoto et al., 2011; Matsushita et al., 2013; Ohtsubo et al., 2015), we analyzed the peak amplitude of the maximal CAP. 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).

2.4. Data analysis

Concentration-dependent curves for the reduction of the peak amplitude of CAP in the sciatic nerve soaked with a drug were analyzed using the following Hill equation:

$$\text{CAP amplitude (\% of control)} = 100/(1 + ([\text{Drugs}]/IC_{50})^{n_H}),$$

where [Drug] is drug concentration, IC_{50} is half-maximal inhibitory concentration and n_H is the Hill coefficient. Data were indicated as mean ± SEM 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.

2.5. Drugs

Drugs used were daikenchuto (TJ-100), rikkosan (TJ-110), kikyoto (TJ-138), rikkunshito (TJ-43), shakuyakukanzoto (TJ-68), kakkonto (TJ-1), ginseng radix, Japanese pepper and processed ginger; they were kindly given by Tsumura & Co. (Tokyo, Japan).