Available online at www.sciencedirect.com
SCIENCE DIRECT®
Life Sciences 78 (2005) 187 – 194
Life Sciences
www.elsevier.com/locate/lifescie

Inhibitory effect of mitragynine, an analgesic alkaloid from Thai herbal medicine, on neurogenic contraction of the vas deferens

Kenjiro Matsumoto [a], Leonardo T. Yamamoto [b], Kazuo Watanabe [b], Shingo Yano [c], Jie Shan [d], Peter K.T. Pang [d], Dhavadee Ponglux [e], Hiromitsu Takayama [f], Syunji Horie [a,b,*]

[a] *Department of Pharmacology, Faculty of Pharmaceutical Sciences, Josai International University, 1 Gumyo, Togane, Chiba 283-8555, Japan*
[b] *Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan*
[c] *Department of Molecular Pharmacology and Pharmacotherapeutics, Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan*
[d] *Department of Physiology, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7*
[e] *Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*
[f] *Department of Molecular Structure and Biological Function, Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*

Received 28 January 2005; accepted 16 April 2005

Abstract

The effect of an indole-alkaloid mitragynine isolated from the Thai medicinal herb kratom (*Mitragyna speciosa*) on neurogenic contraction of smooth muscle was studied in guinea-pig vas deferens. Mitragynine inhibited the contraction of the vas deferens produced by electrical transmural stimulation. On the other hand, mitragynine failed to affect the responses to norepinephrine and ATP. Mitragynine did not reduce KCl-induced contraction in the presence of tetrodotoxin, prazosin and α,β-methylene ATP. Mitragynine inhibited nicotine- or tyramine-induced contraction. By using the patch-clamp technique, mitragynine was found to block T- and L-type Ca^{2+} channel currents in N1E-115 neuroblastoma cells. In the Ca^{2+} measurement by a fluorescent dye method, mitragynine reduced KCl-induced Ca^{2+} influx in neuroblastoma cells. The present results suggest that mitragynine inhibits the vas deferens contraction elicited by nerve stimulation, probably through its blockade of neuronal Ca^{2+} channels.

Keywords: Mitragynine; Morphine; Vas deferens; Ca^{2+} channel; *Mitragyna speciosa*

Introduction

The leaf of *Mitragyna speciosa* (*kratom* in Thai) has been used in Thailand for its opium-like effect (Burkill, 1935) and its coca-like stimulant ability to combat fatigue and enhance tolerance to hard work under a scoring sun (Grewal, 1932; Suwanlert, 1975). There are descriptions of its use as a tonic, cure for fever, treatment for diarrhea and substitute for morphine in treating addicts (Suwanlert, 1975; Jansen and Prast, 1988). From the leaves of *M. speciosa*, mitragynine (Fig. 1) was obtained as the major constituent (66.2% base on the crude base extract) together with its analogues, paynantheine (8.6%), speciogynine (6.6%), 7-hydroxymitragynine (2.0%) and speciociliatine (0.8%) (Takayama, 2004). We studied the pharmacological effects of mitragynine on guinea-pig ileum, radioligand binding assay and the tail-flick test in mice, and found that mitragynine acts on opioid receptors and possesses analgesic effects (Watanabe et al., 1997; Yamamoto et al., 1999; Takayama et al., 2002). Mitragynine-related compounds also express interesting opioid activities; mitragynine pseudoindoxyl and 7-hydroxymitragynine, especially, were found to exhibit potent antinociceptive activity in mice (Takayama et al., 2002; Takayama, 2004; Matsumoto et al., 2004). In addition, some pharmacological studies have revealed that mitragynine has an antinociceptive action through the supraspinal opioid receptors (Matsumoto et al., 1996a; Thongpradichote et al., 1998) and descending noradrenergic and serotonergic systems (Matsumoto et al., 1996b).

* Corresponding author. Department of Pharmacology, Faculty of Pharmaceutical Sciences, Josai International University, 1 Gumyo, Togane, Chiba 283-8555, Japan. Tel.: +81 475 534586; fax: +81 475 538811.
E-mail address: shorie@jiu.ac.jp (S. Horie).

0024-3205/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2005.04.042

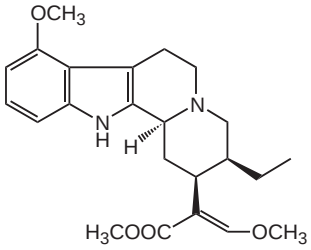

Fig. 1. Chemical structure of mitragynine.

We have so far studied pharmacological properties of alkaloids isolated from the Chinese herbal medicine Uncariae Ramulus et Uncus (*Uncaria rhynchophylla*) that is used clinically for hypertension (Yano et al., 1991; Horie et al., 1992). We are interested in the similarity of the chemical structure of mitragynine to those of Uncaria alkaloids such as hirsutine, which inhibits the voltage-dependent Ca^{2+} channel in smooth muscle.

The knowledge of pharmacological properties of mitragynine is still limited. Thus, it is important for the scientific elucidation of the clinical use of kratom to study the effect of mitragynine in the in vitro assays. The present study was conducted to clarify the effects of mitragynine on neurogenic contraction in guinea-pig vas deferens and on the cytosolic Ca^{2+} level in cultured neuroblastoma cells.

Methods

Materials

The following drugs were used: α,β-methylene ATP, prazosin (Sigma, St. Louis, MO, USA), norepinephrine bitartarate, tyramine (Wako, Osaka, Japan), hexamethonium chloride, nicotine bitartarate (Tokyo Kasei, Japan), tetrodotoxin (Sankyo, Japan), morphine (Takeda Chemical Industries, Osaka, Japan), fura-2 acetoxymethyl ester (Molecular Probes, Eugene, OR, USA). Mitragynine was isolated from the extract of the leaves of *M. speciosa* as described previously (Ponglux et al., 1994), and total synthesis of mitragynine was also established (Takayama et al., 1995). The purity (>99%) of mitragynine was checked by HPLC and ^{1}H NMR (500 MHz) analysis (Takayama et al., 2002). Mitragynine was first dissolved in 100% dimethylsulfoxide to yield a 1 mM solution, and then subsequently diluted with distilled water. Other drugs were dissolved in distilled water.

Animals

All experiments were performed in compliance with the "Guiding Principles for the Care and Use of Laboratory Animals" approved by the Japanese Pharmacological Society and the guidelines approved by the Ethical Committee on Animal Care and Animal Experimentation of the Graduate School of Pharmaceutical Sciences, Chiba University. The number of animals used was kept to the minimum necessary for a meaningful interpretation of the data and the animal discomfort was kept to the minimum. Male albino guinea pigs (300–400 g, Takasugi Lab. Animals, Japan) were killed by CO_2 inhalation.

Vas deferens preparation

The epididymal portion of the vas deferens was dissected from guinea pigs, and placed in Krebs–Henseleit solution of the following composition (mM): NaCl, 112; KCl, 5.9; $CaCl_2$, 2; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25 and glucose, 11; EDTA, 0.03 (pH 7.4). A segment of the vas deferens (10–15 mm long) was placed in a 5-ml organ bath containing the nutrient solution and suspended from an isometric transducer (Toyo Boldwin, T-7-8-240, Orientec, Japan) under a load of 0.5 g. Contractions of the preparation were amplified by DC strain amplifier (San-ei 6M92) and recorded on a pen-writing recorder (Hitachi, Mod 056). The nutrient solution was maintained at 37 °C and saturated with 95% O_2 and 5% CO_2. Contraction was expressed as a percentage of the maximum response to the corresponding stimulation (nicotine 1 mM, tyramine 100 μM). In the experiments of nicotine, desensitization of vas deferens contraction was observed when nicotine was added into the organ bath repeatedly. Therefore, bilateral vasa deferentia were isolated from one animal. Only in the experiments of nicotine, one segment was used for control, and another one was used for the drug treatment. The contraction in the treatment group was evaluated as the percentage of the control contraction in the contralateral vas deferens.

Electrical transmural stimulation

The preparation was transmurally stimulated by a needle-ring platinum electrode. The needle electrode was vertically positioned and inserted in the lower end, and the ring electrode was positioned at the upper end of the preparation. Square-wave pulses (10 Hz, 0.3 ms duration, 50 V) were delivered to the guinea-pig vas deferens every 1 min for 10 s. During electrical stimulation, mitragynine was cumulatively administered to the bath fluid.

Neuroblastoma cell culture

Mouse neuroblastoma cells (N1E-115) were cultured in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY, USA) containing 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. After mechanical agitation, 3×10^4 cells were removed to 35 mm tissue culture dishes containing 4 ml of the medium. After cell attachment, the dish was mounted on the stage of an inverted phase-contrast microscope (Nikon, Tokyo, Japan). These cells expressed predominately T channel currents (Pang et al., 1990). In experiments where L channels were specifically sought, the cells were grown and maintained at confluence for 3–4 weeks under the same culture conditions with the addition of 2% dimethylsulfoxide. These cells expressed predominately long-lasting (L)-channel currents (Pang et al., 1990). The transient (T)-channel component was