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Short communication

Effect of mitragynine, derived from Thai folk medicine, on gastric acid secretion through opioid receptor in anesthetized rats

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Abstract

Mitragynine, an indole alkaloid from Thai folk medicine *Mitragyna speciosa*, exerts agonistic effects on opioid receptors. Gastric acid secretion is proposed to be regulated by opioid receptors in the central nervous system (CNS). Previously, we reported the dual roles (inhibition via μ-opioid receptors and stimulation via κ-opioid receptors) of the opioid system in the central control of gastric acid secretion. We investigated whether mitragynine affects gastric acid secretion via opioid receptors in the CNS. Injection of mitragynine (30 μg) alone into the lateral cerebroventricle did not have a significant effect on basal gastric acid secretion in the perfused stomach of anesthetized rats. Injection of mitragynine (3–30 μg) into the fourth cerebroventricle, like morphine, inhibited 2-deoxy-D-glucose-stimulated gastric acid secretion. The inhibitory effect of mitragynine (30 μg) was reversed by naloxone (100 μg). These results suggest that mitragynine has a morphine-like action on gastric acid secretion in the CNS.

Keywords: Opioid receptor; Central nervous system; Gastric acid secretion; Mitragynine

1. Introduction

Opioid receptors are clearly divided into delta (δ)-, kappa (κ)- and mu (μ)-opioid receptors. Many studies suggest that the opioid system in the central nervous system (CNS) regulates gastric acid secretion, and that centrally injected opioids inhibit gastric acid secretion through a vagal mechanism (Magee, 1975; Taché, 1987; Watanabe et al., 1987; Geoghegan and Pappas, 1997). However, we recently reported on a dual role of the opioid system in the CNS regarding gastric acid secretion in anesthetized rats: μ-opioid receptor agonists do not show any effects on basal acid secretion but inhibit the 2-deoxy-D-glucose-stimulated gastric acid secretion. On the other hand, κ-opioid receptor agonists stimulate gastric acid secretion alone, and a δ-opioid receptor agonist does not have any effects (Ishihara et al., 2001a,b).

The leaves of *Mitragyna speciosa* are known as an opium substitute in traditional use. Despite their use as analgesics, it has been considered that a main component of *M. speciosa*, mitragynine, does not act on opioid receptors (Jansen and Prast, 1988). Recently, we found that mitragynine has agonistic characteristics on opioid receptors in in vitro assays, the guinea-pig ileum and the mouse vas deferens (Watanabe et al., 1997; Yamamoto et al., 1999). In addition, it was shown that mitragynine shows an antinociceptive action through central μ- and δ-opioid receptors in mice (Matsumoto et al., 1996; Thongpradichote et al., 1998). Interestingly, its chemical structure is unrelated to that of the other analgesics. Side effects such as anorexia and weight loss, which may be closely related to acid secretion, are known in persons who take *M. speciosa* (Jansen and Prast, 1988). It has not been found whether mitragynine affects gastric acid secretion via opioid receptors in the CNS.

In the present study, we investigated the effect of centrally injected mitragynine on basal or 2-deoxy-D-glucose-stimulated gastric acid secretion in the continuously perfused stomach of anesthetized rats.

2. Materials and methods

Male Wistar rats (200–300 g, Takasugi Exp. Animals, Japan) were housed under conditions with controlled temper-

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ature (24 ± 2 °C) and a 12-h light/dark cycle (lighting on at 0700 h), for at least 1 week before the start of the experiment. Food and tap water were available ad libitum. The animals were fasted for 18 h before the experiment, but free access to water was maintained.

The rats were anesthetized with urethane (1.35 g/kg, i.p.; Tokyo Kasei, Japan), and were placed in a stereotaxic instrument (SR-6, Narishige, Japan) in the mouth down position (−3.3 mm). A stainless steel cannula for microinjection was positioned unilaterally (right side) through a small hole drilled in the skull, and was secured. Stereotaxic coordinates were taken from the atlas of Paxinos and Watson (1982): lateral cerebroventricle: −1.0 mm anteroposterior and 1.3 mm lateral from the bregma, 3.8 mm dorsoventral from horizontal skull surface; fourth cerebroventricle: −11.5 mm anteroposterior and 0.0 mm lateral from the bregma, 7.5 mm dorsoventral from horizontal skull surface. The femoral vein was cannulated for injection of 2-deoxy-D-glucose.

After implantation of the cannula, the animals were used for the measurement of gastric acid secretion. Gastric acid secretion was determined by the gastric perfusion method as described previously (Watanabe et al., 1987). The esophagus was ligated at the cervical level, and a cannula was inserted into the trachea. After laparotomy, the pylorus was ligated and a dual cannula was inserted through a small incision into the forestomach. The lumen was continuously perfused with saline (1 ml/min, pH 5.0, 37 °C). The intragastric pressure was maintained at 5 cm H_2O. The perfusate was continuously titrated with 0.02 N NaOH to pH 5.0 using an automatic titrator (ABT-101, Toa Electronics, Japan).

Morphine hydrochloride was obtained from Takeda Chemical Industries (Japan). Naloxone hydrochloride was obtained from Sigma (USA). 2-Deoxy-D-glucose was obtained from Nacalai Tesque (Japan). Mitragynine was isolated from extract of the leaves of *M. speciosa* as described previously (Ponglux et al., 1994).

Mitragynine was dissolved in a minimum of 0.1 N HCl and saline, the final pH was about 4.0. The other compounds were dissolved in saline. The volume for i.v. injection was 1 ml/kg, and that for the lateral cerebroventricular or the fourth cerebroventricular was 5 μl.

Acid output is expressed as ΔμEq H^+/10 min of the basal values measured 10 min prior to injection of 2-deoxy-D-glucose. The grouped data for gastric acid secretion were statistically analyzed using one-way analysis of variance followed by the Bonferroni multiple comparison test.

3. Results

We previously reported that the stimulatory effect of κ-opioid receptor agonists was more potent on injection into the lateral cerebroventricle than into the fourth cerebroventricle, but that the inhibitory effect of the μ-opioid receptor agonist, morphine, was more potent after injection into the fourth cerebroventricle than into the lateral cerebroventricle (Ishihara et al., 2001a). First, we investigated the effects of mitragynine alone by injection into the lateral cerebroventricle. Mitragynine (30 μg) did not have a significant effect on basal gastric acid secretion (vehicle: 10.3 ± 9.5 μEq H^+/120 min, $n=4$; mitragynine: 1.3 ± 4.6 μEq H^+/120 min, $n=3$). Next we investigated the effect of mitragynine injected into the fourth cerebroventricle on 2-deoxy-D-glucose-stimulated gastric acid secretion. Mitragynine (3–30 μg) dose dependently inhibited the 2-deoxy-D-glucose-stimulated gastric acid secretion (Fig. 1). The total acid output for 3 h with 2-deoxy-D-glucose was significantly inhibited by mitragynine (10 and 30 μg) or morphine (1–10 μg), as shown in Fig. 1B. Naloxone injected into the fourth cerebroventricle dose dependently reversed the inhibition induced by mitragynine (30 μg) (Fig. 2). Naloxone at 100 μg significantly reversed the inhibition induced by mitragynine of the total acid output for 3 h (Fig. 2B).

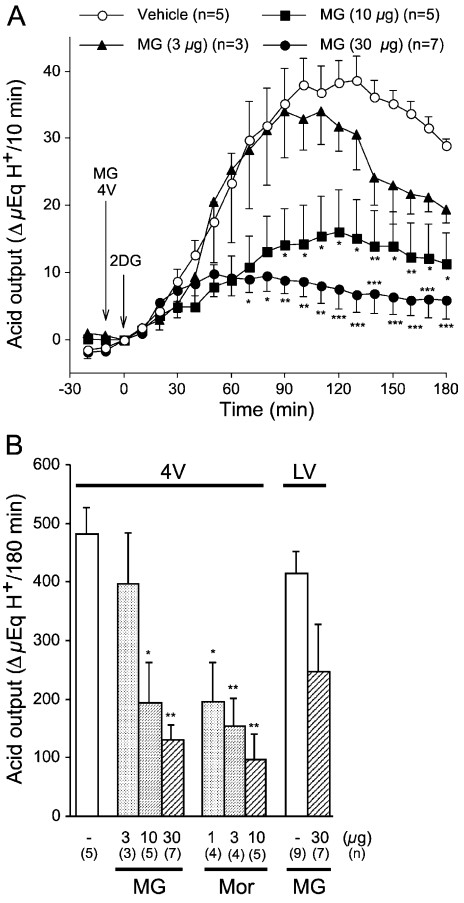

Fig. 1. Inhibitory effects of mitragynine (MG) or morphine (Mor) injected into the fourth cerebroventricle (4V) and the lateral cerebroventricle (LV) on gastric acid secretion induced by 2-deoxy-D-glucose (200 mg/kg, i.v.) in urethane-anesthetized rats. Vehicle, mitragynine or morphine was injected at 10 min before the injection of 2-deoxy-D-glucose. (A) Time course of the acid output measured every 10 min. (B) The total acid output for 180 min. All values represent means ± S.E.M. $^*P < 0.05$ $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the vehicle group.

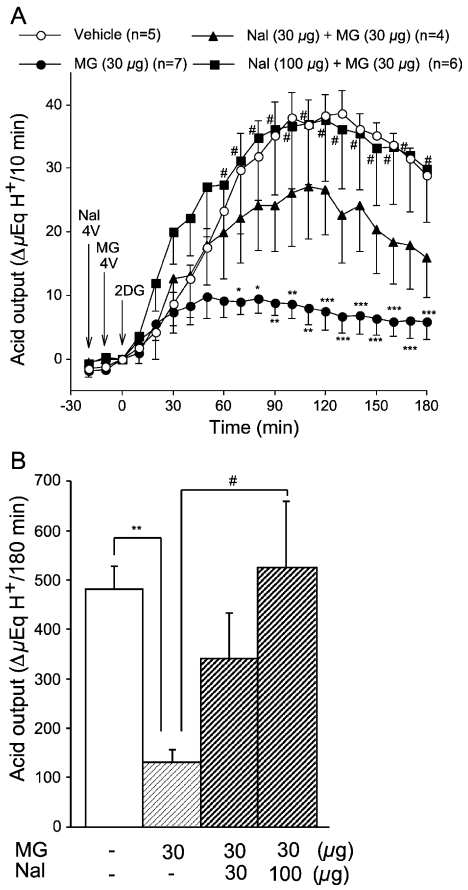

Fig. 2. Antagonistic effect of naloxone (Nal) injected into the fourth cerebroventricle (4V) against the antisecretory effect of mitragynine (MG, 30 μg) on gastric acid secretion induced by 2-deoxy-D-glucose (200 mg/kg, i.v.) in urethane-anesthetized rats. Nal was injected at 10 min before the injection of mitragynine. (A) Time course of the acid output measured every 10 min. (B) The total acid output for 180 min. All values represent means ± S.E.M. $^{*}P < 0.05$ $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with vehicle. $\#P < 0.05$ compared with MG.

For further investigation of the effective site in the CNS, mitragynine was injected into the lateral cerebroventricle. Mitragynine (30 μg) slightly inhibited the 2-deoxy-D-glucose-stimulated gastric acid secretion, but the effect was not significant (Fig. 1B).

4. Discussion

In the present study, we examined the pharmacological effects of mitragynine on gastric acid secretion in anesthetized rats. Injection of mitragynine into the fourth cerebroventricle inhibited the 2-deoxy-D-glucose-stimulated gastric acid secretion, and the inhibitory effect was reversed by naloxone (Figs. 1 and 2). These results suggest that mitragynine exerts its inhibitory effect on gastric acid secretion through opioid receptors. Mitragynine (30 μg) injected into the lateral cerebroventricle did not induce an increase in basal acid secretion. This indicates that mitragynine does not have a κ-opioid receptor-selective agonistic character, because we reported that a κ-opioid receptor agonists stimulate basal gastric acid secretion (Ishihara et al., 2001a).

It has been established that 2-deoxy-D-glucose is a potent vagally mediated stimulant of gastric acid secretion (Hirschowitz and Sachs, 1965). In addition, we previously discussed that the different expression of mRNA and protein of μ-opioid receptors in the CNS might cause the difference in efficacy between injection into the lateral and into the fourth cerebroventricle (Kotz et al., 1997; Ishihara et al., 2001a). In the present study, the lateral cerebroventriculer injection of mitragynine (30 μg) was less effective than the fourth cerebroventriculer injection (Fig. 1B), and its potency order is in agreement with that of morphine. These results suggest that mitragynine acts on the CNS.

Mitragynine showed significant inhibition at more than 10 μg (25.1 nmol) in this study, while morphine showed a significant inhibition at more than 1 μg (3.5 nmol) (Fig. 1B). Our previous study using the isolated guinea-pig ileum showed that the pD_2 value (the negative logarithm of the IC_{50}) of mitragynine is 6.91 ± 0.04, and that of morphine is 7.68 ± 0.11 (Watanabe et al., 1997). From these results, the effective dose ratios of morphine/mitragynine are 7.2 for gastric acid inhibition and 5.9 for isolated guinea-pig ileum. These two ratios are nearly equal.

It is speculated that the mechanism of side effects such as anorexia and weight loss induced by mitragynine is related to that of its inhibitory effect on gastric acid secretion. It is reported that opioid receptor agonists such as morphine generally increase food intake via the rewarding system of ingestion (Mercer and Holder, 1997). On the other hand, it is also reported that morphine or enkephalin reduces food intake via inhibition of neurons in the lateral hypothalamus (King et al., 1979; Sikdar and Oomura, 1985), and that eating disorders such as anorexia are associated with an addictive disorder (Mercer and Holder, 1997). It is considered that anorexia or weight loss elicited by ingestion of *M. speciosa* is associated with a direct inhibition of neurons in the lateral hypothalamus or with an addictive disorder.

In summary, we showed that mitragynine, like morphine, inhibits the 2-deoxy-D-glucose-stimulated gastric acid secretion in urethane-anesthetized rats through the stimulation of opioid receptors.

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