ELSEVIER

European Journal of Pharmacology 337 (1997) 223–226

ejp

Short communication

Antinociceptive effects of (+)-matrine in mice

Junzo Kamei [a,*], Ping Xiao [b], Masahiro Ohsawa [a], Hajime Kubo [b], Kimio Higashiyama [b], Hiroshi Takahashi [b], Jiashi Li [c], Hiroshi Nagase [d], Shigeru Ohmiya [b]

[a] *Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-chome, Shinagawa-ku, Tokyo 142, Japan*

[b] *Department of Drug Manufacturing Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-chome, Shinagawa-ku, Tokyo 142, Japan*

[c] *Department of Pharmacognosy, Beijing University of Traditional Chinese Medicine, Beijing 100029, China*

[d] *Basic Research Laboratories, Toray Industries Inc., Kamakura 248, Japan*

Received 22 May 1997; revised 12 August 1997; accepted 15 August 1997

Abstract

The antinociceptive potency of matridin-15-one ((+)-matrine) was examined using the acetic acid-induced abdominal contraction test and the tail-flick test in mice. (+)-Matrine, at doses of 1 to 10 mg/kg, s.c., produced a marked and dose-dependent inhibition of the number of acetic acid-induced abdominal contractions in mice. The antinociceptive effect of (+)-matrine in the acetic acid-induced abdominal contraction test in mice was identical to that of pentazocine. Indeed, there was no significant difference in the ED_{50} (mg/kg with 95% confidence limits) values for the inhibition of acetic acid-induced abdominal contractions between (+)-matrine (4.7 (4.1–5.3)) and pentazocine (3.3 (2.2–5.0)). Furthermore, in the tail-flick assay, (+)-matrine at doses of 10 and 30 mg/kg, s.c., again produced a dose-dependent antinociceptive effect. When nor-binaltorphimine (20 mg/kg, s.c.), a selective κ-opioid receptor antagonist, was administered 3 h before treatment with (+)-matrine, the antinociceptive effect of (+)-matrine was markedly antagonized. Furthermore, the antinociceptive effect of (+)-matrine was partially antagonized by pretreatment with β-funaltrexamine, a selective μ-opioid receptor antagonist. Naltrindole, a selective δ-opioid receptor antagonist, had no effect on the antinociceptive effect of (+)-matrine. In conclusion, (+)-matrine produced an antinociceptive effect mainly through the activation of κ-opioid receptors and partially through μ-opioid receptors. © 1997 Elsevier Science B.V.

Keywords: Matrine; Antinociception; κ-Opioid receptor; Pentazocine; Nor-binaltorphimine; (Mouse)

1. 1. Introduction

(+)-Matrine (matridin-15-one) is a typical lupine alkaloid along with lupinine, sparteine and cytisine, and has an absolute structure of 5*S*, 6*S*, 7*R*, 11*R* (Okuda et al., 1966) (Fig. 1). This alkaloid occurs in many leguminous plants, especially in the genus *Sophora* and is one of the main basic constituents in both *Sophora flavescens* and *S. tonkinensis* (Ohmiya et al., 1995; Xiao et al., 1996). Dry roots of these plants have been used as important Chinese drugs 'Ku-shen' and 'Shan-dou-gen', respectively, for the treatment of fever, peptic ulcer, eczema, hemorrhoids, asthma, tumors, bacillary dysentery, etc., and as analgesics. The biological activities of (+)-matrine are related to the applications of Ku-shen and Shan-dou-gen. It has been reported that (+)-matrine has pharmacological effects, e.g., antipyretic, antiulcer, non-steroidal anti-inflammatory and antitumor activity (Kojima et al., 1970; Yamazaki et al., 1984; Tan and Zhang, 1985; Cho et al., 1986). However, the antinociceptive effect of (+)-matrine has not yet been examined.

Thus, the first aim of the present study was to investigate the antinociceptive effect of (+)-matrine in mice. An additional aim was to investigate the influence of β-funaltrexamine, a selective μ-opioid receptor antagonist, naltrindole, a selective δ-opioid receptor antagonist and nor-binartorphimine, a selective κ-opioid receptor antagonist, on (+)-matrine-induced antinociception to determine

* Corresponding author. Tel.: (81-3) 5498-5030; Fax: (81-3) 5498-5029; e-mail: kamei@hoshi.ac.jp

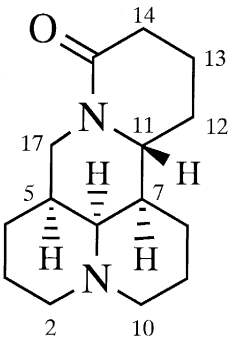

Fig. 1. Chemical structure of (+)-matrine.

the role of the opioid receptor types in the antinociceptive effect of (+)-matrine.

2. Materials and methods

2.1. Animals

Male ICR mice (Tokyo Laboratory Animals Science, Tokyo, Japan), weighing about 30 g (6 weeks old) were used. They had free access to food and water in an animal room that was maintained at 24 ± 1°C with a 12 h light–dark cycle. The studies were carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals as adopted by the committee on care and use of laboratory animals of Hoshi University which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Isolation of (+)-matrine

Dry plant material was cut into small chips and extracted with 75% MeOH several times at room temperature. The aqueous concentrate was acidified with 10% HCl to pH 3 and the resulting precipitate was filtered off. The filtrate was extracted three times with CH_2Cl_2. The aqueous layer was made strongly alkaline with K_2CO_3 and extracted with CH_2Cl_2 three times. The CH_2Cl_2 extracts were combined, dried over K_2CO_3 and concentrated in vacuo to give crude base. The crude alkaloid mixture was separated by silica gel column chromatography and then purified by aluminum oxide column chromatography or preparative HPLC to give (+)-matrine (Xiao et al., 1996).

2.3. Antinociceptive assay

The antinociceptive effect was evaluated using the acetic acid-induced abdominal contraction test. Each mouse was injected i.p. with 0.7% acetic acid in a volume of 10 ml/kg, 30 min after administration of the test drug. After a 10 min delay, the animals were observed for an additional 10 min, during which the abdominal contractions were counted. The number of abdominal contractions in each test period was normalized to the mean number shown by the control group. Percent antinociception was expressed as: 100 × (mean control responses − test responses)/(mean control responses).

In a separate series of experiments, the antinociceptive effect was evaluated by recording the latency in the tail-flick assay using radiant heat as a stimulus. The intensity of the thermal stimulus was adjusted so that the animal flicked its tail in 3–4 s. A cut-off latency of 15 s was used to prevent injury to the tail. Animals which did not respond within 15 s were removed and assigned a score of 15 s. Percent antinociception was calculated for each animal using the formula: 100 × (post-drug latency − pre-drug latency)/(15 − pre-drug latency).

2.4. Drugs

β-Funaltrexamine, naltrindole and nor-binaltorphimine were synthesized by Dr. H. Nagase (Toray Industries, Kamakura, Japan). Pentazocine (Sosegon®) was purchased from Yanamouchi Pharmaceutical (Tokyo, Japan). Drugs were dissolved or diluted in saline. β-Funaltrexamine (20 mg/kg, s.c.) was injected 24 h before testing. Naltrindole (1 mg/kg, s.c.) was injected 10 min before injection of (+)-matrine. Nor-binaltorphimine (20 mg/kg, s.c.) was injected 3 h before (+)-matrine injection. The dose and schedule for each opioid antagonist in this study were determined as described previously (Kamei et al., 1995).

2.5. Statistical analysis

The data are expressed as the means ± S.E. The statistical significance of differences was assessed with the Newman–Keuls test for the comparison of percent antinociception and with the Mann–Whitney *U*-test for comparison of the number of abdominal contraction responses. The ED_{50} values and their 95% confidence intervals for the antinociceptive effect were determined using linear regression techniques. A level of probability of 0.05 or less was accepted as significant.

3. Results

3.1. Effect of (+)-matrine on acetic acid-induced abdominal contraction

Intraperitoneal injection of 0.7% acetic acid induced abdominal contractions in the mice (39.6 ± 3.3/10 min, $n = 11$). Subcutaneous administration of (+)-matrine, at doses of 3 to 30 mg/kg, resulted in a marked and dose-dependent reduction in the number of acetic acid-induced abdominal contractions (Fig. 2A). The antinociceptive potency of (+)-matrine was identical to that of pentazocine.

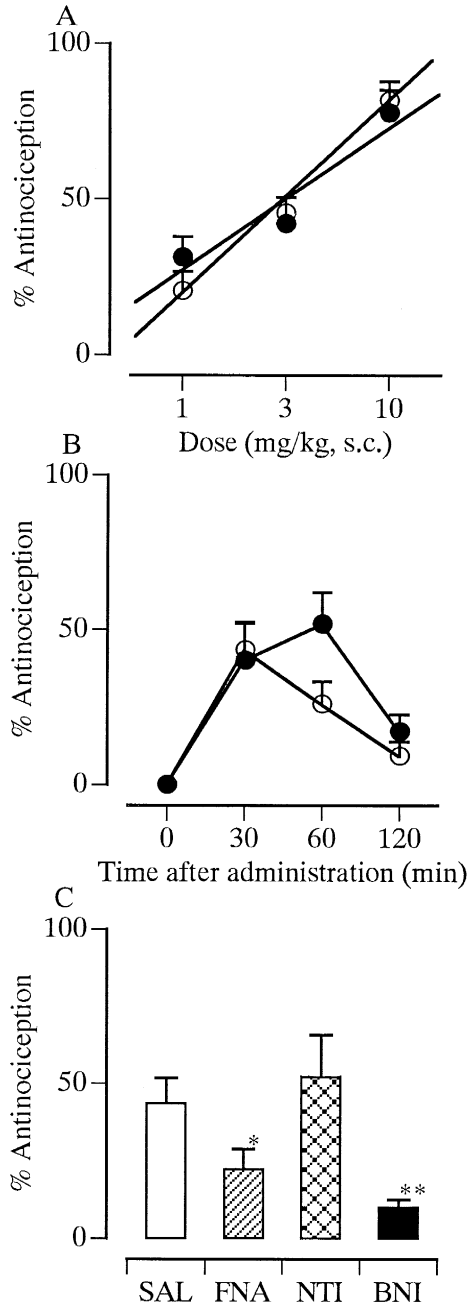

Fig. 2. (A) Dose–response lines for the antinociceptive effect of s.c. administration of (+)-matrine (open circle) and pentazocine (closed circle) in the acetic acid-induced abdominal constriction assay in mice. Each mouse received an i.p. injection of 0.7% acetic acid 30 min after the administration of (+)-matrine or pantazocine. Each point represents the mean ± S.E. for 10 to 11 mice in each group. (B) Antinociceptive effects of (+)-matrine (open circle) and pentazocine (closed circle) in the tail-flick assay. The antinociceptive effects of (+)-matrine and pentazocine were measured in the tail-flick assay 30, 60, 90 and 120 min after injection. Each point represents the mean ± S.E. for 10 mice in each group. (C) Blockade of the antinociceptive effect of (+)-matrine by opioid antagonists in mice. β-Funaltrexamine (FNA, 20 mg/kg) was injected s.c. 24 h before the test. Nor-binaltorphimine (BNI, 20 mg/kg, s.c.) was injected 3 h before administration of (+)-matrine. Naltrindole (NTI, 1 mg/kg) was injected s.c. 10 min before administration of (+)-matrine. Each column represents the mean ± S.E. for 10 mice in each group. $^{**}P < 0.01$, $^{*}P < 0.05$ versus the saline-pretreated group.

There was no difference in ED_{50} values (mg/kg with 95% confidence limits) between (+)-matrine (4.7 (4.1–5.3)) and pentazocine (3.3 (2.2–5.0)).

3.2. Effects of selective opioid-receptor antagonists on the antinociceptive effect of (+)-matrine in the tail-flick assay

Fig. 2B shows the time-courses of the antinociception produced by (+)-matrine and pentazocine in the tail-flick assay. (+)-Matrine at a dose of 30 mg/kg, s.c., produced marked antinociception. This effect reached its peak 30 min after administration and then gradually decreased. Pentazocine at a dose of 30 mg/kg, s.c., also produced a marked antinociception. The antinociceptive effect of pentazocine reached its peak 60 min after administration and then gradually decreased. However, the peak effect of the antinociception induced by (+)-matrine was not significantly different from that produced by pentazocine (Fig. 2B).

The effects of β-funaltrexamine, a selective μ-opioid receptor antagonist, of naltrindole, a selective δ-opioid receptor antagonist and of nor-binaltorphimine, a selective κ-opioid receptor antagonist, on the antinociceptive effect of (+)-matrine in the tail-flick assay are summarized in Fig. 2C. When nor-binaltorphimine was administered 3 h before the administration of (+)-matrine, the antinociceptive effect of (+)-matrine was markedly antagonized. Furthermore, the antinociceptive effect of (+)-matrine was partially antagonized by pretreatment with β-funaltrexamine. However, naltrindole had no significant effect on the antinociceptive effect of (+)-matrine.

4. Discussion

The present study demonstrated that s.c. administration of (+)-matrine to mice reduced the number of acetic acid-induced abdominal contractions and the tail-flick latency in a dose-dependent manner. The antinociceptive potency of (+)-matrine was similar to that of pentazocine in the acetic acid-induced abdominal contraction test. Although the time course of the antinociceptive effect differed, there was no significant difference in the peak effect of the antinociception between (+)-matrine (30 mg/kg, s.c.) and pentazocine in the tail-flick test. Thus, the present study clearly demonstrated that (+)-matrine is an effective antinociceptive agent that is as active as pentazocine.

We were surprised to find that (+)-matrine-induced antinociception was abolished by s.c. pretreatment with nor-binartorphimine, a selective κ-opioid receptor antagonist. Furthermore, β-funaltrexamine, a selective μ-opioid receptor antagonist, partially antagonized the antinociceptive effect of (+)-matrine. However, naltrindole, a selective δ-opioid receptor antagonist, had no effect on the antinociceptive effect of (+)-matrine in the tail-flick test. These results indicating that the antinociceptive effect of

(+)-matrine results from the activation of both κ-opioid receptors and μ-opioid receptors. Since U-50,488 (trans-(±)-3,4-dichloro-*N*-methyl-*N*-(2-(1-pyrrolidinyl)cyclohexyl)-benzeneacetamide), a selective κ-opioid receptor agonist, produced a naloxone-sensitive sedative (Von Voigtlander et al., 1982), it seems likely that sedative activity is characteristic of κ-opioid agonists. Although the data are not shown, (+)-matrine has a sedative action, in contrast to the well-known stimulant effects of morphine in mice. Furthermore, the antinociceptive effect of (+)-matrine in mice was as potent as that of U-50,488 (Von Voigtlander et al., 1982). These results further support the notion that the effects of (+)-matrine are mediated mainly by κ-opioid receptors.

Several opioid agonists, such as morphine, codeine and tebaine, originated from plant materials and these opioid alkaloids do not show an affinity selective for the various opioid receptor types. These opioid alkaloids produce an antinociceptive effect mainly through the activation of μ-opioid receptors (Reisine and Pasternak, 1996). In our study, the antinociceptive effect of (+)-matrine was partially but significantly antagonized by a μ-opioid receptor antagonist. It seems likely that alkaloids which produce opioid receptor-mediated antinociception may have a common affinity for μ-opioid receptors. To the best of our knowledge, (+)-matrine is the first alkaloid to show an antinociceptive action through the activation of κ-opioid receptors. On the other hand, it has been reported that (+)-matrine has several stereoisomers (Ueno et al., 1975) and the biochemical and pharmacological properties of these stereoisomers have not been clearly demonstrated. The affinity of (+)-matrine for κ-opioid receptors was not clearly proved. Thus, additional pharmacological and biochemical studies of these stereoisomers may lead to the identification of more κ-opioid receptor-selective alkaloids. Such studies are currently underway.

In conclusion, the findings of the present study clearly indicate that (+)-matrine produced antinociception mainly through the activation of κ-opioid receptors and partially through μ-opioid receptors.

References

Cho, C., Chuang, C., Chen, C., 1986. Study of the antipyretic activity of matrine. A lupine alkaloid isolated from *Sophora subprostrata*. Planta Med. 52, 343–345.

Kamei, J., Suzuki, T., Misawa, M., Nagase, H., Kasuya, Y., 1995. Antinociceptive effect of dihydroetorphine in diabetic mice. Eur. J. Pharmacol. 275, 109–113.

Kojima, R., Fukushima, S., Ueno, A., Saiki, Y., 1970. Antitumor activity of Leguminosae plants constituents. I. Antitumor activity of constituents of *Sophora subprostrata*. Chem. Pharm. Bull. 18, 2555–2563.

Okuda, S., Yoshimoto, M., Tsuda, K., Utzugi, N., 1966. Über die absolute Konfiguration des Matrins. Chem. Pharm. Bull. 14, 314–318.

Ohmiya, S., Saito, K., Murakoshi, I., 1995. Lupin alkaloids. In: Cordell, G.A. (Ed.), The Alkaloids, vol. 47. Academic Press, New York, NY, pp. 1–114.

Reisine, T., Pasternak, G., 1996. Opioid analgesics and antagonists. In: Hardman, J.G., Goodman Gilman, A., Limbird, L.E. (Eds.), The Pharmacological Basis of Therapeutics, 9th ed. McGraw-Hill, New York, NY, pp. 521–555.

Tan, H., Zhang, B., 1985. Antiinflammatory effects of matrine. Chin. J. Integrat. Trad. West. Med. 5, 108–110.

Ueno, A., Morinaga, K., Fukushima, S., Iitaka, Y., Koiso, Y., Okuda, S., 1975. Studies on lupin alkaloids. VI. Isolation and structure of (+)-isomatrine. Chem. Pharm. Bull. 23, 2560–2566.

Von Voigtlander, P.F., Lahti, R.A., Ludens, J.H., 1982. U-50,488: A selective and structurally novel non-mu (kappa) opioid agonist. J. Pharmacol. Exp. Ther. 224, 7–12.

Xiao, P., Li, J., Kubo, H., Saito, K., Murakoshi, I., Ohmiya, S., 1996. (-)14b-Hydroxymatrine, A new lupine alkaloid from the roots of *S. tonkinensis*. Chem. Pharm. Bull. 44, 1951–1953.

Yamazaki, M., Arai, A., Suzuki, S., Takeuchi, T., 1984. Protective effects of matrine and oxymatrine on stress ulcer in relation to their effects on the central nervous system. J. Pharm. Soc. Jpn. 104, 293–301.