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The involvement of central cholinergic system in (+)-matrine-induced antinociception in mice

Lin-Lin Yin, Xing-Zu Zhu*

Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, China

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Abstract

The antinociceptive effect of (+)-matrine was examined in mice by writhing, tail-pressure and hot-plate tests. (+)-Matrine (5, 10 and 20 mg/kg s.c.) produced antinociception in a dose-dependent manner. In hot-plate test, the antinociception produced by (+)-matrine (10 mg/kg s.c.) was attenuated by muscarinic receptor antagonists atropine (5 mg/kg i.p.) and pirenzepine (0.1 μg/mouse i.c.v.) and acetylcholine depletor hemicholinium-3 (HC-3) (1 μg/mouse i.c.v.), but not by opioid receptor antagonist naloxone (2 mg/kg i.p.), dopamine D_2 receptor agonist (−)-quinpirole (0.1 mg/kg i.p.) or catecholamine depletor reserpine (2.5 mg/kg i.p.). Radioligand binding assay demonstrated that (+)-matrine had no affinity for μ-, κ- or δ-opioid receptors in a wide concentration range (1×10^{-11}–1×10^{-3} M). The results suggest that (+)-matrine exerts its antinociceptive effect through multiple mechanism(s) such as increasing cholinergic activation in the CNS rather than acting on opioid receptors directly.

Keywords: (+)-Matrine; Antinociception; Central cholinergic system; Atropine; Opioid receptors; Binding assay

1. Introduction

The plant *Sophora alopecuroides* L. is a commonly used Chinese herbal drug. It possesses antipyretic, anti-inflammatory and analgesic effects. (+)-Matrine (Fig. 1) is one of the major active alkaloids isolated from the plant (Zhao, 1980). It was previously reported that (+)-matrine had antinociceptive effect and that it exerted its antinociceptive effect mainly through the activation of κ-opioid receptors and partially through μ-opioid receptors in mice (Kamei et al., 1997). Since the structure of the (+)-matrine differs from those of conventional κ-opioid receptor agonists, such as ethylketocyclazocine and U-50488 (Fig. 1), it is interesting to see whether (+)-matrine induces antinociception by interfering, in addition to opioid system, other physiological system such as cholinergic system (Costa and Murphy, 1986; Dussor et al., 2004), catecholamine system (Loomis et al., 1987) and dopamine system (Hernandez et al., 1986), all of which are involved in the transmission of nociceptive information.

In the present studies, the antinociceptive profiles of (+)-matrine were examined by use of writhing, tail-pressure and hot-plate tests in mice. In addition, the possible mechanism(s) underlying its antinociceptive action were studied.

2. Materials and methods

2.1. Animals

Male or female Kunming strain mice (18–20 g) were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences. All animals were housed in a 12/12 h light–dark cycle, temperature and humidity controlled rooms with food and water ad libitum. The study was performed in compliance with National Institutes of Health (NIH) guideline for the Care and Use of Laboratory

* Corresponding author. Tel./fax: +86 21 5080 6096.
E-mail address: xzzhu@mail.shcnc.ac.cn (X.-Z. Zhu).

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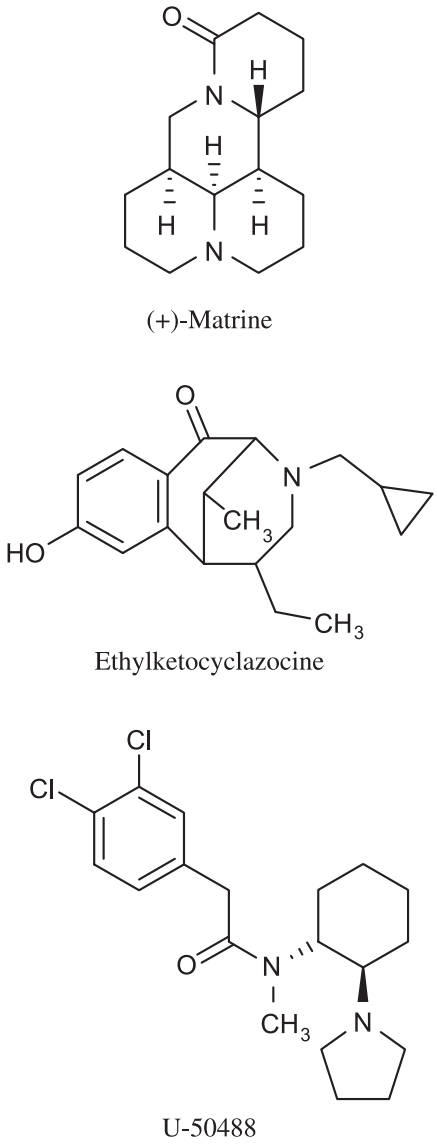

Fig. 1. Chemical structure of (+)-matrine, ethylketocyclazocine and U-50488.

Animals and was approved by Animal Care and Use Committee, Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

2.2. Drugs

(+)-Matrine was purchased from NingXia ZiJingHua Pharmacy (purity >98.3%). *Trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide (U-50,488H), naloxone hydrochloride, atropine sulfate, (−)-quinpirole hydrochloride, pirenzepine dihydrochloride and hemicholinium-3 hydrobromide (HC-3) were products of Sigma (St. Louis, MO, USA). Reserpine was purchased from Merck, Germany. D-Pen^2, D-Pen^5 enkephalin (DPDPE) and ohmefentanyl were gifts from Prof. Zhi-Qiang Chi, Shanghai Institute of Materia Medica, Chinese Academy of Sciences. [^{3}H]Diprenorphine (specific activity 50 Ci/mmol) was product of PekinElmer, Inc. All drugs for animal experiments were dissolved in saline solution immediately before use, except reserpine, which was dissolved in a 20% solution of ascorbic acid. Drug concentrations were prepared so that the necessary dose could be injected systematically in a volume of 10 ml/kg.

2.3. Nociceptive procedure

Writhing test was performed in mice according to Koster et al. (1959) with modification. Briefly, mice received an i.p. injection of 0.6% acetic acid solution (0.1 ml/10 g) 30 min after administration of (+)-matrine. Ten minutes after acetic acid administration, the animals were placed in individual transparent containers and the number of writhes per animal was measured for 10 min. Tail-pressure test was modified from the original method (Green et al., 1951). Briefly, pressure was applied to the tail 1 cm distant from its root using a pressure anti-nociception meter (XZC-A, Shandong Academy of Medical Sciences, China) with a wedge-shaped piston at a loading rate of 16 g s^{-1}. The weight (g) at which animals struggled or withdrew was considered the nociceptive threshold. To avoid tissue damage, pressure was limited to a maximum of 250 g unless the animals started to bite or vocalize before this weight was reached. Only mice with a baseline between 80 and 120 g were selected. Antinociceptive effects of (+)-matrine were measured 10, 20, 30, 45 and 60 min after administration. Antinociceptive response was expressed as a percentage of the maximum possible effect (% MPE): % MPE=[(post-drug reaction pressure)−(pre-drug reaction pressure)]/[250−(pre-drug reaction pressure)]×100. Hot-plate test is a modification of that described by Chao and Tsoh (1956). Mice were placed into a 15-cm wide plexiglas cylinder on a hot plate (55±0.2 °C) (YLS-6A, Shandong Academy of Medical Sciences, China). The latency to the first sign of licking paw or jumping to avoid the heat was taken as an index of the pain threshold. Each mouse was tested at least 10 min apart twice before drugs administration. Only mice with latency between 10 and 20 s were used. A cut-off of 45 s was used to avoid any paw damage. Antinociceptive effects of (+)-matrine were measured 10, 20, 30, 45 and 60 min after administration. The antinociceptive response was calculated as the percentage of the maximum possible effect (% MPE) according to the following formula: % MPE=[(post-drug reaction time)−(pre-drug reaction time)]/[45−(pre-drug reaction time)]×100.

The minimum effective dose, defined as the lowest dose that produced a statistically significant effect across all of the tests, was chosen for the antagonism test. The area-under-the-curve (AUC), depicting % MPE vs. time, was calculated by the trapezoidal rule in order to express the overall magnitude and duration of effect for tail-pressure and hot-plate test.

2.4. Treatment schedule

To test the possible involvement of opioid system in (+)-matrine-induced antinociception, naloxone (2 mg/kg i.p.) was injected 15 min before (+)-matrine administration (Ghelardini et al., 1997). To test the possible involvement of cholinergic system in the antinociception induced by (+)-matrine, atropine (5 mg/kg i.p.) was given 15 min before (+)-matrine administration (Bartolini et al., 1987), pirenzepine (10 mg/kg i.p. or 0.1 μg/mouse i.c.v.) given simultaneously with (+)-matrine (Ghelardini et al., 1997) and HC-3 (1 μg/mouse i.c.v.) given 5 h before (+)-matrine administration (Bartolini et al., 1987). To test the possible involvement of monoamine system, reserpine (2.5 mg/kg i.p.) was given 16 to 20 h before (+)-matrine administration (Nakazawa et al., 1991). To test whether dopamine D_2 system was involved in the (+)-matrine-induced antinociception, (−)-quinpirole (0.1 mg/kg i.p.) was given 15 min before (+)-matrine administration (Ghelardini et al., 1997). Intracerebroventricular (i.c.v.) administration was performed under slight ether anesthesia using isotonic saline as a solvent according to a modification of the method described by Haley and McCormick (1957). Each experiment consisted of two-groups (pre- or co-treatment alone and treatment followed by (+)-matrine): saline–saline/(+)-matrine, naloxone–naloxone/(+)-matrine, reserpine–reserpine/(+)-matrine, (−)-quinpirole–(−)-quinpirole/(+)-matrine, atropine–atropine/(+)-matrine, pirenzepine (i.p.)–pirenzepine (i.p.)/(+)-matrine, pirenzepine (i.c.v.)–pirenzepine (i.c.v.)/(+)-matrine, HC-3–HC-3/(+)-matrine.

2.5. Mice whole brain membrane preparation

Homogenization of male mouse brain was performed as described (Jin et al., 1996). Briefly, the mice were decapitated and the brains were rapidly removed. Brains without cerebellum were homogenized in 0.32 M ice-cold sucrose. The homogenate was centrifuged at 1000×*g* for 10 min. Then the supernatants were centrifuged at 40,000×*g* for 30 min. The pellets were resuspended in ice-cold 50 mM Tris–HCl buffer (pH 7.4) and centrifuged at 40,000×*g* for another 30 min. The pellets were resuspended in ice-cold Tris–HCl buffer (pH 7.4).

2.6. Receptor binding assay

The binding of (+)-matrine for μ-, κ- and δ-opioid receptors was determined with non-selective opioid receptors ligand [^{3}H]diprenorphine. The membrane homogenate was incubated with [^{3}H]diprenorphine in a final volume of 200 μl for 20 min at 37 °C in the presence of increasing concentrations of tested drugs. Non-specific binding was defined in the presence of naloxone (10 μM). After incubation, the mixture was immediately cooled in an ice bath and then filtered rapidly through Whatman GF/B glass fiber filter in 24-well cell harvester. The filters were washed three times with ice-cold 50 mM Tris–HCl buffer (pH 7.4), dried and transferred to Eppendorf tubes and then 1 ml lipophilic scintillation cocktail was added to each tube. The radioactivity was counted with Beckman LS6500 liquid scintillation analyzer.

All experiments were performed three times, each in triplicate, and the values represented the means of three determinations. The error of the triplicate determinations was less than 10%.

2.7. Data analysis

Data were expressed as mean±S.E.M. and were analyzed using one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test. $P<0.05$ was considered significant in this study.

3. Results

3.1. Antinociceptive effects of (+)-matrine

(+)-Matrine produced a dose-dependent antinociceptive effect regardless of what noxious stimulus was used. In the writhing test, the numbers of abdominal constrictions of (+)-matrine groups (5, 10 and 20 mg/kg s.c.) were decreased dose-dependently as compared with the saline group (Fig. 2). (+)-Matrine (5, 10 and 20 mg/kg s.c.) also produced a time- and dose-dependent antinociceptive effect in tail-pressure test (Fig. 3). The maximum effect was obtained 20 min after s.c. administration of (+)-matrine. In hot-plate test, (+)-matrine (5, 10 and 20 mg/kg s.c. or 5, 10 and 20 μg/mouse i.c.v.) produced a time- and dose-

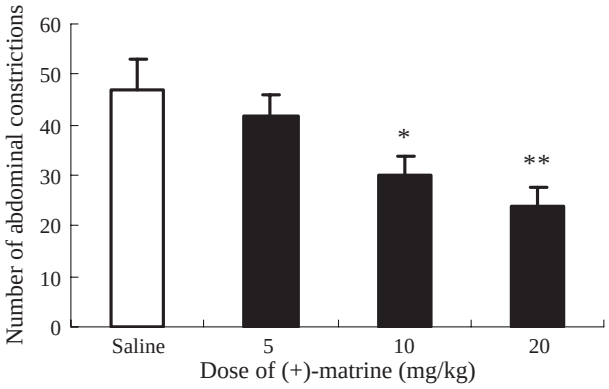

Fig. 2. The antinociceptive effect of (+)-matrine in mouse writhing test. Each mouse received an i.p. injection of 0.6% acetic acid solution (0.1 ml/10 g) 30 min after (+)-matrine (5, 10 and 20 mg/kg s.c.) administration. Ten minutes after acetic acid administration, the animals were placed in individual transparent containers and the number of writhes per animal was measured for 10 min. Data are presented as mean±S.E.M. (n=8–10). *$P<0.05$, **$P<0.01$ compared with saline group by one-way ANOVA followed by the Student–Newman–Keuls test.

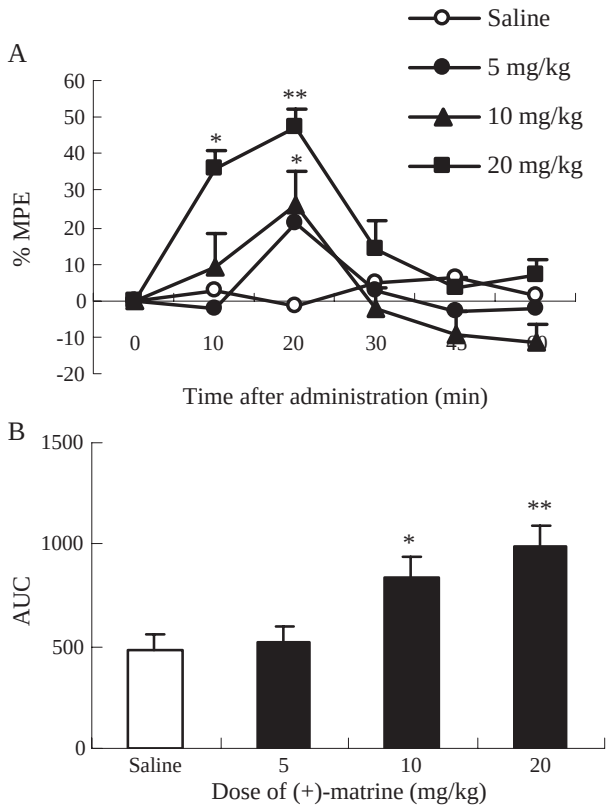

Fig. 3. The antinociceptive effect of (+)-matrine in tail-pressure test. Antinociceptive effects of (+)-matrine (5, 10 and 20 mg/kg s.c.) were measured 10, 20, 30, 45 and 60 min after administration. Time-course of (+)-matrine are shown in A and AUC are shown in B. The data are presented as means±S.E.M. (n=8–10). *P<0.05, **P<0.01 compared with saline group by one-way ANOVA followed by the Student–Newman–Keuls test.

dependent increase in the pain threshold (Fig. 4). The antinociceptive effect of (+)-matrine peaked 20 min and 10 min after s.c. and i.c.v. administration, respectively. Then the effects diminished slowly.

3.2. Receptor binding assay

In mice whole brain membranes displacement studies, ohmefentanyl, U-50,488H or DPDPE produced a concentration-dependent displacement of [^{3}H]Diprenorphine binding to opioid receptors (Fig. 5). However, (+)-matrine had no affinity to opioid receptors even at high concentration (1×10^{-3} M) (Fig. 5).

3.3. Antagonism of (+)-matrine-induced antinociception

(+)-Matrine with dose of 10 mg/kg (s.c.), which gave a consistent analgesia and no significant modification of behavioral and motor function, was used for the antagonism tests in the hot plate assay. As shown in Fig. 6, the antinociceptive effect of (+)-matrine (10 mg/kg s.c.) was not antagonized by naloxone (2 mg/kg i.p.), reserpine (2.5 mg/kg i.p.), (−)-quinpirole (0.1 mg/kg i.p.) or pirenzepine (10 mg/kg i.p.). However, the antinociception of (+)-matrine were attenuated by pretreatment with atropine (5 mg/kg i.p.) or HC-3 (1 μg/mouse i.c.v.) or co-treatment with pirenzepine (0.1 μg/mouse i.c.v.) (Fig. 6). The treatments with naloxone (2 mg/kg i.p.),

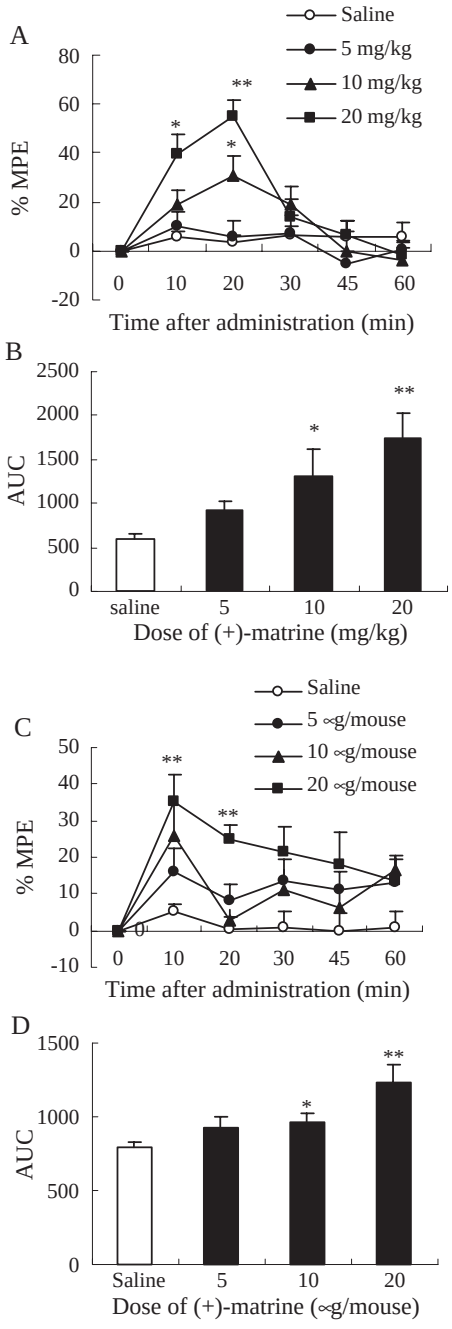

Fig. 4. The antinociceptive effect of (+)-matrine in hot-plate test. Antinociceptive effects of (+)-matrine (5, 10 and 20 mg/kg s.c. or 5, 10 and 20 μg/mouse i.c.v.) were measured 10, 20, 30, 45 and 60 min after administration. Time-course of (+)-matrine are shown in A (s.c.) and C (i.c.v.), and AUC were shown in B (s.c.) and D (i.c.v.). Data are presented as means±S.E.M. (n=8–10). *P<0.05, **P<0.01 compared with saline group by one-way ANOVA followed by the Student–Newman–Keuls test.

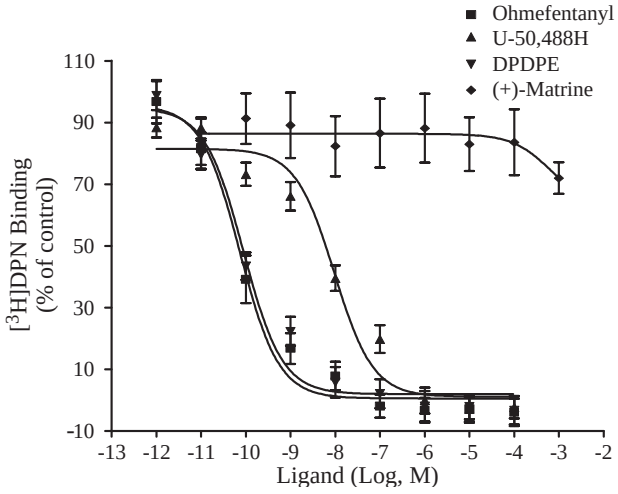

Fig. 5. Competition of [^{3}H]diprenorphine binding in the presence of opioids agonists or (+)-matrine in mice whole brain membranes. Brain membranes were incubated at 37 °C for 20 min with [^{3}H]diprenorphine (0.56 nM) with increasing concentration of ohmefentanyl, U-50,488H and DPDPE (1×10^{-12}–1×10^{-4} M) or (+)-matrine (1×10^{-11}–1×10^{-3} M). Non-specific binding was defined in the presence of naloxone (10 μM). Each data point represents the mean±S.E.M. of three independent experiments conducted in triplicate.

reserpine (2.5 mg/kg i.p.), (−)-quinpirole (0.1 mg/kg i.p.), atropine (5 mg/kg i.p.), pirenzepine (10 mg/kg i.p.), pirenzepine (0.1 μg/mouse i.c.v.) or HC-3 (1 μg/mouse i.c.v.) alone had no significant effect on the pain threshold in the studies (Fig 6).

4. Discussion

In the present studies, we demonstrated that (+)-matrine-induced antinociception in mice regardless of what noxious stimulus was used: chemical (writhing test), mechanical (tail-pressure test) or thermal (hot-plate test). (+)-Matrine may exert its antinociceptive effect by acting centrally since the antinociceptive dose of (+)-matrine for i.c.v. injection (5–20 μg/mouse) was 1000 times lower than that for i.p. administration.

Previous studies suggested that the antinociception of (+)-matrine was exerted mainly through the activation of κ-opioid receptors and partially through μ-opioid receptors (Kamei et al., 1997; Xiao et al., 1999). Thus, compounds with structure similar to matrine-type alkaloids were synthesized as promising new κ-opioid receptor agonists for the treatment of pain without undesirable morphine-like side effects (Kobashi et al., 2002, 2003). However, our studies demonstrated that the opioid system might not be involved in (+)-matrine-induced antinociception directly since (+)-matrine had no affinity for opioid receptors in receptor binding assays. In addition, the (+)-matrine-induced antinociceptive activity was not reversed by naloxone at the dose which prevented antinociceptive effect of morphine (Yang et al., 2001).

Modulation of antinociception can occur via different physiological systems. These distinct neuronal systems, if activated selectively, could provide effective control of nociception arising from a variety of causes (see review by Yaksh and Hammond, 1982). Many neuromediators, such as enkephalins, γ-aminobutyric acid, catecholamines, 5-HT and histamine, are able to induce a change in pain threshold. It seems that the central cholinergic activation may involve in (+)-matrine-induced antinociception in mice since (+)-matrine-induced antinociception was prevented by muscarinic antagonist atropine (i.p.) and pirenzepine (i.c.v.) and cholinergic depletor HC-3 (i.c.v.). The fact that pirenzepine and HC-3 antagonized (+)-matrine-induced antinociception after i.c.v. injection suggests that (+)-matrine produces its antinociceptive effect centrally since pirenzepine and HC-3, which cross the blood brain barrier with difficulty, did not antagonize the (+)-matrine-induced antinociception when these two compounds were given by i.p. injection. It seems that (+)-matrine exerted its antinociception by enhancing presynaptic acetylcholine release in the CNS. However, the AUC was only reduced by less than 50% when atropine (5 mg/kg i.p.) was given 15 min before (+)-matrine administration or pirenzepine (10 mg/kg i.p. or 0.1 μg/mouse i.c.v.) was given simultaneously with (+)-matrine or HC-3 (1 μg/mouse i.c.v.) was given 5 h before (+)-matrine administration (Fig. 6). The lack of complete reversal suggests that multiple mechanisms may be involved, not just presynaptic release of acetylcholine.

To explore other possible system which may involve in the (+)-matrine-induced antinociception, following studies were preformed in the present studies. Nakazawa et al.

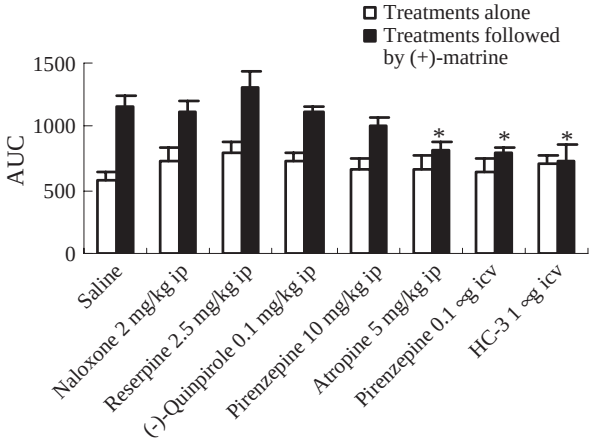

Fig. 6. Antagonism of (+)-matrine-induced antinociception in hot-plate test. Naloxone (2 mg/kg i.p.), atropine (5 mg/kg i.p.) or (−)-quinpirole (0.1 mg/kg i.p.) was given 15 min before (+)-matrine administration. Pirenzepine (10 mg/kg i.p. or 0.1 μg/mouse i.c.v.) was given simultaneously with (+)-matrine administration. HC-3 (1 μg/mouse i.c.v.) was given 5 h before (+)-matrine administration. Reserpine (2.5 mg/kg i.p.) was given 16 to 20 h before (+)-matrine administration. Data are presented as means±S.E.M. (n=8–10). *P<0.01 compared with pre-treatment with saline by one-way ANOVA followed by the Student–Newman–Keuls.

(1991) reported that reserpine (2.5 mg/kg i.p.) could deplete both norepinephrine and 5-HT in mice spinal cord 16 to 20 h after it was administered. The same treatment with reserpine prevented the antinociception induced by morphine and U-50,488H. However, reserpine treatment had no effect on (+)-matrine-induced antinociception in the present studies. Thus, the possible involvement of catecholaminergic mechanism in (+)-matrine-induced antinociception could be excluded.

The role of the dopaminergic system in producing antinociception is still controversial. Dopamine D_2 receptor antagonist sulpiride, but not D_1 receptor antagonist SCH-23390, has been found to produce antinociceptive effect at the spinal level (Liu et al., 1992). However, it was also found that D_2 agonists had antinociceptive effect (Morgan and Franklin, 1991). In the present studies, we found that dopamine D_2 receptor agonist (−)-quinpirole failed to prevent (+)-matrine-induced antinociception, although the same dose of (−)-quinpirole prevented the antinociception induced by haloperidol (Ghelardini et al., 1992), suggesting that dopamine D_2 antagonism was not involved in the (+)-matrine-induced antinociception.

In present studies, we found that (+)-matrine induced no visible effect on animal behavior at low dose; however, (+)-matrine at high doses caused overt signs of cholinergic activity potential such as salivation, tremors and diarrhoea. In addition, it was recently found that patients exposed to overdoses of (+)-matrine showed complex neurological manifestation including convulsion, mental changes and dystonia syndromes (Wang and Yang, 2003). The acetylcholine-like effects of (+)-matrine might be responsible for this movement disorders.

The results from the present study are opposite to the previous finding that (+)-matrine exerts antinociceptive effect mainly through the activation of κ-opioid receptors and partially through μ-opioid receptors (Kamei et al., 1997). The previous studies demonstrated that (+)-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. 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. In addition, the antinociceptive effect of (+)-matrine was partially antagonized by pretreatment with beta-funaltrexamine, a selective μ-opioid receptor antagonist. Naltrindole, a selective δ-opioid receptor antagonist, had no effect on the antinociceptive effect of (+)-matrine. Thus, it was concluded that (+)-matrine produced an antinociceptive effect mainly through the activation of κ-opioid receptors and partially through μ-opioid receptors. However, the affinity of (+)-matrine for κ-opioid receptors was not clearly proved as indicated in the previous studies (Kamei et al., 1997). In the present studies, radioligand binding assay was used to determine the affinity of (+)-matrine for κ- and μ-opioid receptors. We demonstrated that (+)-matrine had no affinity for μ-, κ- or δ-opioid receptors in a wide concentration range. In addition, we found that the antinociception produced by (+)-matrine was not attenuated by opioid receptor antagonist naloxone, suggesting that the opioid system might not be involved in the (+)-matrine-induced antinociception.

The reason why our in vivo results are opposite to the literature is unclear. It might due to the difference in the animal species used. While Kunming strain mice were used in the present studies, ICR mice were used in the previous studies (Kamei et al., 1997).

In conclusion, the present study demonstrated that (+)-matrine produced a dose-dependent antinociception in several nociceptive tests in mice. A key finding of the present study was that (+)-matrine exerted its antinociception through multiple mechanism(s) such as increasing cholinergic activation in the CNS rather than acting on opioid receptors directly.

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