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Research Paper

Serotonergic mechanisms are involved in antidepressant-like effects of bisbenzylisoquinolines liensinine and its analogs isolated from the embryo of *Nelumbo nucifera* Gaertner seeds in mice

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Keywords
antidepressant-like effects; isoliensinine; Liensinine; neferine; *Nelumbo nucifera* Gaertner

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Abstract

Objectives We attempted to ascertain if bisbenzylisoquinoline alkaloids, liensinine and isoliensinine from *Nelumbo nucifera* Gaertner have antidepressant-like effects and compare the effects with those previously obtained by their analogue neferine.

Methods Using mice, the forced swimming test (FST) was carried out after treatment with liensinine, isoliensinine and neferine.

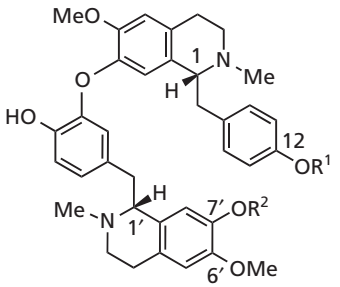
Key findings Liensinine and isoliensinine elicited antidepressant-like effects in mice after the FST. Anti-immobility effects of liensinine and isoliensinine were antagonized by the 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor antagonist WAY 100635, but not by the α_1-adrenoceptor antagonist prazosin. The anti-immobility effects of liensinine, isoliensinine and neferine were blocked by pretreatment with p-chlorophenylalanine (PCPA), which depletes serotonin (5-HT).

Conclusions These data suggest that liensinine and isoliensinine from *Nelumbo nucifera* Gaertner have antidepressant-like effects and that antidepressant-like effects of liensinine and its analogues are closely related to serotonergic mechanisms.

Introduction

Natural compounds that may be effective for the treatment of psychiatric disorders such as depression and anxiety have been isolated from medicinal plants.[1] *Nelumbo nucifera* Gaertner is distributed in all parts of the world. Several parts of this plant (e.g., leaves, flowers, rhizomes) contain useful compounds such as flavonoids and alkaloids.[2] Embryos of the seeds of *Nelumbo nucifera* Gaertner are used widely as Chinese traditional medicines presenting sedative, antipyretic and hypothermic effects in humans.[3] Embryos of these seeds contain the bisbenzylisoquinoline alkaloids neferine, liensinine or isoliensinine (Figure 1).[4–6] However, the detailed effects and mechanisms of pharmacologic action of bisbenzylisoquinoline alkaloids on the central nervous system (CNS) have not been investigated. Previously, we reported that the major alkaloid neferine reduces locomotor activity, elicits hypothermia and potentiates thiopental-induced sleep in mice.[7] Furthermore, we reported that neferine has anxiolytic effects similar to those of diazepam in the elevated plus maze test.[7] In addition, neferine was shown to have antidepressant-like effects in mice when using the forced swimming test (FST).[8]

It has been reported that liensinine has anti-arrhythmic effects in rats as a result of the blockade of influx of Ca^{2+} and Na^{+}.[9] Isoliensinine has been shown to inhibit bleomycin-induced pulmonary fibrosis in mice.[10] Recently, we found that liensinine and isoliensinine reduce locomotor activity (i.e., have sedative effects) in mice.[11] These results



$R^1 = R^2$ = Me: neferine
R^1 = H, R^2 = Me: liensinine
R^1 = Me, R^2 = H: isoliensinine

Figure 1 Chemical structures of alkaloids from the embryos of the seeds of *Nelumbo nucifera* Gaertner.

raise the possibility that liensinine and isoliensinine may have antidepressant effects similar to those of neferine. However, there are differences in the involvement of adenosine triphosphate-binding cassette transporters in the absorption of these alkaloids.[12] Thus, it is not clear whether liensinine and isoliensinine show antidepressant-like effects as observed with neferine treatment. Hence, we studied the effects of liensinine and isoliensinine on immobility in the FST and compared these effects with those of neferine. Further, the involvement of serotonergic and noradrenergic systems in antidepressant-like effects of liensinine, isoliensinine as well as neferine was examined.

Materials and Methods

Experiments were carried out in accordance with the *Guiding Principles for the Care and Use of Laboratory Animals* approved by the Japanese Pharmacological Society.

The study protocol was approved by the Ethics Committee of the University of Yasuda Women's University (protocol number 1412).

Extraction and isolation from plant materials

Embryos of the seeds of *Nelumbo nucifera* Gaertner (Nelumbonaceae, Chinese folk medicine 'Lian zi xin') collected in China were purchased from the Longhua Hospital (Shanghai University of Traditional Chinese Medicine, Shanghai, China). A voucher specimen (KPUY-031) is deposited in the laboratory of Kobe Pharmaceutical University. Liensinine, isoliensinine and neferine were isolated and identified from the embryos of the seeds of *Nelumbo nucifera* Gaertner according to the method described in our previous report.[13] Isolated bisbenzylisoquinoline alkaloids were prepared as the hydrochloride salt.

Animals

Male ICR mice (25–30 g, 5 weeks) were purchased from SLC Japan (Shizuoka, Japan). Mice had free access to food and water and were maintained on a 12-h dark–light cycle in a room with controlled temperature (23 ± 1°C) and humidity (55 ± 5%).

Forced swimming test

The FST was carried out according to the methods described by Porsolt *et al*.[14] and our previous reports.[8,15] Each mouse was placed in a 25-cm glass cylinder (diameter, 10 cm) containing 10 cm of water at 23 ± 1°C. Immobility was recorded during a 6-min swimming test. A mouse was judged to be immobile if it floated and its hindlimbs were immobile, and if only small movements of the forepaws were made to keep its head above water.

Drug treatment

N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide maleate (WAY 100635), imipramine HCl, prazosin HCl and p-chlorophenylalanine (PCPA) methyl ester HCl were obtained from Sigma-Aldrich (St Louis, MO, USA). All drugs were dissolved in physiologic (0.9%) saline and administered via the intraperitoneal route. The volume of administration was 0.1 ml/10 g body weight. Mice in the control group received saline. WAY 100635 was given 30 min before administration of liensinine or isoliensinine. PCPA (300 mg/kg, intraperitoneally) was given 72, 48 and 24 h before administration of liensinine, isoliensinine and neferine. The FST was carried out 30 min after treatment with liensinine, isoliensinine and neferine.

Statistical analyses

Results are mean ± standard error (SE) of five to nine mice in the behavioural studies. Differences between groups of animals treated with 0.9% saline or drugs alone were assessed using one-way analysis of variance (ANOVA) followed by the Dunnett's multiple comparison post-hoc test. Other data were analyzed by two-way ANOVA. Pairwise follow-up comparisons of individual treatment groups were analyzed by the Tukey's multiple comparison post-hoc test.

Results

Effects of liensinine, isoliensinine and neferine on the immobility time of mice

Figure 2 shows the effects of liensinine, isoliensinine, neferine and imipramine on the time of immobility of mice. Anti-immobility effects of liensinine, isoliensinine

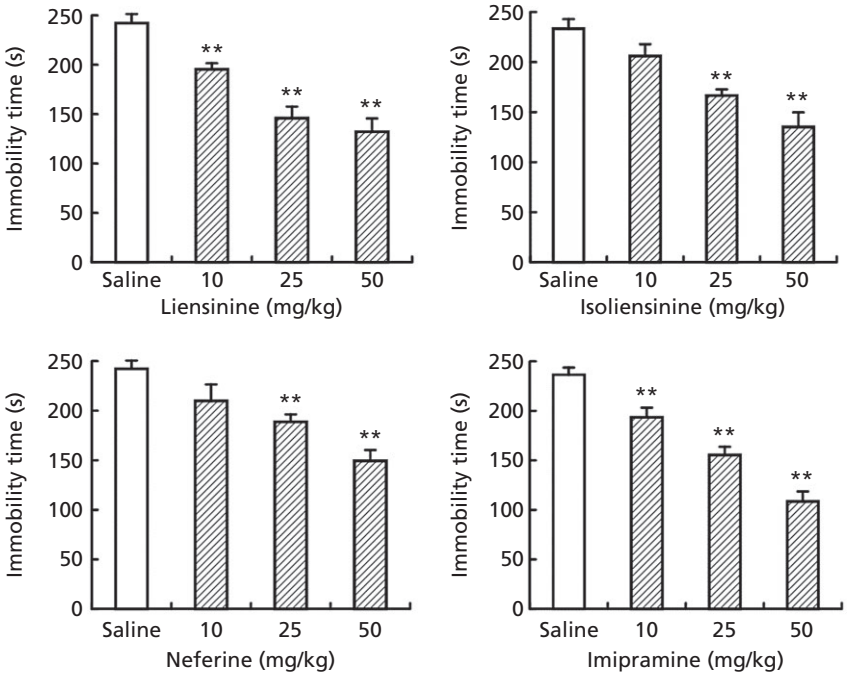


Figure 2 Effects of liensinine, isoliensinine, neferine and imipramine on immobility in the forced swimming test in mice. Results are the mean ± SE (n = 6–9). *P < 0.05, **P < 0.01. White bar graph shows the saline-treated group. Striped bar graph shows the respective drug-treated group.

and neferine were examined 30 min after administration based on previous studies on its sedative effects and anxiolytic effects of neferine.[7,11] Liensinine elicited apparent antidepressant-like effects at 10, 25 and 50 mg/kg. Isoliensinine elicited significant anti-immobility effects at 25 and 50 mg/kg. Neferine elicited significant anti-immobility effects at 25 and 50 mg/kg. Imipramine produced significant anti-immobility effects at 10, 25 and 50 mg/kg.

Effects of WAY 10063 on liensinine and isoliensinine-induced anti-immobility effects in mice

Figure 3 demonstrates the effects of 5-HT_{1A} receptor antagonist WAY 106335 on the antidepressant-like effects elicited by liensinine and isoliensinine. WAY 100635 (0.5 and 1 mg/kg) significantly attenuated the anti-immobility effects of liensinine and isoliensinine. Doses of WAY 100635 were chosen based on information on how they block the 5-HT_{1A} receptors.[16,17]

Effects of PCPA on liensinine, isoliensinine and neferine-induced anti-immobility effects in mice

Figure 4 shows the effect of PCPA (which depletes 5-HT) on bisbenzylisoquinolines-induced anti-immobility effects. PCPA significantly attenuated the anti-immobility effects of liensinine, isoliensinine and neferine. Previous reports suggested that the treatment with PCPA in this study could decrease brain 5-HT levels (over 60%), whereas noradrenaline and dopamine levels are not altered.[18,19]

Effects of prazosin on liensinine, isoliensinine and neferine-induced anti-immobility effects in mice

Effects of the α_1-adrenoceptor antagonist prazosin on the antidepressant-like effects elicited by liensinine, isoliensinine and neferine (Figure 5). Prazosin did not affect anti-immobility effects of three alkaloids.

Discussion

Recently, a multitude of pharmacologic effects of bisbenzylisoquinoline alkaloids from the embryos of the seeds of *Nelumbo nucifera* Gaertner have been reported.[2] Pharmacologic analyses of bisbenzylisoquinoline alkaloid neferine in mice revealed that it has sedative, anxiolytic effects and, using the FST, it has antidepressant-like effects.[7,8] Moreover, it was reported that neferine improves anti-amnesic effects in rats.[20]

We reported that the other alkaloids from the embryos of the seeds of *Nelumbo nucifera* Gaertner liensinine and isoliensinine have sedative effects in mice.[11] However, it

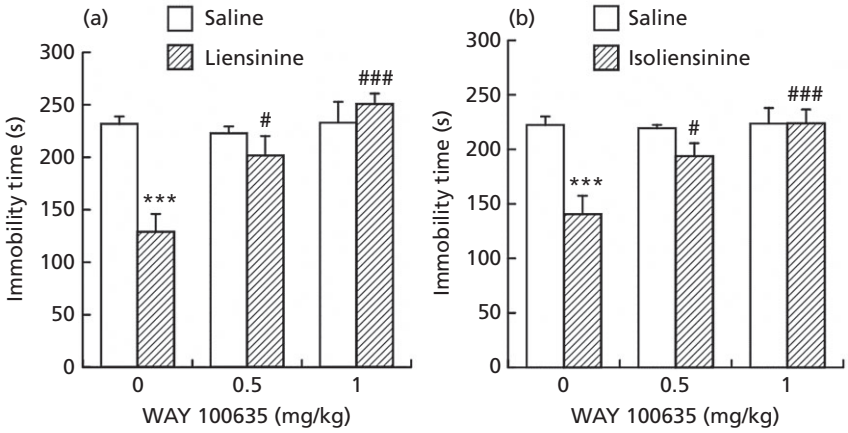


Figure 3 Effects of WAY 100635 on liensinine and isoliensinine-induced anti-immobility in mice. Results are the mean ± SE ($n = 5$). (a) Liensinine, (b) Isoliensinine. WAY 100635 was injected 30 min before liensinine (50 mg/kg) or isoliensinine (50 mg/kg). ***$P < 0.001$ vs saline of the respective group. #$P < 0.05$, ###$P < 0.001$ vs saline + liensinine or isoliensinine-treated group.

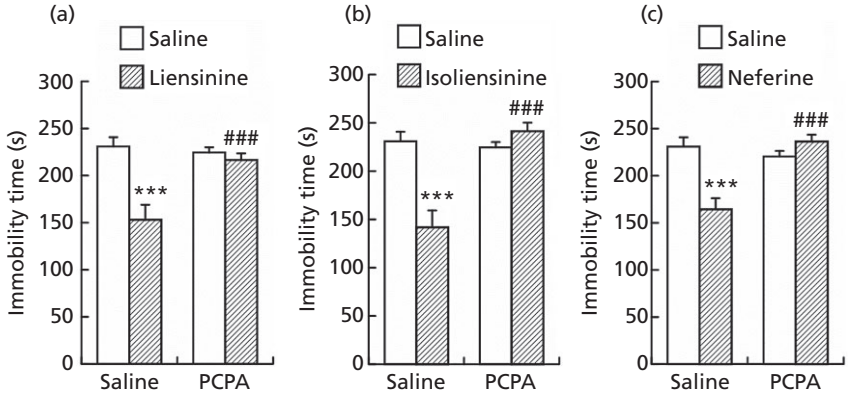


Figure 4 Effects of p-chlorophenylalanine (PCPA) on bisbenzylisoquinolines-induced anti-immobility in mice. Results are the mean ± SE ($n = 5$–7). (a) Liensinine, (b) Isoliensinine, (c) Neferine. PCPA (300 mg/kg) was given 72, 48 and 24 h before liensinine (50 mg/kg), isoliensinine (50 mg/kg) and neferine (50 mg/kg). ***$P < 0.001$ vs saline of the respective group. ###$P < 0.001$ vs saline + respective alkaloid-treated group.

was not clear whether liensinine and isoliensinine are effective against depression. Therefore, we examined if liensinine and isoliensinine have antidepressant-like effects in mice.

In this study, the typical antidepressant imipramine elicited dose-dependent anti-immobility effects, which reveals that the FST in this study can be used for evaluation of antidepressant effects. Liensinine and isoliensinine elicited significant anti-immobility effects in mice. Liensinine showed significant antidepressant effects at lower doses than those of isoliensinine and neferine. Our previous report demonstrated that liensinine and isoliensinine decreased locomotor activity over 30 min but did not increase general activity.[11] Therefore, it is unlikely that liensinine and isoliensinine elicit anti-immobility effects by enhancing locomotor activity. Although some antidepressants like imipramine or maproltiline show anti-immobility effects, they also reduce locomotor activity similar to liensinine and isoliensinine.[8] It suggests that sedation and antidepressant effects may be caused through different mechanisms.

The serotonergic and noradrenergic system has been implicated in the mechanism of action of several antidepressants.[21] Selective serotonin reuptake inhibitors inhibit 5-HT transporter proteins and reuptake of 5-HT, which elevates 5-HT levels in the synaptic cleft.[21,22] Mirtazapine inhibits the hetero α_2 receptor on serotonergic neurons to facilitate 5-HT release.[23] We found that the selective serotonin reuptake inhibitors fluvoxamine or paroxetine elicit antidepressant-like effects in mice and that these effects are mediated by 5-HT_{1A} receptors.[24,25] It has been reported that mirtazapine stimulates 5-HT_{1A} receptors, which contributes

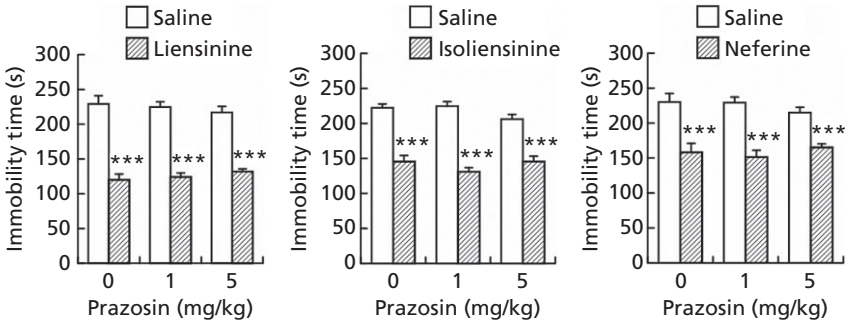


Figure 5 Effects of prazosin on bisbenzylisoquinolines-induced anti-immobility in mice. Results are the mean ± SE (n = 5–8). Prazosin was given 30 min before liensinine (50 mg/kg), isoliensinine (50 mg/kg) and neferine (50 mg/kg). ***P < 0.001 vs saline of the respective group. ###P < 0.001 vs saline + respective alkaloid-treated group.

to its antidepressant effects.[23] Our previous report demonstrated that antidepressant-like effects induced by neferine were antagonized by pretreatment with the 5-HT_{1A} receptor antagonist.[8] Thus, we examined the involvement of 5-HT_{1A} receptors in the antidepressant-like effects of liensinine and isoliensinine.

The selective 5-HT_{1A} receptor antagonist WAY 100635 significantly reduced the anti-immobility effects of liensinine and isoliensinine. These results suggest that liensinine and isoliensinine elicit antidepressant-like effects through 5-HT_{1A} receptors.

Evidence of the involvement of 5-HT_{1A} receptors in the antidepressant-like effects of several drugs has been accumulated.[22,26,27] Selective 5-HT_{1A} receptor agonists such as 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and gepirone elicit anti-immobility effects in mice and rats.[8,26,28] These agonists stimulate the 5-HT_{1A} receptor directly and induce antidepressant-like effects. We formerly demonstrated that 8-OH-DPAT elicits anti-immobility effects in mice and its effects were antagonized by the 5-HT_{1A} receptor antagonist WAY 100635.[8] The present study further supports the importance of 5-HT_{1A} receptors in the antidepressant-like effects in the FST. Bisbenzylisoquinoline alkaloids cause antidepressant-like effects via the 5-HT_{1A} receptor. However, it is not clear whether they cause antidepressant-like effects as direct 5-HT_{1A} receptor agonists. Therefore, we examined the influence of PCPA on the antidepressant-like effects of liensinine, isoliensinine and neferine to assess their association with serotonergic neurons.

PCPA inhibits 5-HT synthesis and decreases brain levels of 5-HT.[29] PCPA attenuated the antidepressant-like effects of liensinine, isoliensinine and neferine. Although PCPA decreases brain 5-HT contents, PCPA itself did not alter immobility time. In previous studies with PCPA, immobility was not significantly changed, but the anti-immobility effects of drugs affecting serotonergic mechanisms were reduced.[30–32]

The finding with PCPA suggested that bisbenzylisoquinolines were not direct 5-HT_{1A} receptor agonists and that they modify neurotransmission at presynaptic sites. Modification of 5-HT neurons may be derived by facilitation of 5-HT release from nerve terminals, inhibition of 5-HT reuptake at 5-HT transporter sites, or inhibition of breakdown of 5-HT by monoamine oxidase. Studies on detailed mechanisms on 5-HT neurotransmission are required.

It has been reported that α_1-adrenoceptor is involved in depression. The antidepressant-like effects induced by several drugs, including noradrenaline reuptake inhibitors, are mediated by the α_1-adrenoceptor[33–35] because these effects are antagonized by α_1-adrenoceptor antagonists. We therefore studied the effects of the α_1-adrenoceptor α-antagonist prazosin on the anti-immobility effects of bisbenzylisoquinolines. Prazosin did not affect liensinine, isoliensinine and neferine-induced anti-immobility effects. These results suggest that α_1-adrenoceptor is not related to anti-immobility effects of these alkaloids.

Our previous study showed that liensinine, isoliensinine and neferine induce sedation in mice.[11] Previously, we demonstrated the relationship between the chemical structure and sedative effects of bisbenzylisoquinoline alkaloids from the seeds of *Nelumbo nucifera* Gaertner. A hydroxyl group at C-12 or C-7′ of these alkaloids (Figure 1) may contribute to their sedative effects.[11] Liensinine and isoliensinine, which have a hydroxyl group at C-12 and C-7′, respectively, produced more potent sedative effects than neferine.[11] Because liensinine shows antidepressant-like effects at the lower dose, hydroxyl group at C-12 may enhance central effects of bisbenzylisoquinolines. Previous studies suggest that bisbenzylisoquinolines elicited sedation without toxicological signs.[7,11] Although effects of the higher doses are required to examine, our present findings suggest that bisbenzylisoquinoline alkaloids from the embryos of the seeds of *Nelumbo nucifera* Gaertner may be drug candidates for the treatment of depression.

Conclusions

We found that the bisbenzylisoquinoline alkaloids liensinine and isoliensinine-induced antidepressant-like effects in mice. Our results suggest that the antidepressant-like effects of bisbenzylisoquinoline alkaloids are closely associated with serotonergic mechanisms.

Declaration

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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