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Nelumbinis Semen reverses a decrease in hippocampal 5-HT release induced by chronic mild stress in rats

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Abstract

Depression is associated with a dysfunctional serotonin system. Recently, several lines of evidence have suggested that a very important evoking factor in depression may be a serotonin deficit in the hippocampus. This study assessed the antidepression effects of Nelumbinis Semen (NS) through increasing serotonin concentrations under normal conditions and reversing a decrease in serotonin concentrations in rat hippocampus with depression-like symptoms induced by chronic mild stress (CMS). Using an in-vivo microdialysis technique, the serotonin-enhancing effect of NS on rat hippocampus was investigated and its effects compared with those of two well-known antidepressants, *Hypericum perforatum* (St John's wort) and fluoxetine (Prozac). Rats were divided into five groups: saline-treated normal, without CMS; saline-treated stress control; NS-, St John's wort- and fluoxetine-treated rats under CMS for 8 weeks or no stress treatment. NS and fluoxetine significantly increased serotonin in normal conditions and reversed a CMS-induced decrease in serotonin release in the hippocampus ($P < 0.05$ compared with normal group or control group under CMS). These results suggest that NS increases the serotonin levels normally decreased in depression, resulting in an enhancement of central serotonergic transmission and possible therapeutic action in depression. It is suggested that NS may present an antidepressant effect through enhancement of serotonin.

Introduction

Pathologies of the central nervous system (CNS) are generally associated with changes in the concentrations of neurotransmitters in specific brain regions (Crespi et al 2004). Assessment of neurotransmitter levels is important, therefore, in evaluating the efficacy of new pharmacological treatments. There is considerable clinical evidence that serotonin-containing pathways in the CNS play a significant role in the pathological development of major depression (van der Stelt et al 2004). Chronic stress is thought to impair the hippocampus, leading to a deficiency of serotonin in the hippocampus and the outbreak of depression (Penalva et al 2002; Dremencov et al 2003; Malberg & Duman 2003). In line with this notion, selective serotonin reuptake inhibitors (SSRIs) are a current mainstay for the treatment of major depression (van der Stelt et al 2004). The main action of antidepressants is to increase the amounts of such neurotransmitters in the synaptic space. SSRIs are highly effective and produce milder side effects than do tricyclic antidepressants (Khawaja et al 2004).

Nelumbinis Semen has been widely used in Korean traditional medicine as a remedy for insomnia, anxiety and women's depression following the menopause. We recently found this herbal medicine to have an antidepressant effect on rats under a forced swim-induced depression-like symptom (Kang et al 2005) as well as a chronic mild stress (CMS)-induced depression-like symptom (Jang et al 2004). There has been no direct indication, however, of an antidepressant effect through measurement of extracellular serotonin release by treatments with Nelumbinis Semen. This study assessed the direct increasing effect of Nelumbinis Semen on hippocampal serotonin release under normal and CMS conditions in rats using an in-vivo microdialysis technique coupled with HPLC. The results were then compared with the two well-known antidepressants *Hypericum perforatum* (St John's wort) and fluoxetine (Prozac).

Materials and Methods

Animals

Male Sprague–Dawley rats (Samtaco, Kyunggi-do, Korea), weighing 220–250 g at the start of the experiment, were used. Rats were kept on a 12-h light–dark cycle in individual home cages with food and water freely available. All experiments were approved by the KHU Institutional Animal Care and Use Committee and abided by International Animal Care and Use Committee regulation.

Stress procedures

Rats were randomly divided into two groups, one exposed to a variety of chronic mild stressors (CMS group) and the other an unstressed normal group (control group). CMS procedures, similar to those we have previously reported, were employed for this study (Kim et al 2003). Briefly, CMS rats were subjected for eight weeks to a weekly regimen of stressors, including two periods of water and food deprivation (20 h), one period of either water (14 h) or food deprivation (2 h), two periods (7 h and 17 h) of 45° cage tilt, one period in a soiled cage (100 mL of water in sawdust bedding per individual cage), two periods (3 h and 5 h) of white noise (85 dB), three periods (9 h) of stroboscope light (300 flashes/min) and one period (17 h) of group housing (5 rats per cage). Normal rats were housed in a separate room and received a daily oral administration of 0.9% saline, but did not receive any stress (Normal group (C), $n = 10$). Stressed groups received a daily oral administration of 0.9% saline (Stress control group (S), $n = 8$), NS 100 mg kg^{-1} ($n = 6$), NS 400 mg kg^{-1} ($n = 7$), NS 1000 mg kg^{-1} ($n = 6$) or St John's wort (500 mg kg^{-1}, $n = 7$) or intraperitoneal fluoxetine (10 mg kg^{-1}, $n = 6$) during the stress treatment.

Surgery, microdialysis and analytical procedure

At least one week before the start of the experiment, rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.) and, using aseptic techniques, guide cannulae (CMA/Microdialysis, Solna, Sweden) aimed to terminate in the hippocampus (AP = 1.8, DV = 5.7, L = 4.8 from bregma) were stereotaxically implanted and attached to the skull using skull screws and dental cement as previously described by Paxinos & Watson (1986). After the last day of the CMS schedule, all rats were left without any treatment for at least 24 h. On the following day, a 3-mm vertical dialysis probe (CMA12, CMA/Microdialysis) connected via a dual liquid swivel to a syringe pump (CMA100, CMA/Microdialysis) was inserted into the guide cannula and perfused at a constant rate of 1.0 μL min^{-1} with artificial cerebrospinal fluid (ACSF; composition in mM: 145 NaCl, 2.7 KCl, 1.2 $CaCl_2$, 1.0 $MgCl_2$ and 2.0 Na_2HPO_4, pH 7.4). Rats were then placed in the cage and the outlet tubing connected to a microfraction collector (CMA142, CMA/Microdialysis). The dialysate was collected during 20-min sampling intervals in plastic microvials on the fraction collector. Three baseline samples were collected before treatments. Dialysate samples were collected every 20 min for 2 h following all injections. Samples (injection volume, 20 μL) were assayed for serotonin using an HPLC system equipped with an electrochemical detector (ESA Coulochem II- 5200B). Separation of serotonin was performed on an LC-8-DB 3-μm column (150 × 4.6 mm; Supecol, Bellefonte, PA). The mobile phase (0.05 M monobasic sodium phosphate, 0.1 N sodium acetic acetate, 1% methanol, pH 4.4 with H_3PO_4) was pumped at a flow rate of 1.0 mL min^{-1}. Serotonin content in the various dialysates was expressed as a percentage of baseline release measured as the mean of the final 3 samples.

Drug administration

After 2 h of baseline data had been collected, rats received either 100, 400 or 1000 mg kg^{-1} of NS, 500 mg kg^{-1} of St John's wort, 10 mg kg^{-1} of fluoxetine, or the saline vehicle under normal or stress conditions. Each drug was dissolved in isotonic sodium chloride (0.9%) and administered orally (Nelumbinis Semen and St John's wort) or intraperitoneally (fluoxetine) to rats for 1 day before assessment of serotonin or for 8 weeks CMS before assessment of serotonin.

Histological procedure

At the end of the experiment, rats were perfused transcardially, under deep pentobarbital anaesthesia, with normal saline followed by a 10% formalin solution. The brains were removed from the skulls and stored in 10% formalin for at least 2 weeks, after which 50-μm cryostat sections were cut through the sites of the microdialysis probes and subsequently stained with cresyl violet to identify the location of the probe.

Statistical analysis

Data were statistically analysed by one-way analysis of variance. Differences among the groups were further analysed by post-hoc LSD test.

Results

Effect of Nelumbinis Semen on extracellular serotonin release in the hippocampus under normal conditions

Figures 1A and 1B compare the effect, on rat hippocampal serotonin concentration, of normal, NS, St John's wort and fluoxetine without CMS. The mean values of dialysate serotonin in the hippocampus at 20 min after drug injections were 19.5 ± 2.9, 20.1 ± 2.2, 23.3 ± 1.8, 23.6 ± 1.9, 21.8 ± 1.9 and 28.3 ± 4.1 pg/20 μL for the normal control (C), NS 100, NS 400, NS 1000, St John's wort (JWE) and fluoxetine (F)-treated groups, respectively. Oral administration of 400 and 1000 mg kg^{-1} of

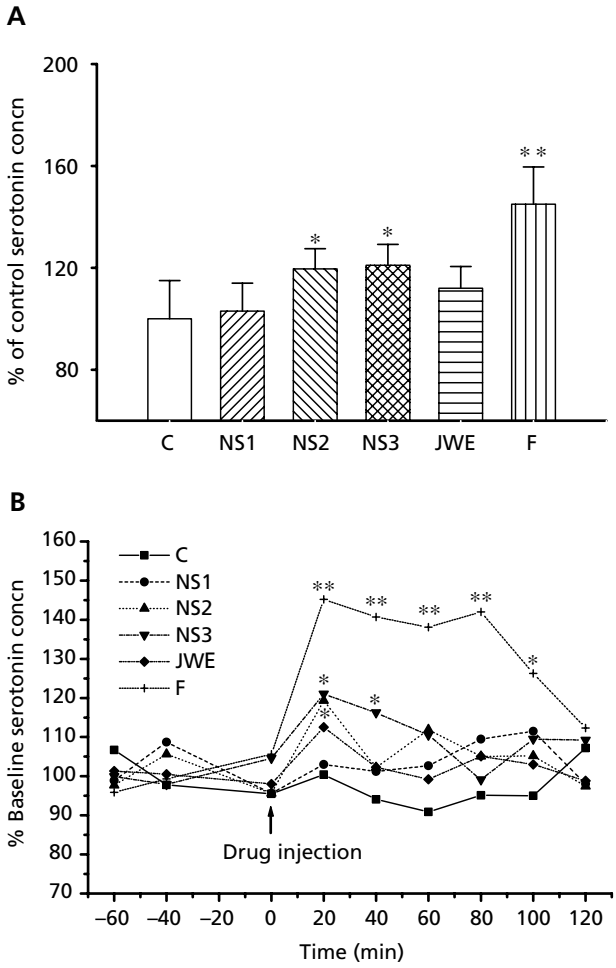

Figure 1 Effect of Nelumbinis Semen on extracellular serotonin concentrations in the hippocampus at the peak, 20 min (A), and time course of its changes (B), after drug injections, as measured by microdialysis in rats under normal conditions. Each bar represents the mean value ± s.e.m. from ten rats per group for the six treatment groups: saline-treated normal group without any drug treatment (C), Nelumbinis Semen treatment group (NS1 (100 mg kg^{-1} p.o.), NS2 (400 mg kg^{-1} p.o.), NS3 (1000 mg kg^{-1} p.o.)), St John's wort treatment group (JWE) and fluoxetine treatment group (F). *$P < 0.05$, **$P < 0.01$, compared with normal control group (C) based on one-way analysis of variance followed by post-hoc LSD test.

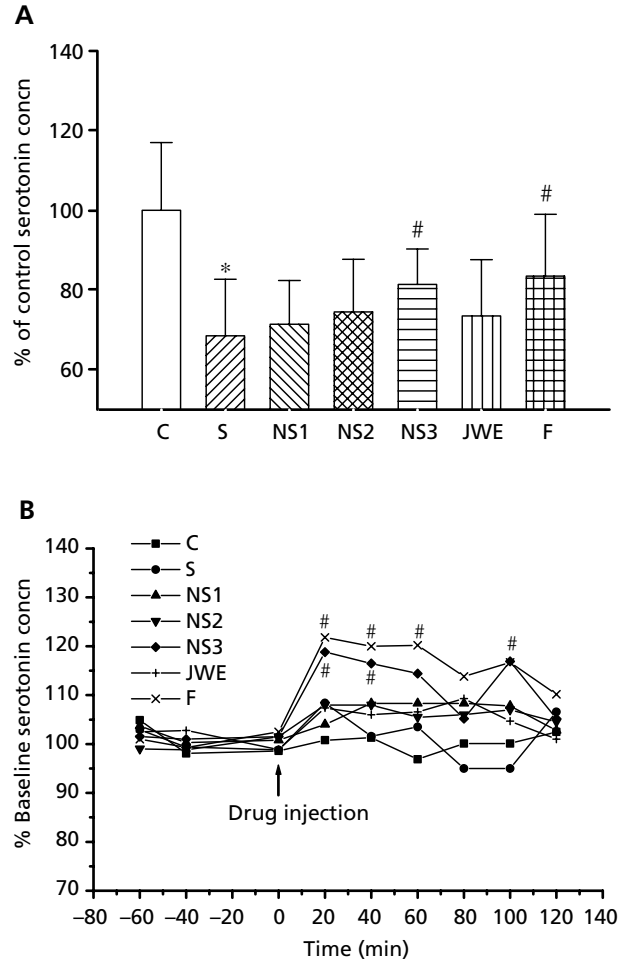

Figure 2 Effects of Nelumbinis Semen on extracellular serotonin concentrations in the hippocampus at the peak, 20 min (A), and time course of its changes (B), after drug injections, as measured by microdialysis in rats under chronic mild stress (CMS) conditions. Each bar represents the mean value ± s.e.m. from 6–10 rats per group for the seven treatment groups: saline-treated normal group without any treatment (C), saline-treated stress group under CMS (S), Nelumbinis Semen treatment group under CMS (NS1 (100 mg kg^{-1} p.o.), NS2 (400 mg kg^{-1} p.o.), NS3 (1000 mg kg^{-1} p.o.)), St John's wort treatment group under CMS (JWE), and fluoxetine treatment group under CMS (F). *$P < 0.05$ compared with C group; or $^{\#}P < 0.05$ compared with S group based on one-way analysis of variance followed by post-hoc LSD test.

NS significantly increased the concentration of normal serotonin ($P < 0.05$ for normal controls (100 ± 15%, n = 10) vs NS 400 (119 ± 5.9%, 19% increase compared with normal controls, n = 10) and for normal control vs NS 1000 (121 ± 8.2%, 21% increase compared with normal controls, n = 10)). A similarly significant increase was seen in the fluoxetine-treated group, although St John's wort treatment did not significantly increase the concentration of normal serotonin ($P < 0.05$ for normal controls (100 ± 15%, n = 10) vs fluoxetine (145 ± 14.6%, 45% increase compared with normal controls, n = 10) and for normal controls vs St John's wort (112 ± 8.5%, 12% increase compared with normal controls, n = 10)), as seen in Figures 1A and 1B. These results suggest that NS has a significant antidepressant effect by causing an increase in serotonin concentration even in the normal condition.

Effect of Nelumbinis Semen on extracellular serotonin release in the hippocampus under CMS

Figures 2A and 2B compare the effect, on changes of extracellular serotonin concentrations in the rat hippocampus, of saline-treated normal without CMS (C) and stress control without drug treatment (S), NS, St John's wort (JWE) and fluoxetine (F) under CMS for

8 weeks. As seen in Figure 2A, the mean values of dialysate 5-HT in the hippocampus at 20 min after drug injections were 20.0 ± 3.4, 13.7 ± 1.9, 14.3 ± 1.6, 14.9 ± 2.0, 16.3 ± 1.4, 14.7 ± 2.1 and 16.7 ± 2.6 pg/20 μl for the normal, stress control, NS 100, NS 400, NS 1000, St John's wort, and fluoxetine-treated groups, respectively. The basal serotonin levels in the stress group were lower than in the saline-treated normal group, and this effect was statistically significant in either group ($P < 0.05$ for saline-treated control ($68.5 \pm 14.2\%$, $n = 8$) vs normal control ($100.0 \pm 17.0\%$, $n = 10$)) as shown in Figure 2A. Oral administration of 1000 mg kg^{-1} NS significantly reversed the decrease in serotonin concentration induced by CMS ($P < 0.05$ for saline-treated control ($68.5 \pm 14.2\%$, $n = 8$) vs NS 1000 ($81.4 \pm 8.9\%$, $n = 6$)). A similar reversal by NS is presented in the fluoxetine treatment but not in the St John's wort treatment ($P < 0.05$ for saline-treated control ($68.5 \pm 14.2\%$, $n = 8$) vs fluoxetine ($83.5 \pm 15.5\%$, 15.5% increase compared with saline-treated control, $n = 6$); $P > 0.05$ for saline-treated control ($68.5 \pm 14.2\%$, $n = 8$) vs St John's wort ($73.5 \pm 14.1\%$, 5% increase compared with saline-treated control, $n = 7$)) at the peak, relative to baseline, as seen in Figures 2A and 2B. These results suggest that NS has a significant antidepressant effect by increasing serotonin concentration under CMS, similar to the action of fluoxetine.

Discussion

Major depression is a severe disorder that involves disturbances of autonomic, cognitive, endocrine and emotional functions. Depressive disorders affect a large population (an estimated 9–10% of adults) in the United States (Regier et al 1993). Because of its severity, a quick and effective treatment of the illness is often required. Exposure to chronic stress is thought to precipitate or exacerbate depression, and several studies suggest that repeated, weak stressors are effective methods of inducing depression-like symptoms in an animal model. Such animal models reflect symptoms of depression in man commonly attributed to weak, consistent and chronic stress in modern society. The model of chronic mild stress (CMS) developed by Willner and colleagues is one of the most widely accepted animal models of depression, characterized by a high degree of validity and reliability (Katz 1981; Willner 1991).

In a typical experiment involving the Willner model, rats (Willner et al 1987) or mice (Monleon et al 1995) are consistently exposed to various mild stressors, such as overnight illumination, food or water deprivation, cage tilt and change of cage mate. This procedure induces anhedonia. Furthermore, it decreases consumption and preference for palatable weak (1–2%) sucrose solution, which is concrete behaviour of anhedonia present in the animals under CMS. Treatment with most antidepressants causes the consumption of sucrose solution to return to normal (Muscat et al 1992). The CMS model was chosen for this study because of the ease with which a depression-like symptom is induced.

In a recent study, we found that Nelumbinis Semen (NS) had a distinct antidepressant effect in that it reduced the immobility time of rats in the forced swim (Kang et al 2005), reversing decreases of sucrose intake and serotonin $(5\text{-HT})_{1A}$ receptor binding in hippocampus (5-HT1A hetero-receptors) induced by CMS (Jang et al 2004). It is known that NS contains various alkaloids (Table 1) (Zelenski 1977; Wang et al 1991). Of these components, anonaine, asimilobine, isoquercitrin, hyperoside, lirinidine and nornuciferine have been most widely recognized as having antidepressant effects, as evaluated by neurotransmitter reuptake inhibition and the forced swimming test (Shoji et al 1987; Protais et al 1995; Hasrat et al 1997; Butterweck et al 2000).

It has been established that depressed patients have a dysfunctional serotonin system (Leitch et al 2003). Such a dysfunction is thought to cause loss in postsynaptic signalling mechanisms and a stress-induced impairment in the hypothalamic–pituitary–adrenal (HPA) axis, a major receptive site of stress. This results in impairment of the hippocampus and its very well developed serotonergic nerve system, the most sensitive area for impairment of the HPA axis (Dremencov et al 2003). The hippocampus also controls many of the brain functions that, when altered, disturb patients. These include regulation of neuroendocrine and autonomic functions, mood and cognition difficulties, adverse responses to stressful stimuli, and others. Hippocampal functions are highly regulated by serotonergic systems (Hjorth et al 2000). The hippocampus is thus suggested to play a critical role in depressive disorders. It should be noted that fluoxetine, as a potent SSRI, significantly increased the concentrations of normal serotonin, but St John's wort treatment did not. These results suggest that fluoxetine increases serotonin release and, in turn, produces an antidepressant effect, whereas St John's wort may produce antidepressant

Table 1 The known components of Nelumbinis Semen

(−)-Nornuciferine	Lirinidine
4′-*O*-Methyl-*N*-methylcoclaurine	Liriodenine
	Lotusine
Anonaine	Methylcorypalline
Armepavine	*N*-Methylasimilobine (*R*-form)
Asimilobine (*R*-form)	*N*-Methylcoclaurine (*R*-form)
Dehydroanonaine	*N*-Methylisococlaurine
Dehydronuciferine	*N*-Norarmepavine
Dehydroroemerine	Neferine
Demethylcoclaurine	Nornuciferine
Hyperoside	Nuciferine (*R*-form)
Isoliensinine	Phytol
Isoquercitrin	Pronuciferine (*R*-form)
Kaempferol-3-*O*-beta-D-glucuronide	Quercetin-3-*O*-beta-D-diglucopyranoside
	Quercetin-3-*O*-beta-D-glucuronide
Liensinine	Quercetin
Linalool	Roemerine (*R*-form) beta-Sitosterol

This table was referred from www.tradimed.com

effects through other neurotransmitter systems such as dopamine or noradrenaline, which are as critical as serotonin in depression (Butterweck et al 1997). This suggestion is strengthened by the fact that St John's wort preferentially increased extracellular dopamine release in the rat brain using in-vivo microdialysis (Yoshitake et al 2004). In the other way, it is reported that acute stress increases serotonin release (Linthorst et al 1994, 1997; Merali et al 1997; Broderick 2002), while chronic stress decreases serotonin release in the brain (Meltzer 1989; Risch & Nemeroff 1992; Li et al 2003). Extending the arguments above, the effect of NS on extracellular serotonin concentrations was assessed by microdialysis in rat hippocampus under normal without any stress or CMS conditions. Our results suggest that NS may have an antidepressant effect through enhancement of serotonin concentrations, in normal without any stress, as well as CMS, conditions. These effects are likely mediated by ingredients in NS, such as the known neurotransmitter reuptake inhibitor anonaine (Hasrat et al 1997). These results support the development of a novel antidepressant based on the pharmacological action of NS. The molecular mechanism underlying the antidepressant effect of NS requires further study, however, before firm conclusions can be drawn.

Conclusions

This study assessed the antidepressant effects of Nelumbinis Semen (NS) through increasing serotonin concentrations under normal conditions without any stress and reversing a decrease in serotonin concentrations in rat hippocampus with depression-like symptoms induced by chronic mild stress. NS significantly increased serotonin in normal conditions without any stress and reversed a CMS-induced decrease in serotonin release in the hippocampus. These results suggest that NS may increase the serotonin levels normally decreased in depression, resulting in an enhancement of central serotonergic transmission and possible therapeutic action for depression. These results support the development of a novel antidepressant based on the pharmacological action of Nelumbinis Semen.

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