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Effect of chronic *Albizzia julibrissin* treatment on 5-hydroxytryptamine$_{1A}$ receptors in rat brain

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Abstract

Quantitative receptor autoradiography and behavioral studies were employed to investigate whether the aqueous extract of *Albizzia julibrissin* (AEAJ) specifically targets serotonergic systems in rat brain. AEAJ was orally administered at 50 and 200 mg/kg to adult male SD rats for 7 days. Treatment with AEAJ (200 mg/kg) significantly increased time-spent in open arms and the number of open arm entries in an elevated plus-maze (EPM) versus saline controls ($P<0.05$). Moreover, those effects of AEAJ were blocked by WAY 100635, a 5-HT$_{1A}$ receptor antagonist. Following behavioral evaluation, the binding of [^{3}H]8-hyroxy-2-(di-*n*-propylamino) tertalin ([^{3}H]8-OH-DPAT) to 5-HT$_{1A}$ receptors in rat brain was investigated. [^{3}H]8-OH-DPAT binding after AEAJ (200 mg/kg) treatment showed a marked increase in the frontal cortex, hippocampus (CA2 and CA3 regions) and in the lateral septum versus vehicle-treated controls. No changes of [^{3}H]8-OH-DPAT binding were observed in the caudate putamen, dentate gyrus and CA1 areas of the hippocampus or in the hypothalamus. In the dorsal raphe region, [^{3}H]8-OH-DPAT binding was significantly reduced by AEAJ (50 mg/kg) treatment but was unchanged by AEAJ (200 mg/kg). These results suggest that the anxiolytic-like effect of *A. julibrissin* is mediated by the changes of serotonergic nervous system, especially 5-HT$_{1A}$ receptors.

Keywords: Anxiety; *Albizzia julibrissin*; Elevated plus-maze; Serotonin; WAY 100635; Receptor-binding autoradiography

1. Introduction

The 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor is viewed as a relevant target for the treatment of psychiatric disorders, notably anxiety and depression (File, 1996). 5-HT$_{1A}$ receptors are located at presynaptic and postsynaptic sites (Blier et al., 1993). Somatodendritic autoreceptor, which when activated by systemic stimulation, is believed to exert anxiolytic-like effects and to reduce 5-HT release both in the cell body and in the terminal regions of serotonergic neurons (Lanfumey et al., 1997). The other 5-HT$_{1A}$ receptor is localized postsynaptically to serotonergic neurons in the hippocampus, septum, amygdala, and cortex, where it increases signal transfer, which leads to an inhibition of firing activity (Okazawa et al., 1999). Indeed, potential anxiolytic properties have been attributed to full and partial 5-HT$_{1A}$ receptor agonists (Schreiber and De Vry, 1993).

Since the introduction of benzodiazepines in the 1960s, they remained the most commonly prescribed treatment for anxiety. Although these compounds remain the mainstay of drug treatment in anxiety disorders, their side-effects are prominent, such as sedation, myorelaxation, ataxia, amnesia, and pharmacological dependence (Lader and Morton, 1991). Recently, research has been conducted to investigate safer, more specific, and perhaps lower cost therapies. Natural anxiolytic agents feature in such research because herbs have been used to treat psychiatric disorders and generally have fewer harmful effects (Carlini, 2003).

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The stem bark of *Albizzia julibrissin* Durazz (Leguminosae) is widely used for the traditional treatment of insomnia, traumatic injuries and for calming the mind (Zhu, 1998). Recently, we observed that the aqueous extract of *A. julibrissin* stem bark (AEAJ) exhibits good anxiolytic-like activity in the rat using the elevated plus-maze (EPM) (Kim et al., 2004). Moreover, this activity was blocked by the 5-$HT_{1A/1B}$ receptor antagonist, pindolol. Therefore, we hypothesize that AEAJ administered in vivo, may affect serotonergic transmission directly or indirectly in the brain. In order to test this hypothesis, quantitative receptor autoradiography was employed to investigate whether AEAJ specifically targets serotonergic systems in rat brain. In addition, the anxiolytic-like effects of AEAJ were examined using an EPM.

2. Methods

2.1. Materials

Buspirone, WAY 100635, and serotonin–HCl was obtained from the Sigma Chemical Co. (USA), and [^{3}H]8-hyroxy-2-(di-*n*-propylamino) tertalin ([^{3}H]8-OH-DPAT, 170 Ci/mmol) and [^{3}H]Microscales™ were purchased from NEN Life Science (Boston, MA, USA) and Amersham Biosciences (Piscataway, NJ, USA), respectively. *A. julibrissin* stem bark was obtained from a herbalist in Seoul, Korea, and voucher specimens (KHUOPS2001-14) were deposited at the herbarium of the College of Pharmacy, Kyung Hee University (Seoul, Korea). The material was authenticated by Prof. C.S. Yook of the Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University. All other materials were of the highest grade commercially available.

2.2. Animals

Male SD rats, weighing 260–270 g, were purchased from the Orient Co., Ltd. (a branch of Charles River in Seoul, Korea). Animals were housed 4 or 5 per cage, allowed free access to water and food, and maintained under constant temperature (23 ± 1 °C) and humidity (60 ± 10%) under a 12 h light/dark cycle (light on 07.30–19.30 hours). Animal treatment and maintenance were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Kyung Hee University, Korea.

2.3. Sample preparation and drug administration

AEAJ was prepared by boiling dried bark of *A. julibrissin* in 10 volumes of water for 2 h. The aqueous solution obtained was filtered, concentrated on a water bath under vacuum, frozen and lyophilized (Eyela, model FDU-2000, Japan) (yield 3.4%), and stored at −20 °C until required. AEAJ was freshly resuspended in saline and administered per os (po) for 7 days, once per day at 50 or 200 mg/kg (po).

2.4. Elevated plus-maze test

The EPM consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 10 cm) with 40 cm high walls, extending from the central platform (10 × 10 cm). The arms were connected to a central square, 10 × 10 cm, to give the apparatus a plus sign appearance. The maze was raised to a height of 50 cm above floor level in a dimly lit room (20 lx) and a video camera was suspended above the maze to record movements for analysis. The maze floor and walls were constructed from dark opaque polyvinylplastic. Each rat was placed on the center of the platform facing an enclosed arm. Animals were tested individually and only once for 5 min. The maze was cleaned following each trial to remove any residue or odors. The following measurements were taken using the video-based Ethovision System and analyzed: the number of entries into open and closed arms, time spent in each arm, and the total distance moved in the EPM.

Rats were placed in the EPM 1 h after treatment. Rats in the control group were treated with vehicle only, and these animals were also tested individually and only once for 5 min. In a separate antagonism experiment, rats were co-administered AEAJ and WAY 100635. Thirty minutes after AEAJ (50 and 200 mg/kg, po) treatment, rats were intraperitoneally injected with WAY 100635 (0.3 mg/kg). Rats were treated with buspirone (1 mg/kg, i.p.) as a positive control.

2.5. Tissue preparation for autoradiography

The rats were immediately decapitated after the EPM test, and brains were removed and frozen on dry ice and stored at −80 °C until sectioned. Serial coronal sections (20 μm thickness) were prepared at −20 °C using a microtome cryostat (Leica, Germany) and thaw-mounted onto gelatin-coated glass slides (Superfrost®/Plus, Fisher Scientific, USA) and stored at −20 °C until required.

2.6. Quantitative autoradiography of 5-HT_{1A} receptor binding sites

Autoradiographic assay for 5-HT_{1A} receptors was performed using [^{3}H]8-OH-DPAT, as described previously (Ase et al., 1999) with some modifications. Briefly, for 5-HT_{1A} receptors, the sections were pre-incubated for 15 min in 50 mM Tris–HCl buffer (pH 7.4), and then incubated for 60 min in the same pre-incubation buffer with 2 nM [^{3}H]8-OH-DPAT (170 Ci/mmol) at room temperature. Non-specific binding was assessed in the presence of 10 μM of serotonin–HCl (Radja et al., 1993). After incubation, both sets of slides were rinsed three times in buffer (Tris–HCl, 50 mM at 4 °C) for 1 min each, once briefly in cold distilled water, and then immediately dried in a stream of cool air. Dried tissue sections were exposed to Kodak BioMax MR

film (Eastman Kodak Co., Rochester, NY, USA) for 5 weeks. Films were developed at room temperature for 3 min and then fixed for 3 min. Autoradiograms were analyzed using a digital scanning densitometer (Personal Densitometer, Molecular Dynamics, Sunnyvale, CA, USA) using the image acquisition and analysis program (ImageQuant 3.3, Molecular Dynamics, Sunnyvale, CA, USA). A standard curve obtained from standard [^{3}H]Microscales™ was used to convert density levels into femtomoles per milligram. The specific binding of [^{3}H]8-OH-DPAT was obtained by subtracting nonspecific binding from total binding.

2.7. Statistics

Values are expressed as mean ± S.E.M. Data were analyzed by a one-way analysis of variance (ANOVA) followed by Dunnett's test or Student Newman–Keuls test for multiple comparisons. For antagonism study, the interactions between the agonist and antagonist were analyzed separately with a two-way ANOVA [factors: agonist versus antagonist]; pairwise comparisons for the assessment of the antagonist influence on the agonist effects were conducted by Tukey's test. Statistical significance was set at $P<0.05$.

3. Results

3.1. Effect of chronic AEAJ treatment on EPM performance

As shown in the saline treated group, rats typically avoided spending time in or entering open arms (Fig. 1).

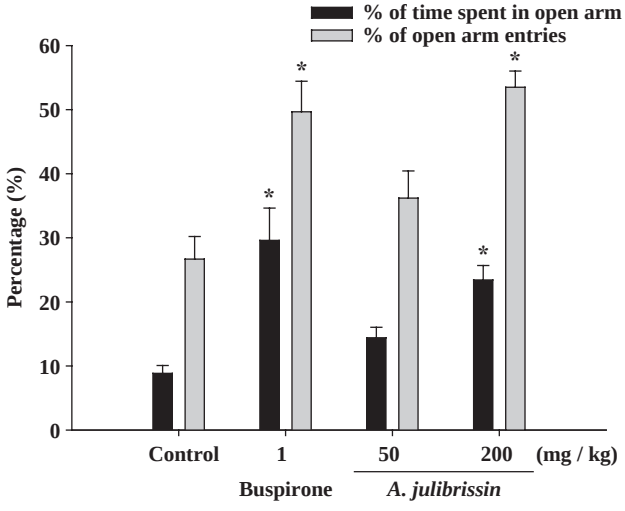

Fig. 1. Effect of chronic treatment of the water extract of *Albizzia julibrissin* Durazz. (*A. julibrissin*) on the percentage of the time spent in and the number of entries into open arms of the elevated plus-maze over a 5 min test period in the rat. Each bar represents mean ± S.E.M. of 10–12 rats. *P* values for group comparisons were obtained by one-way ANOVA followed by the Student Newman–Keuls test (*$P<0.001$ as compared with the saline treated control group: Time, F (3, 41)=12.80, $P<0.001$; Frequency, F (3, 41)=11.48, $P<0.001$).

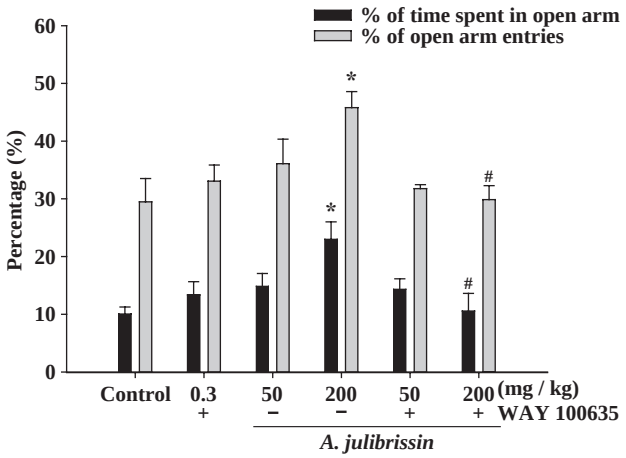

Fig. 2. Anxiolytic-like effects of *Albizzia julibrissin* Durazz. (*A. julibrissin*) were blocked by WAY 100635. The water extract of *Albizzia julibrissin* (AEAJ) was orally administered at 50 and 200 mg/kg to adult male SD rats for 7 days. Thirty minutes after the last oral administration, WAY 100635 (0.3 mg/kg) or vehicle were intraperitoneally treated; $N=10–12$ rats per group. The data are expressed as the means (±S.E.M.) of the percentage of the time spent in and the number of entries into the open arms of the elevated plus-maze over 5 min test period. *, $P<0.001$ versus the saline treated controls (one-way ANOVA followed by the Student Newman–Keuls test): Time, F (2, 30)=14.64, $P<0.001$; Frequency, F (2, 30)=11.31, $P<0.001$. $^{\#}$, $P<0.001$ compared to the corresponding effect of agonist (two-way ANOVA followed by the Tukey's test): Time, F (1, 38)=42.14, $P<0.001$; Frequency, F (1, 38)=20.18, $P<0.001$. The AEAJ × WAY 100635 interaction was as follows: Time, F (1, 38)=2.21, $P<0.15$; Frequency, F (1, 38)=0.96, $P<0.34$.

Chronic AEAJ treatment (200 mg/kg) for 7 days significantly increased the time spent in open arms versus the control (Fig. 1; $P<0.001$). In addition, AEAJ treated rats (200 mg/kg) made significantly more entries into open arms than the controls (Fig. 1; $P<0.001$). However, no significant changes were observed in terms of the time spent and the number of entries made into open arms at 50 mg/kg of AEAJ. The buspirone-treated (1 mg/kg) positive controls entered open arms more frequently and spent more time in these arms than saline treated group ($P<0.001$).

3.2. The effect of WAY 100635 antagonism on the anxiolytic-like activity of AEAJ

To investigate whether the anxiolytic effect of AEAJ is exerted via the serotonergic nervous system, AEAJ (50 or 200 mg/kg for 7 days) treated rats were co-treated with WAY 100635, a specific 5-HT$_{1A}$ receptor antagonist. As shown in Fig. 2, the anxiolytic-like effects of AEAJ (200 mg/kg) were antagonized by WAY 100635 (0.3 mg/kg). However, treatment with 50 mg/kg of AEAJ or WAY 100635 (0.3 mg/kg) caused no significant changes in the number of open arm entries or in the time spent in open arms.

3.3. Quantitative autoradiography of 5-HT$_{1A}$ receptor binding sites after chronic AEAJ treatment

The autoradiograms in Fig. 3 showed representative binding of [^{3}H]8-OH-DPAT at frontal cortex (panel A1-3), striatum (panel B1-3), hippocampus (panel C1-3), and dorsal raphe (panel D1-3) levels. As shown in Fig. 3 (panel A1-3) and Table 1, [^{3}H]8-OH-DPAT binding 7 days after treating with AEAJ (200 mg/kg) revealed a marked increase in binding to the frontal cortex compared to the vehicle-treated group. Similarly, the binding of [^{3}H]8-OH-DPAT was also increased in the hippocampus (CA2 and CA3 regions) and in the lateral septum (intermediate and dorsal parts) by AEAJ (50 and 200 mg/kg) treatment (Fig. 3B1-3, C1-3, and Table 1). No changes in [^{3}H]8-OH-DPAT binding were observed in the caudate putamen, dentate gyrus and CA1 areas of the hippocampus or hypothalamus. In the dorsal raphe region, [^{3}H]8-OH-DPAT binding was significantly reduced by 50 mg/kg of AEAJ (Fig. 3D2 and Table 1). However, no changes were observed in the binding of [^{3}H]8-OH-DPAT due to treatment with 200 mg/kg of AEAJ (Fig. 3D3 and Table 1).

4. Discussion

The main findings of this study were that chronic high doses of AEAJ significantly increased time spent in the open arms and the frequency of open arm entries, and that these effects were antagonized by WAY 100635, thus demonstrating the involvement of the 5-HT$_{1A}$ receptors. Moreover, chronic treatment with AEAJ produced a significant and region-specific change in 5-HT$_{1A}$ receptors in the cortex, lateral septum, and in a number of limbic regions. In addition, significant changes in 5-HT$_{1A}$ receptor binding were observed after low dose AEAJ treatment, however no significant anxiolytic-like effects was induced by 50 mg/kg regimens.

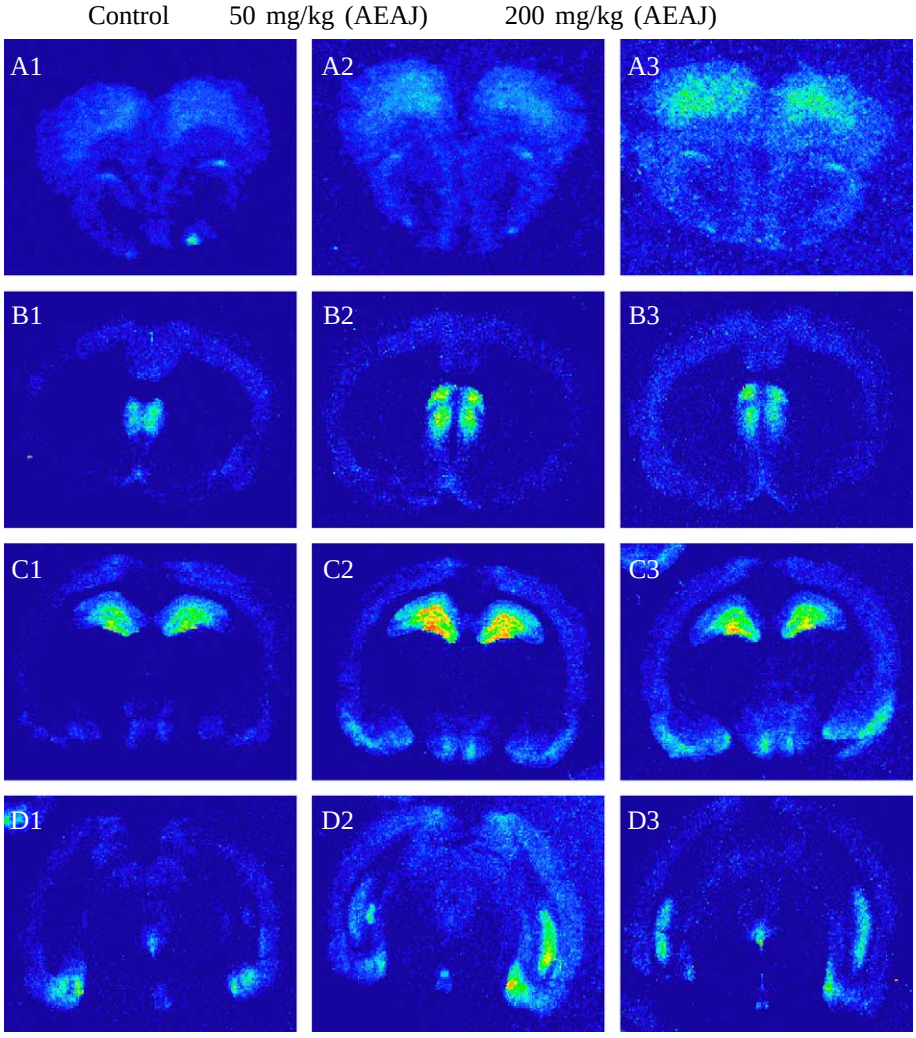

Fig. 3. Representative photographs showing changes in 5-HT$_{1A}$ receptor binding sites labeled with [^{3}H]8-OH-DPAT 7 days after treatment with vehicle (column A1–D1), the water extract of *Albizzia julibrissin* (AEAJ) at 50 (column A2–D2) or 200 mg/kg (column A3–D3). The row figures represent levels in the cortex (row A1–A3), striatum and lateral septal nucleus (row B1–B3), hippocampus (row C1–C3), and dorsal raphe nucleus (row D1–D3).

Table 1
Changes in [^{3}H]8-OH-DPAT (fmol/mg tissue) binding sites of discrete brain regions of rats after treating with aqueous extract of *Albizzia julibrissin* (AEAJ)

Regions	Control	AEAJ 50 mg/kg	AEAJ 200 mg/kg
Frontal cortex	20.47±2.96	24.78±1.85	28.74±1.45*
Caudate putamen	10.19±2.16	9.46±1.68	7.36±1.19
Hippocampus			
CA1	48.61±3.35	54.27±1.88	47.88±2.54
CA2	38.28±3.08	53.37±2.17***	49.93±2.82*
CA3	21.40±2.19	31.11±1.96***	32.35±1.54***
DG	60.01±2.70	65.93±2.82	59.68±3.20
Hypothalamus	16.46±2.65	20.79±1.47	19.32±1.21
Dorsal raphe	45.18±6.11	24.78±5.07*	54.06±5.61
Lateral septal nucleus			
Dorsal part	47.68±3.50	74.56±2.44***	64.34±3.84***
Intermediate	55.47±4.17	66.56±2.01*	63.78±1.93*

AEAJ was orally administered at 50 and 200 mg/kg to adult male SD rats for 7 days. Following a behavioral evaluation, the binding of [^{3}H]8-hyroxy-2-(di-*n*-propylamino) tertalin ([^{3}H]8-OH-DPAT) to 5-HT$_{1A}$ receptors were investigated in rat brain (n=5 for each group). The tissue sections were incubated with 2 nM [^{3}H]8-OH-DPAT. Binding capacity is expressed as femtomole per milligram tissue. Each value is the mean±S.E.M of 4–6 determinations for each region. *, $P<0.05$; ***, $P<0.001$, compared to the control group (ANOVA followed by Dunnett's test).

5-HT$_{1A}$ receptors play important roles in the mediation of 5-HT neurotransmission in the CNS, and changes in their functional state are implicated in human anxiety. Indeed, local microinjection of 5-HT$_{1A}$ receptor agonists directly into the dorsal raphe nucleus, to stimulate somatodendritic 5-HT$_{1A}$ autoreceptors, reproduces anxiolytic effects (Schreiber and De Vry, 1993). Moreover, the anxiolytic effect induced by systemic administration of 8-OH-DPAT was blocked by WAY 100635 given into the dorsal raphe nucleus (Remy et al., 1996). Therefore, 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus are probably a key target whereby 5-HT$_{1A}$ receptor agonists exert their anxiolytic properties. In addition, postsynaptic 5-HT$_{1A}$ receptors located in limbic forebrain areas might also be involved, as some have reported a reduction in anxiety-driven behavior in rats that have been administered 5-HT$_{1A}$ receptor agonists directly into the hippocampus (Kataoka et al., 1991). We observed that chronic AEAJ (200 mg/kg) treatment produced good anxiolytic-like activities by EPM testing. Furthermore, these anxiolytic-like behaviors were completely blocked by WAY 100635, a specific 5-HT$_{1A}$ receptor antagonist. Previously, we also observed that a single AEAJ treatment produced anxiolytic-like behavior, and that these effects were blocked by pindolol, a 5-HT$_{1A/1B}$ receptor and β-receptor antagonist (Kim et al., 2004). Thus, we concluded that the anxiolytic-like activity of AEAJ is mediated via the activation of the 5-HT$_{1A}$ receptor and that AEAJ could be useful as a 5-HT$_{1A}$ receptor agonist.

Several lines of evidence suggest that chronic treatment with 5-HT$_{1A}$ receptor agonist induces receptor desensitization, as measured by electrophysiological and neurochemical indexes (Blier and de Montigny, 1987; Kreiss and Lucki, 1997; Le Poul et al., 1999; Okazawa et al., 1999). However, the capacity of 5-HT$_{1A}$ receptor agonists to desensitize or to down-regulate 5-HT$_{1A}$ receptors in vivo remains a topic of debate (Godbout et al., 1991). Le Poul et al. (1999) reported that chronic treatment with the 5-HT$_{1A}$ receptor agonist, alnespirone, did not change [^{3}H]8-OH-DPAT bindings in various regions, including the dorsal raphe. Casanovas et al. (1999) reported that alnespirone significantly reduced [^{3}H]8-OH-DPAT binding in the dorsal raphe region, but that it was unchanged in other regions, such as, the hippocampal and cortical regions. In the present study, we observed that [^{3}H]8-OH-DPAT bindings was significantly increased in the frontal cortex, lateral septum, and hippocampal CA2 and CA3 regions by AEAJ treatment at both low and high doses. Moreover, no changes of [^{3}H]8-OH-DPAT binding were observed in the dorsal raphe region after treating with high dose AEAJ (200 mg/kg). However, significant [^{3}H]8-OH-DPAT binding reduction was observed in the dorsal raphe after treating with low dose AEAJ (50 mg/kg).

The up-regulation of the 5-HT$_{1A}$ receptor found in the present study is not consistent with other reports (Casanovas et al., 1999; Fanelli and McMonagle-Strucko, 1992). It is generally accepted that postsynaptic receptors are up-regulated by the presynpatic inhibition or denervation of presynaptic neurons. The density of 5-HT$_{1A}$ receptors was increased by the destruction of ascending serotonergic neurons by 5,7-dihydroxytryptamine treatment (Patel et al., 1996). It is likely that the presynaptic inhibition of serotonergic neurons by AEAJ treatment caused an increase in [^{3}H]8-OH-DPAT bindings in postsynaptic 5-HT$_{1A}$ receptors. Interestingly, at high AEAJ doses, no changes were observed in [^{3}H]8-OH-DPAT binding in the dorsal raphe region. These results suggest that the functional desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors is induced by the repeated administration of this exogenous agonist. 5-HT$_{1A}$ autoreceptors desensitization has already been described after repeated treatment with 5-HT$_{1A}$ agonists (Kreiss and Lucki, 1997; Le Poul et al., 1999). Collectively, it seems likely that anxiolytic-like activity is mediated by the 5-HT$_{1A}$ receptor at a high AEAJ dose. However, a significant decrease in [^{3}H]8-OH-DPAT binding in the dorsal raphe regions was observed after chronic treatment with low dose AEAJ. Welner et al. (1989) reported that chronic treatment with gepirone, a 5-HT$_{1A}$ agonist, significantly reduced the binding of [^{3}H]8-OH-DPAT in the dorsal raphe. Fanelli and McMonagle-Strucko (1992) reported similar results in the dorsal raphe nucleus. These findings show that chronic low dose AEAJ treatment did not cause the functional desensitization of somatodendritic 5-HT$_{1A}$ receptors. However, changes in 5-HT$_{1A}$ receptor bindings were not correlated with anxiolytic-like behavior at a low dose (50 mg/kg). Further research is needed to determine why 5-HT$_{1A}$ receptor binding is changed without anxiolytic-like behavior at a low dose (50 mg/kg).

The identity of the AEAJ constituent that exerts its anxiolytic effect remains unknown. Several reports have

been published on compounds isolated from *A. julibrissin* stem bark. These include flavone derivatives, unsaturated acid, and lignan glycosides (Kinjo et al., 1991; Jung et al., 2003a,b). Previously, various synthetic flavone derivatives were found to have anxiolytic-like activities in the EPM, which was attributed to benzodiazepine receptor activation (Griebel et al., 1999). Liao et al. (2003) also reported that baicalein or baicalin isolated from *Scutellaria baicalensis* activate the benzodiazepine binding site of $GABA_A$ receptors. Thus, if the active principle in AEAJ is a flavone derivative, the anxiolytic-like effects of AEAJ may be mediated by the activation of benzodiazepine binding sites. Previously, the anxiolytic-like effect of AEAJ (200 mg/kg) was not found to be antagonized by flumazenil, a selective $GABA_A$ receptor antagonist (Kim et al., 2004). Recently, during the bioactivity-guided fractionation of AEAJ, we observed that butanol soluble compounds might have the anxiolytic-like activity (data not shown). We believe that the anxiolytic-like effect of AEAJ is mediated primarily by the 5-HT_{1A} receptor. However, the nature of its underlying mode of action remains to be elucidated.

In conclusion, the present investigations suggested that the anxiolytic-like effect of AEAJ be mediated by the serotonergic nervous system, especially by 5-HT_{1A} receptors. Although our findings indicate that the herb extracts may not in itself provide a clinically useful treatment, our findings may be of importance because they confirm the medicinal properties of *A. julibrissin*.

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References

Ase AR, Amdiss F, Hebert C, Huang N, van Gelder NM, Reader TA. Effects of antipsychotic drugs on dopamine and serotonin contents and metabolites, dopamine and serotonin transporters, and serotonin$_{1A}$ receptors. J Neural Transm 1999;106:75–105.

Blier P, de Montigny C. Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: electrophysiological studies in the rat brain. Synapse 1987;1:470–80.

Blier P, Lista A, De Montigny C. Differential properties of pre- and postsynaptic 5-hydroxytryptamine$_{1A}$ receptors in the dorsal raphe and hippocampus: II Effect of pertussis and cholera toxins. J Pharmacol Exp Ther 1993;265:16–23.

Carlini EA. Plants and the central nervous system. Pharmacol Biochem Behav 2003;75:501–12.

Casanovas JM, Vilaro MT, Mengod G, Artigas F. Differential regulation of somatodendritic serotonin 5-HT_{1A} receptors by 2-week treatments with the selective agonists alnespirone (S-20499) and 8-hydroxy-2-(Di-*n*-propylamino)tetralin: microdialysis and autoradiographic studies in rat brain. J Neurochem 1999;72:262–72.

Fanelli RJ, McMonagle-Strucko K. Alteration of 5-HT_{1A} receptor binding sites following chronic treatment with ipsapirone measured by quantitative autoradiography. Synapse 1992;12:75–81.

File SE. Recent developments in anxiety, stress, and depression. Pharmacol Biochem Behav 1996;54:3–12.

Godbout R, Chaput Y, Blier P, de Montigny C. Tandospirone and its metabolite, 1-(2-pyrimidinyl)-piperazine—I Effects of acute and long-term administration of tandospirone on serotonin neurotransmission. Neuropharmacology 1991;30:679–90.

Griebel G, Perrault G, Tan S, Schoemaker H, Sanger DJ. Pharmacological studies on synthetic flavonoids: comparison with diazepam. Neuropharmacology 1999;38:965–77.

Jung MJ, Chung HY, Kang SS, Choi JH, Bae KS, Choi JS. Antioxidant activity from the stem bark of *Albizzia julibrissin*. Arch Pharm Res 2003;26:458–62.

Jung MJ, Kang SS, Choi JS. A new (E)4-hydroxy-dodec-2-enedioic acid from the stem bark of *Albizzia julibrissin*. Arch Pharm Res 2003;26:207–9.

Kataoka Y, Shibata K, Miyazaki A, Inoue Y, Tominaga K, Koizumi S, et al. Involvement of the dorsal hippocampus in mediation of the antianxiety action of tandospirone, a 5-hydroxytryptamine$_{1A}$ agonistic anxiolytic. Neuropharmacology 1991;30:475–80.

Kim WK, Jung JW, Ahn NY, Oh HR, Lee BK, Oh JK, et al. Anxiolytic-like effects of extracts from *Albizzia julibrissin* bark in the elevated plus-maze in rats. Life Sci 2004;75:2787–95.

Kinjo J, Fukui K, Higuchi H, Nohara T. The first isolation of lignan tri- and tetra-glycosides. Chem Pharm Bull 1991;39:1623–5.

Kreiss DS, Lucki I. Chronic administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT differentially desensitizes 5-HT_{1A} autoreceptors of the dorsal and median raphe nuclei. Synapse 1997;25:107–16.

Lader M, Morton S. Benzodiazepine problems. Br J Addict 1991;86:823–8.

Lanfumey L, Haj-Dahmane S, Laporte AM, Martin P, Hamon M, Gozlan H. Effects of chronic diazepam treatment on pre- and postsynaptic 5-HT_{1A} receptors in the rat brain. Eur J Pharmacol 1997;323:137–48.

Le Poul E, Laaris N, Doucet E, Fattaccini CM, Mocaer E, Hamon M, et al. Chronic alnespirone-induced desensitization of somatodendritic 5-HT_{1A} autoreceptors in the rat dorsal raphe nucleus. Eur J Pharmacol 1999;365:165–73.

Liao JF, Hung WY, Chen CF. Anxiolytic-like effects of baicalein and baicalin in the Vogel conflict test in mice. Eur J Pharmacol 2003;464:141–6.

Okazawa H, Yamane F, Blier P, Diksic M. Effects of acute and chronic administration of the serotonin$_{1A}$ agonist buspirone on serotonin synthesis in the rat brain. J Neurochem 1999;72:2022–31.

Patel TD, Azmitia EC, Zhou FC. Increased 5-HT_{1A} receptor immunoreactivity in the rat hippocampus following 5,7-dihydroxytryptamine lesions in the cingulum bundle and fimbria-fornix. Behav Brain Res 1996;73:319–23.

Radja F, Descarries L, Dewar KM, Reader TA. Serotonin 5-HT_1 and 5-HT_2 receptors in adult rat brain after neonatal destruction of nigrostriatal dopamine neurons: a quantitative autoradiographic study. Brain Res 1993;606:273–85.

Remy SM, Schreiber R, Dalmus M, De Vry J. Somatodendritic 5-HT_{1A} receptors are critically involved in the anxiolytic effects of 8-OH-DPAT. Psychopharmacology (Berl) 1996;125:89–91.

Schreiber R, De Vry J. Neuronal circuits involved in the anxiolytic effects of the 5-HT_{1A} receptor agonists 8-OH-DPAT ipsapirone and buspirone in the rat. Eur J Pharmacol 1993;249:341–51.

Welner SA, De Montigny C, Desroches J, Desjardins P, Suranyi-Cadotte BE. Autoradiographic quantification of serotonin$_{1A}$ receptors in rat brain following antidepressant drug treatment. Synapse 1989;4:347–52.

Zhu YP, 1998. Chinese materia medica chemistry, pharmacology and applications. Netherlands: Harwood Academic Publishers; 1998. p. 519–20.