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The antidepressant effects of curcumin in the forced swimming test involve 5-HT_1 and 5-HT_2 receptors

Rui Wang, Ying Xu, Hong-Li Wu, Ying-Bo Li, Yu-Hua Li, Jia-Bin Guo, Xue-Jun Li *

Department of Pharmacology, School of Basic Medical Sciences and State Key Laboratory of Natural & Biomimetic Drugs, Peking University, Beijing 100083, China

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Abstract

Curcuma longa is a main constituent of many traditional Chinese medicines, such as Xiaoyao-san, used to manage mental disorders effectively. Curcumin is a major active component of *C. longa* and its antidepressant-like effect has been previously demonstrated in the forced swimming test. The purpose of this study was to explore the possible contribution of serotonin (5-HT) receptors in the behavioral effects induced by curcumin in this animal model of depression. 5-HT was depleted by the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (PCPA, 100 mg/kg, i.p.) prior to the administration of curcumin, and the consequent results showed that PCPA blocked the anti-immobility effect of curcumin in forced swimming test, suggesting the involvement of the serotonergic system. Moreover, pre-treatment of pindolol (10 mg/kg, i.p., a β-adrenoceptors blocker/5-$HT_{1A/1B}$ receptor antagonist), 4-(2′-methoxy-phenyl)-1-[2′-(*n*-2″-pyridinyl)-*p*-iodobenzamino-]ethyl-piperazine (*p*-MPPI, 1 mg/kg, s.c., a selective 5-HT_{1A} receptor antagonist), or 1-(2-(1-pyrrolyl)-phenoxy)-3-isopropylamino-2-propanol (isamoltane, 2.5 mg/kg, i.p., a 5-HT_{1B} receptor antagonist) was found to prevent the effect of curcumin (10 mg/kg) in forced swimming test. On the other hand, a sub-effective dose of curcumin (2.5 mg/kg, p.o.) produced a synergistic effect when given jointly with (+)-8-hydroxy-2-(di-*n*-propylamino)tetralin, (8-OH-DPAT, 1 mg/kg, i.p., a 5-HT_{1A} receptor agonist), anpirtoline (0.25 mg/kg, i.p., a 5-HT_{1B} receptor agonist) or ritanserin (4 mg/kg, i.p., a 5-$HT_{2A/2C}$ receptor antagonist), but not with ketanserin (5 mg/kg, i.p., a 5-$HT_{2A/2C}$ receptor antagonist with higher affinity to 5-HT_{2A} receptor) or R(-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 1 mg/kg, i.p., a 5-HT_{2A} receptor agonist). Taken together, these results indicate that the antidepressant-like effect of curcumin in the forced swimming test is related to serotonergic system and may be mediated by, at least in part, an interaction with 5-$HT_{1A/1B}$ and 5-HT_{2C} receptors.

Keywords: Curcumin; Depression; Antidepressant; Forced swimming test; 5-$HT_{1A/1B}$ receptors; 5-$HT_{2A/2C}$ receptors

1. Introduction

Depression, as a widespread incapacitating psychiatric ailment, imposes a substantial health burden on society (Nemeroff, 2007; Rosenzweig-Lipson et al., 2007). In spite of its prevalence and severe impact, the efficacy of currently available antidepressants is often inconsistent and many of them exert undesirable side effects. With a growing number of herbal medicines being introduced to psychiatric practice, many of them have been chosen as alternative therapies for severe depression (Kessler et al., 2001; Thachil et al., 2007). Thus, developing safe and effective agents from traditional herbs may provide us a good way to lessen the side effects as well as improve the efficacy.

Curcuma longa has been used medically for thousands of years in China. As a main component in several traditional Chinese medicines used to manage mental disorders, such as Xiaoyao-san, it has been frequently used in the treatment of depression since ancient time (Chen an and Tang, 2004). Previously, we demonstrated that curcumin, the major active ingredient isolated from *C. longa*, produced an antidepressive-like effect in the tail suspension test and forced swimming test in mice (Xu et al., 2005a), two predictive models widely used for assessing the antidepressant efficacy. In addition, chronic administration of curcumin could reverse the behavioral deficit

* Corresponding author. Tel.: +86 10 82802863; fax: +86 10 62179119.
E-mail address: xjli@bjmu.edu.cn (X.-J. Li).

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in the Olfactory Bulbectomy rat model (Xu et al., 2005b), which not only further supported the antidepressive effect of curcumin, but indicated the involvement of monoamines (serotonin, noradrenaline and dopamine) in its possible pharmacological mechanisms. It is well known that serotonergic system plays a key role in the etiology and pharmacotherapy of depression (Pineyro and Blier, 1999). Moreover, many herbal medicines were found to inhibit the monoamine oxidase and improve the amount of brain monoamines (Viana et al., 2005; Machado et al., 2007). Our previous data also demonstrated that administration of curcumin produced an increase in concentrations of central monoamines, especially the serotonin levels, in the hippocampus and frontal cortex of mice (Xu et al., 2005a). This enhancement in the monoamines might result from the inhibition of monoamine oxidase activity, which will lead to an increase in the amount of monoamine stored and released from the nerve terminals (Dar and Khatoon, 2000). Currently, large scales of studies about the antidepressant mechanisms start to focus on the monoamine receptors and the post-receptor actions. In the tail suspension test, selective serotonin reuptake inhibitors fail to reduce the immobility time in 5-HT_{1A} receptor knock-out mice, suggesting a critical role of 5-HT_{1A} receptor in the antidepressant effect of SSRIs (Mayorga et al., 2001). Furthermore, there is accumulating evidence that some other 5-HT receptors, such as 5-HT_{1B}, 5-$HT_{2A/2C}$ receptors, are also involved in the mediation of the behavioral effects of the antidepressant compounds (O'Neill and Conway, 2001; Cryan and Leonard, 2000).

Considering that there has been only sparse information about the interaction between 5-HT receptors and the antidepressant-like effect of curcumin, the present study was designed to further evaluate the involvement of serotonin system, particularly 5-HT_1 and 5-HT_2 receptors, in the antidepressant effect of curcumin in the forced swimming test, by using specific receptor agonists and antagonists.

2. Materials and methods

2.1. Animals

Male ICR mice, weighing 20–25 g, were obtained from the Department of Laboratory Animal Science, Peking University Health Science Center (Beijing, China). Animals were housed 10 per cage at constant room temperature (22–27 °C) with a 12-h light/12-h dark cycle and ad libitum food and water. The manipulations were carried out in the light phase between 9:00 am and 15:00 pm, with each mouse used only once. All procedures in this study were performed in accordance with the National Institutes of Health Guide for the Care.

2.2. Drugs and treatment

The following drugs were used: curcumin, fluoxetine, *p*-chlorophenylalanine HCl (PCPA), (+)-8-hydroxy-2-(di-*n*-propylamino)tetralin HBr (8-OH-DPAT), 4-(2′-methoxy-phenyl)-1-[2′-(*n*-2″-pyridinyl)-*p*-iodobenzamino-]ethyl-piperazine hydrochloride (*p*-MPPI), R(-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI), ketanserin tartarate, pindolol, ritanserin (All from Sigma, USA), anpirtoline, (Tocris Cookson, UK), 1-(2-(1-pyrrolyl)-phenoxy)-3-isopropylamino-2-propanol (isamoltane; Tocris Cookson, UK).

For oral (p.o.) administration, curcumin was dissolved in peanut oil and diluted to the desired concentrations also by peanut oil on the day of experiment, while other drugs were dissolved in saline except pindolol and ritanserin which were diluted in saline with 1% Tween 80. All the receptor agonists and antagonists were injected intraperitoneally (i.p.) in a constant volume of 10 ml/kg body weight, except *p*-MPPI which was administered subcutaneously (s.c.). Control animals received vehicles (saline or peanut oil) only. When curcumin was given in combination with other drugs, appropriate control groups receiving both vehicles (saline and peanut oil) were assessed simultaneously.

In this study, mice were administered with curcumin or fluoxetine 45 min and 30 min before the test respectively. In PCPA pre-treated tests, mice received the injection of PCPA (100 mg/kg, i.p.) once a day for 4 consecutive days. After the last injection of PCPA, mice were treated with fluoxetine (30 mg/kg, i.p.) or curcumin (10 mg/kg, p.o.) and tested in forced swimming test 30 and 45 min later respectively.

In a separate series of experiments, mice were pre-treated with pindolol (10 mg/kg, i.p.), *p*-MPPI (1 mg/kg, s.c.) or isamoltane (2.5 mg/kg, i.p.) 15 min before the administration of curcumin (10 mg/kg, p.o.), and were submitted to the test 45 min later.

Alternatively, 8-OH-DPAT (1 mg/kg, i.p.), anpirtoline (0.25 mg/kg, i.p.), ritanserin (4 mg/kg, i.p.), ketanserin (5 mg/kg, i.p.) or DOI (1 mg/kg, i.p.) were given to mice 15 min before the administration of sub-effective doses of curcumin (2.5 mg/kg), and the test was carried out 45 min later.

Doses and administration schedules of the agents used here were chosen on the basis of our previous results as well as the literature data and were reported not to increase locomotor activity (Redrobe and Bourin, 1999, Dias Elpo Zomkowski et al., 2004; Kaster et al., 2005; Yalcin et al., 2005; Guilloux et al., 2006).

2.3. Forced swimming test

The forced swimming test adopted here is a modification of the method described by Porsolt et al. (1977). As described previously (Xu et al., 2005a), mice were individually forced to swim for 15 min in glass cylinders (height: 25 cm, diameter: 10 cm), containing 19 cm of water at 25 °C, which is a pre-test, and then mice were removed and dried before being returned to cages. Twenty-four hours later, mice were placed in the cylinders again for a 6-min test in the same system depicted above. The duration of immobility was recorded during the last 4 min of the 6-min testing period.

2.4. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) or two-way ANOVA followed by Student–Newman–Keuls analysis for multiple comparisons when appropriate ($P<0.05$

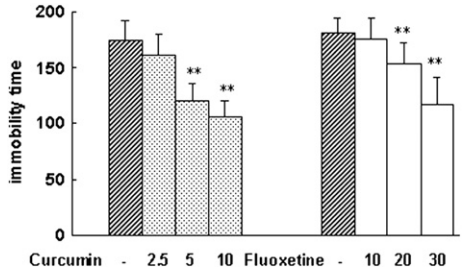

Fig. 1. Effect of administration of curcumin and fluoxetine in forced swimming test. Mice were given vehicles, curcumin (2.5, 5, 10 mg/kg, p.o.) or fluoxetine (10, 20, 30 mg/kg, i.p.) respectively. All values are presented as mean±S.E.M. (n=10–12) **P<0.01, compared with the control group. Curcumin: F (3, 44)=46.54, P<0.01; fluoxetine: F (3, 40)=7.45, P<0.01.

and 0.01). All statistical procedures were carried out using SPSS version 13.0.

3. Results

3.1. The effect of curcumin and fluoxetine on the duration of immobility in the forced swimming test

Fig. 1 shows the effect of administration of curcumin (2.5, 5, 10 mg/kg) and fluoxetine (10, 20, 30 mg/kg) in the forced swimming test. Post hoc analysis revealed that curcumin, at doses of 5 and 10 mg/kg, and fluoxetine, at doses of 20 and 30 mg/kg, led to a significant reduction in time spent immobile compared to the control group respectively (P<0.01).

3.2. Effect of pre-treatment with PCPA on the antidepressant-like effect of curcumin in the forced swimming test

The results in Fig. 2 show that PCPA alone (100 mg/kg, once a day, for 4 consecutive days) did not modify the immobility time, while pre-treatment of mice with PCPA significantly blocked the reduction in the immobility time elicited by fluoxetine (30 mg/kg, i.p.) and curcumin (10 mg/kg, p.o.) in the forced swimming test (P<0.01).

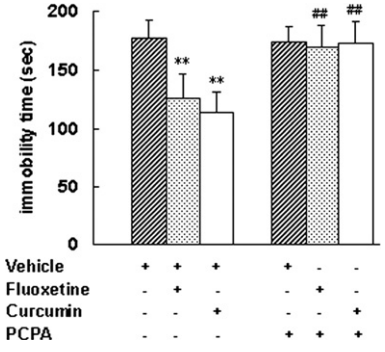

Fig. 2. Effect of pre-treatment of mice with PCPA (5-HT synthesis inhibitors, 100 mg/kg, i.p., for 4 consecutive days) on the fluoxetine (30 mg/kg, i.p.) and curcumin (10 mg/kg, p.o.) induced decrease in the immobility time in the forced swimming test. All values are presented as mean±S.E.M. (n=10–12) **P<0.01, compared with the control group; $^{\#\#}P$<0.01, compared with the same group pre-treated with only curcumin or fluoxetine. Pre-treatment (PCPA or saline): F (1, 66)=67.30, P<0.01; treatment (fluoxetine or curcumin): F (2, 66)=22.93, P<0.01; interaction: F (2, 66)=21.19, P<0.01.

3.3. Interaction of curcumin with 5-HT$_{1A}$ receptors in the forced swimming test

Fig. 3A shows that pre-treatment of mice with pindolol (10 mg/kg, i.p.) antagonized the anti-immobility effect of

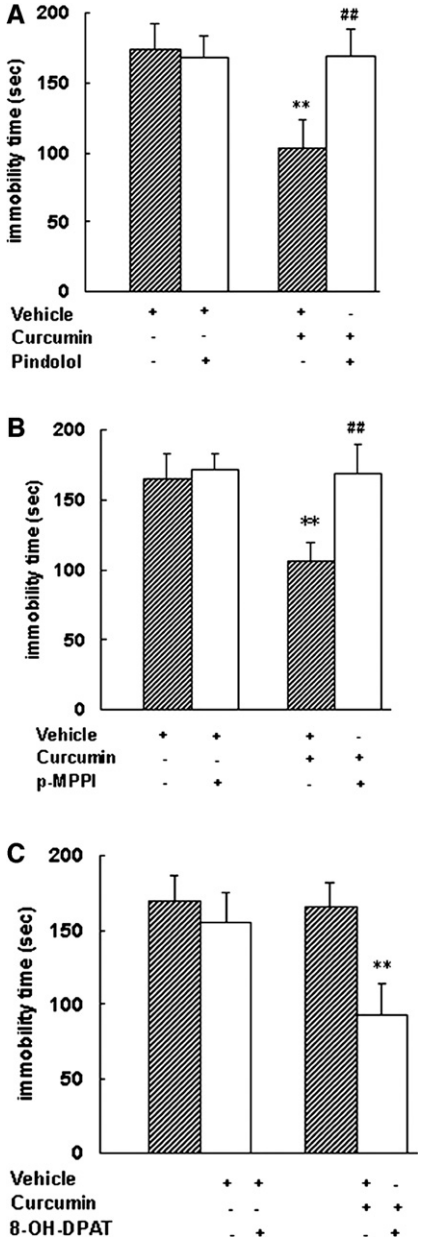

Fig. 3. Effect of pre-treatment of mice with pindolol (10 mg/kg, i.p., a 5-HT$_{1A/1B}$ receptor antagonist, panel A) or p-MPPI (1 mg/kg, s.c., a selective 5-HT$_{1A}$ receptor antagonist, panel B) on the curcumin-elicited (10 mg/kg, p.o.) decrease in the immobility time in the forced swimming test. Effect of 8-OH-DPAT (1 mg/kg, i.p., a 5-HT$_{1A}$ receptor agonist, panel C) combined with a sub-effective dose of curcumin (2.5 mg/kg, p.o.) in the forced swimming test. **P<0.01, compared with the control group; $^{\#\#}P$<0.01, compared with the same group pre-treated with vehicles only. A: pre-treatment: F (1, 44)=42.33, P<0.01; treatment: F (1, 44)=33.28, P<0.01; interaction: F (1, 44)=46.32, P<0.01. B: pre-treatment: F (1, 44)=40.67, P<0.01; treatment: F (1, 44)=52.10, P<0.01; interaction: F (1, 44)=33.77, P<0.01. C: pre-treatment: F (1, 44)=63.42, P<0.01; treatment: F (1, 44)=37.15, P<0.01; interaction: F (1, 44)=30.48, P<0.01.

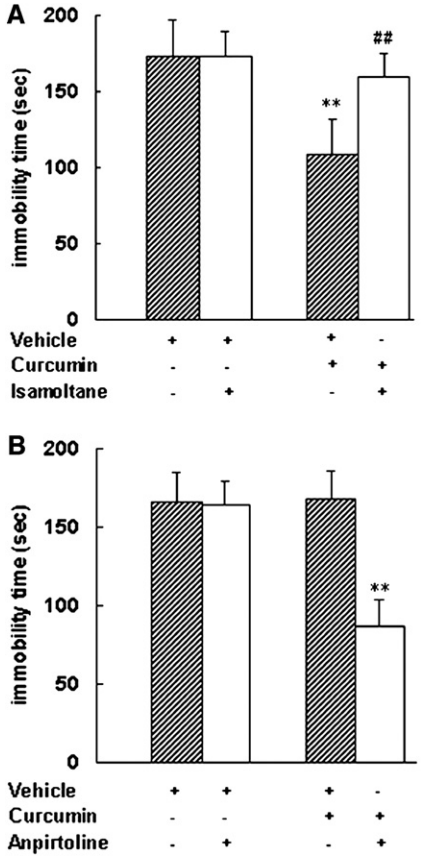

Fig. 4. Effect of pre-treatment of mice with isamoltane (2.5 mg/kg, i.p., a 5-HT_{1B} receptor antagonist, panel A) on the curcumin (10 mg/kg, p.o.) induced reduction in the forced swimming test. The potentiation of anpirtoline (0.25 mg/kg, i.p., a 5-HT_{1B} receptor agonist, panel B) on the effect of a sub-effective dose of curcumin (2.5 mg/kg, p.o.) in the forced swimming test was shown. Values are expressed as mean±S.E.M. (n=10–12). **P<0.01 compared with the control group; $^{\#\#}P$<0.01, compared with the same group pre-treated with saline only. A: pre-treatment: F (1, 42)=18.61, P<0.01; treatment: F (1, 42)=41.85, P<0.01; interaction: F (1, 42)=19.87, P<0.01. B: pre-treatment: F (1, 44)=72.47, P<0.01; treatment: F (1, 44)=60.10, P<0.01; interaction: F (1, 44)=63.76, P<0.01.

curcumin (10 mg/kg, p.o.) in the forced swimming test. Also, the reversal of the antidepressant-like effect of curcumin by pre-treatment of mice with *p*-MPPI (1 mg/kg, s.c.) in the forced swimming test is shown in Fig. 3B.

Fig. 3C shows that prior administration of mice with a sub-effective dose of 8-OH-DPAT (1 mg/kg, i.p.) could produce a synergistic effect with curcumin (2.5 mg/kg, p.o.) in the forced swimming test.

3.4. Interaction of curcumin with 5-HT_{1B} receptors in the forced swimming test

As presented in Fig. 4A, pre-treatment of isamoltan (2.5 mg/kg, i.p.) blocked the effect of curcumin (10 mg/kg, p.o.) in the forced swimming test. On the other hand, the results depicted in Fig. 4B indicate that the administration of a sub-effective dose of anpirtoline (0.25 mg/kg, i.p.) produced a synergistic effect when combined with a sub-effective dose of curcumin (2.5 mg/kg, p.o.) in the forced swimming test.

3.5. Interaction of curcumin with 5-HT_2 receptors

The results presented in Fig. 5A show that administration of ritanserin (4 mg/kg, i.p.) could potentiate the effect of a sub-effective dose of curcumin (2.5 mg/kg, p.o.) in the forced swimming test. However, in Fig. 5B, the results show that pre-treatment of ketanserin (5 mg/kg, i.p.) didn't exert influence on the effect of a sub-effective dose of curcumin (2.5 mg/kg, p.o.)

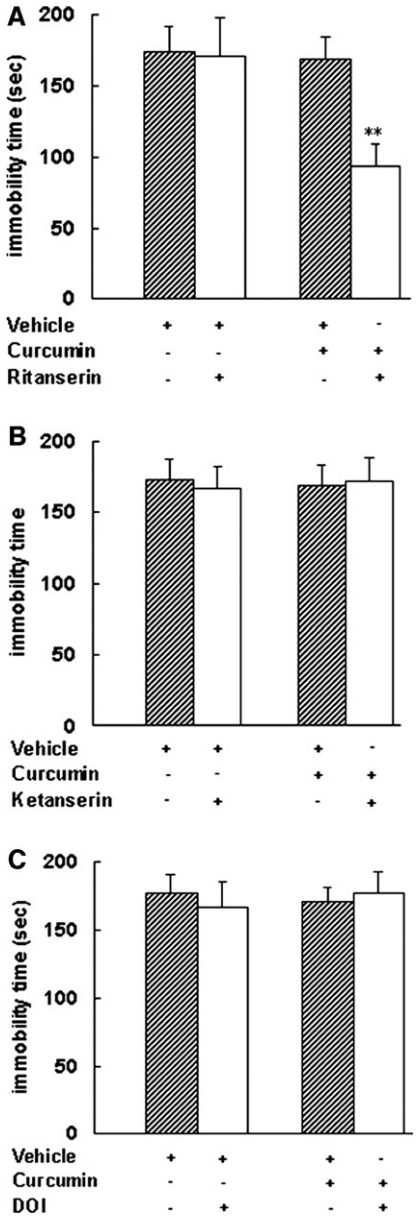

Fig. 5. Effect of pre-treatment of ritanserin (4 mg/kg, i.p., a 5-$HT_{2A/2C}$ receptor antagonist, panel A), ketanserin (5 mg/kg, i.p., a 5-$HT_{2A/2C}$ receptor antagonist, panel B) or DOI (1 mg/kg, s.c., a 5-HT_{2A} receptor agonist, panel C) at the presence of sub-active doses of curcumin (2.5 mg/kg, p.o.) in the forced swimming test. Values are expressed as mean±S.E.M. (n=10–12). **P<0.01 compared with the control group. A: pre-treatment: F (1, 44)=46.74, P<0.01; treatment: F (1, 44)=50.91, P<0.01; interaction: F (1, 36)=38.66, P<0.01). B: pre-treatment: F (1, 44)=0.72, P=0.719; treatment: F (1, 44)=0.93, P=0.01; interaction: F (1, 44)=1.04, P=0.58. C: pre-treatment: F (1, 36)=0.23, P=0.633; treatment: F (1, 36)=0.12, P=0.745; interaction: F (1, 28)=3.17, P=0.083.

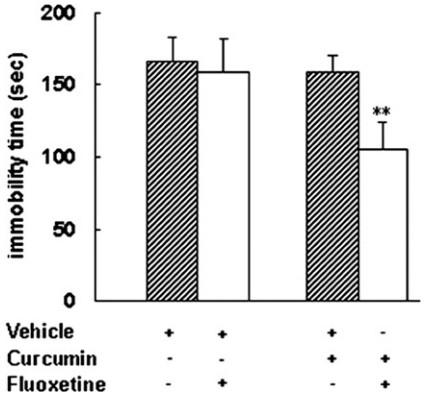

Fig. 6. Effect of prior administration of mice with a sub-active dose of curcumin (2.5 mg/kg, p.o.) on the action of an ineffective dose of fluoxetine (10 mg/kg, i.p.) in the forced swimming test. Values are expressed as mean±S.E.M. (n=10–12). **P<0.01 compared with the control group. Pre-treatment: F (1, 40)=32.07, P<0.01; treatment: F (1, 40)=32.22, P<0.01; interaction: F (1, 40)=18.61, P<0.01.

in the forced swimming test. Similarly, as shown in Fig. 5C, prior treatment of DOI (1 mg/kg, i.p.) was not able to augment the effect of a sub-effective dose of curcumin (2.5 mg/kg, p.o.).

3.6. Interaction of curcumin with fluoxetine in the forced swimming test

As presented in Fig. 6, pre-treatment of mice with a sub-effective dose of curcumin (2.5 mg/kg, p.o.) could potentiate the effect of a sub-optimal dose of fluoxetine (10 mg/kg, i.p.) in the forced swimming test (P<0.01).

4. Discussion

Our previous studies have demonstrated that curcumin produces a significant inhibition of the duration of immobility in mice in the forced swimming test without changing locomotor activity (Xu et al., 2005a). Considering the central role played by serotonin in the pathology of depression and the relative importance of 5-HT_1 and 5-HT_2 receptors to the serotonergic antidepressant responses (Hoyer et al., 2002; Cryan et al., 2005), we designed the present experiments to study the involvement of 5-HT system in the antidepressant-like effect of curcumin in the forced swimming test. Based on the results, the antidepressant-like effect of curcumin in the forced swimming test may involve 5-HT receptors, especially 5-$HT_{1A/1B}$ and 5-HT_{2C} subtypes.

The forced swimming test, also known as the "behavior despair" test, is world-widely used as a reliable animal model of depression to screen new antidepressants as well as to investigate the mechanisms underlying the action of antidepressants (Borsini, 1995). A wide variety of studies have shown that this test is highly sensitive to major classes of the clinically effective antidepressants, including the selective serotonin reuptake inhibitors, the tricyclic antidepressants and the monoamine oxidase inhibitors (Detke et al., 1995). The present result replicated our previous report about the antidepressant-like effect of curcumin in the forced swimming test (Xu et al., 2005a). However, this effect was blocked by the treatment of the tryptophan hydroxylase inhibitor PCPA. Although we did not measure the brain 5-HT level after depletion, according to previous literatures and our behavioral studies, administrating PCPA at the present dose for four consecutive days is able to deplete the endogenous store of serotonin successfully without affecting the noradrenaline and dopamine levels (Eckeli et al., 2000; Dias Elpo Zomkowski et al., 2004). In our experiments, PCPA alone did not affect the immobility time of mice in the forced swimming test, but pre-treatment with PCPA could prevent the anti-immobility effect of curcumin and fluoxetine dramatically. These results are in conformity with earlier studies showing that PCPA treatment completely prevented the antidepressant effect of fluoxetine, a serotonin reuptake inhibitor, while it only partially reversed the effect of imipramine, an inhibitor of serotonin and noradrenaline (Page et al., 1999; Eckeli et al., 2000). Therefore, our results demonstrate that serotonergic system may be implicated in the antidepressant-like effect of curcumin in the forced swimming test.

To further investigate the mechanism of the action of curcumin in the forced swimming test, we pre-treated the mice with specific receptor antagonists/agonists to different 5-HT receptor subtypes. Among the 14 known 5-HT receptor subtypes, 5-HT_{1A} receptors get the most attention mainly due to their pivotal role in the pathogenesis of depression as well as the antidepressant response. The clinical reports have pointed out that low levels of 5-HT_{1A} receptors represent a risk factor in mood disorders (O'Neill and Conway, 2001; Cryan et al., 2005). Additionally, in wild type mice, fluoxetine significantly reduces the immobility in the tail suspension test while the drug has no effect on the 5-HT_{1A} receptor knock-out mice (Toth, 2003), indicating that 5-HT_{1A} receptor may be related to the antidepressant responses of selective serotonin reuptake inhibitors in behavioral models of depression. In order to study the possible involvement of 5-HT_{1A} receptors in the antidepressant-like effect of curcumin, pindolol, a 5-$HT_{1A/1B}$ receptor antagonist, and p-MPPI, a selective 5-HT_{1A} receptor antagonist, were used in the present study.

As a 5-HT $_{1A/1B}$ receptor antagonist, pindolol fully blocked the antidepressant-like effect of curcumin in the present study. Although pindolol is also known to block β-adrenoceptor, it is unlikely that this property is related to the effect of curcumin, as β-adrenoceptor inhibitor has been reported to increase the incidence of depression (Avorn et al., 1986). More importantly, the pre-treatment of mice with p-MPPI could reverse the antidepressant-like effect of curcumin in the forced swimming test, which further reinforced the implication of 5-HT_{1A} receptor in the action of curcumin. p-MPPI is a selective 5-HT_{1A} receptor antagonist with high affinity for 5-HT_{1A} receptors, and it has no agonist activity for pre- and post-synaptic 5-HT_{1A} receptors in both in vivo and in vitro tests (Kung et al., 1994, 1995; Thielen et al., 1996; Martin et al., 1999). Furthermore, 8-OH-DPAT, a selective 5-HT_{1A} receptor agonist, produced a synergistic antidepressant-like effect with a sub-effective dose of curcumin. In this experiment, we chose the sub-effective doses of both agents to study the interaction, as 8-OH-DPAT has significant anti-immobility effect in the forced

swimming test and has been found to enhance the activity of antidepressants in the test (O'Neill and Conway, 2001; Redrobe et al., 1996). Taken together, it appears that 5-HT_{1A} receptor is intimately involved in the antidepressant-like effect of curcumin. Since the pre-treatment of neurotoxins PCPA or 5, 7-DHT, which are believed to act pre-synaptically, has no effect on the anti-immobility effect of 8-OH-DPAT in mice in the forced swimming test, the anti-immobility response of 8-OH-DPAT is proposed to be mediated through post-synaptic 5-HT_{1A} receptor (Luscombe et al., 1993). Thus, the synergistic effect of 8-OH-DPAT observed here may indicate the involvement of post-synaptic 5-HT_{1A} receptors in the action of curcumin in the forced swimming test.

In the present study, an additional experiment was conducted to explore the possible participation of 5-HT_{1B} receptors in the effect of curcumin in the forced swimming test. 5-HT_{1B} receptors act as terminal receptors and are implicated in the control of synaptic neurotransmission, serotonin included (Martin et al., 1992; Bosker et al., 1995). Multiple studies have suggested that 5-HT_{1B} receptors are involved in the mediation of antidepressant-like effects in behavioral tests (Davidson and Stamford, 1995; O'Neill et al, 1996; O'Neill and Conway, 2001). In our experiments, it was found that the pre-treatment of 5-HT_{1B} receptor antagonist isamoltane antagonized the anti-immobility effect of curcumin in the test. Previously, it has been reported that 5-HT_{1B} receptor antagonists reverse the antidepressant effects of imipramine and paroxetine in mice in the forced swimming test (O'Neill and Conway, 2001; Gardier et al., 2001). In the 5-HT_{1B} knock-out mice, the anti-immobility effect of selective serotonin reuptake inhibitors disappears, suggesting that their antidepressant effects might be mediated by the activation of 5-HT_{1B} receptors in the forced swimming test (Gardier et al., 2001). Moreover, the combination of sub-optimal doses of 5-HT_{1B} receptor agonist anpirtoline and curcumin was found to produce a significant increase in the swimming time. Indeed, this finding is consistent with the previous results showing that anpirtoline was able to potentiate the effect of lithium in mice (Redrobe and Bourin, 1999). Pre-treatment of mice with 5-HT_{1B} receptor agonist RU24969 was also found to potentiate the effect of compounds known to act on the serotonergic system, such as imipramine and fluoxetine (Redrobe et al., 1996). In the current study, inactive doses of curcumin and anpirtoline were chosen for use in interaction studies. Although anpirtoline can increase the motor activity, the dose adopted here (0.25 mg/kg) has been demonstrated not to affect the performance of mice in a locomotor activity paradigm (Redrobe and Bourin, 1999). Therefore, this synergistic effect in the current study seems to be mediated via 5-HT_{1B} receptor activation. Taken together, the obtained results provide strong evidence that 5-HT_{1B} receptors seem to play a role in the antidepressant-like effect of curcumin in the forced swimming test.

Recent preclinical and clinical studies have reported a key role for 5-HT_2 receptors in the pathology of depression as well as the action of many antidepressants (Cryan and Leonard, 2000; Cryan and Lucki, 2000; Boothman et al., 2006). Moreover, many established antidepressants are effective 5-HT2 receptor antagonists (Deakin, 1988) and 5-$HT_{2A/2C}$ receptor antagonists are found to enhance the antidepressant-like effects of selective serotonin reuptake inhibitors when given jointly (Redrobe and Bourin, 1997; Redrobe and Bourin, 1998), which suggests that the antagonism of these receptors may be implicated in the action of such antidepressants. In the present study, activation of 5-HT_{2A} receptors by DOI, a preferential 5-HT_{2A} receptor agonist, did not produce any additive effect in the presence of curcumin in the forced swimming test, indicating that the antidepressant-like effect of curcumin may not be mediated by an agonistic activity at the 5-HT_{2A} receptors. Indeed, earlier study has already suggested that agonism at such receptors is not important in the anti-immobility effects of antidepressants (Redrobe and Bourin, 1997). On the other hand, pre-treatment with ritanserin, a 5-$HT_{2A/2C}$ receptor antagonist, but not ketanserin, a 5-$HT_{2A/2C}$ receptor antagonist with higher affinity for 5-HT_{2A} receptor than for 5-HT_{2C} receptor, was able to potentiate the anti-immobility effect of curcumin. These results suggest that the effect of curcumin in reducing the immobility time in forced swimming test can be partially attributed to 5-HT_{2C} rather than 5-HT_{2A} receptors. Actually, the potentiating effect could be explained by the functional interaction, previously described, between 5-HT_{2C} and 5-HT_{1A} receptors (Darmani et al., 1990; Borsini, 1994). It has already been reported that the blockade of 5-HT_2 receptors induces effects similar to those seen after 5-HT_{1A} receptors activation (Berendsen, 1995). Moreover, a disturbed balance between 5-HT_{2C} and 5-HT_{1A} receptors may contribute to the etiology of depression and this balance has also been emphasized in the action of antidepressants in the forced swimming test (Berendsen, 1995; Redrobe and Bourin, 1997). Taking into account that inconsistent data have been reported about the responses when antidepressants are given in combination with 5-$HT_{2A/2C}$ receptor antagonists (Cryan and Lucki, 2000; Dias Elpo Zomkowski et al., 2004), we also evaluated the effects of an active dose of curcumin (10 mg/kg, p.o.) in the test in combination with ritanserin and ketanserin, and were not able to observe any blocking effect (date was not shown). Together, these results may not only suggest that 5-HT_{2C} antagonism has a significant role in the mechanism underlying the antidepressant-like effect of curcumin, but also, to some extent, confirm our hypothesis about the involvement of 5-HT_{1A} receptors in the action of curcumin.

It should also be noted that curcumin administered at a sub-effective dose was able to potentiate the action of fluoxetine in the forced swimming test. The synergistic effect further reinforces that the effects of curcumin in the forced swimming test are related to 5-HT receptor subtypes and curcumin is possibly to share a similar mechanism of producing antidepressant-like effect with selective serotonin reuptake inhibitors. Since selective serotonin reuptake inhibitors are a mainstay in the treatment of depression, this synergistic effect may also indicate an important therapeutic value of curcumin.

In summary, the present data indicate that the antidepressant-like effect of curcumin in the forced swimming test seems to be mediated through an interaction with 5-$HT_{1A/1B}$ and 5-HT_{2C} receptors. Moreover, the synergistic effect induced by co-

administration of sub-effective doses of curcumin and fluoxetine also provides us a clue to the potential therapeutic value of curcumin. Further studies are recently being conducted in our laboratory to examine detailed mechanisms, such as the post-receptor action and signal transduction pathway, underlying the antidepressant-like effects of curcumin.

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References

Avorn, J., Everitt, D.E., Weiss, S., 1986. Increased antidepressant use in patients prescribed beta-blockers. J. Am. Med. Assoc. 255, 357–360.

Berendsen, H.H., 1995. Interactions between 5-hydroxytrptamine receptor subtypes: is a disturbed receptor balance contributing to the symptomatology of depression in humans? Pharmacol. Ther. 66, 17–37.

Borsini, F., 1994. Balance between cortical 5-HT_{1A} and 5-HT_2 receptor function: hypothesis for a faster antidepressant action. Pharmacol. Res. 30, 1–11.

Borsini, F., 1995. Role of serotonergic system in the forced swimming test. Neurosci. Biobehav. Rev. 19, 377–395.

Boothman, L.J., Mitchell, S.N., Sharp, T., 2006. Investigation of the SSRI augmentation properties of 5-HT_2 receptor antagonists using in vivo microdialysis. Neuropharmacology 50, 726–732.

Bosker, F.J., van Esseveldt, K.E., Klompmakers, A.A., Westenberg, H.G., 1995. Chronic treatment with fluvoxamine by osmotic minipumps fails to induce persistent functional changes in central 5-HT_{1A} and 5-HT_{1B} receptors, as measured by in vivo microdialysis in dorsal hippocampus of conscious rats. Psychopharmacology (Berl) 117, 358–363.

Chen, J.X., Tang, Y.T., 2004. Effect on Xiaoyao powder on changes of relative brain zone CRF gene expression in chronic restrained stress rats. Chin. J. Appl. Physiol. 207, 1–4.

Cryan, J.F., Leonard, B.E., 2000. 5-HT_{1A} and beyond: the role of serotonin and its receptors in depression and the antidepressant response. Hum. Psychopharmacol. 15, 113–135.

Cryan, J.F., Lucki, I., 2000. Antidepressant-like behavior effects mediated by 5-hydroxytryptamine (2C) receptors. J. Pharmacol. Exp. Ther. 295, 1120–1126.

Cryan, J.F., Valentino, R.J., Lucki, I., 2005. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci. Biobehav. Rev. 29, 547–569.

Dar, A., Khatoon, S., 2000. Behavioral and biochemical studies of dichloromethane fraction from the *Areca catechu* Nut. Pharmacol. Biochem. Behav. 65, 1–6.

Darmani, N.A., Martin, B.R., Pandey, U., Glennon, R.A., 1990. Do functional relationships between 5-HT_{1A} and 5-HT_2 receptors? Pharmacol. Biochem. Behav. 36, 901–906.

Davidson, C., Stamford, J.A., 1995. The effect of paroxetine on 5-HT efflux in the rat dorsal raphe nucleus is potentiated by both 5-HT_{1A} and 5-$HT_{1B/D}$ receptor antagonists. Neurosci. Lett. 188, 41–44.

Deakin, J.F., 1988. 5-HT_2 receptors, depression and anxiety. Pharmacol. Biochem. Behav. 29, 819–820.

Detke, M.J., Richels, M., Lucki, I., 1995. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmachology 121, 66–72.

Dias Elpo Zomkowski, A., Oscar Rosa, A., Lin, J., Santos, A.R., Calixto, J.B., Lucia Severo Rodrigues, A., 2004. Evidence for serotonin receptor subtypes involvement in agmatine antidepressant-like effect in the mouse forced swimming test. Brain Res. 1023, 253–263.

Eckeli, A.L., Dach, F., Rodrigues, A.L., 2000. Acute treatment with GMP produce antidepressant-like effects in mice. NeuroReport 11, 1839–1843.

Gardier, A.M., Trillat, A., Malagie, I., David, D., HascoJt, M., Colombel, M., Jolliet, P., Jacquot, C., Hen, R., Bourin, M., 2001. Recepteurs 5-HT_{1B} de la serotonine et effets antidepresseurs des inhibiteurs derecapture selectifs de la serotonine. C. R. Acad. Sci., Ser. III 324, 433–441.

Guilloux, J.P., David, D.J., Guiard, B.P., Chenu, F., Reperant, C., Toth, M., Bourin, M., Gardier, A.M., 2006. Blockade of 5-HT1A receptors by (+/−)-pindolol potentiates cortical 5-HT outflow, but not antidepressant-like activity of paroxetine: microdialysis and behavioral approaches in 5-HT_{1A} receptor knockout mice. Neuropsychopharmacology 31, 2162–2172.

Hoyer, D., Hannon, J.P., Martin, G.R., 2002. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol. Biochem. Behav. 71, 533–554.

Kaster, M.P., Santos, A.R., Rodrigues, A.L., 2005. Involvement of 5-HT_{1A} receptors in the antidepressant-like effect of adenosine in the mouse forced swimming test. Brain Res. Bull. 67, 53–61.

Kessler, R.C., Soukup, J., Davis, R.B., Foster, D.F., Wilkey, S.A., Van Rompay, M.I., Eisenberg, D.M., 2001. The use of complementary and alternative therapies to treat anxiety and depression in the United States. Am. J. Psychiatry 158, 289–294.

Kung, H.F., Kung, M.P., Clarke, W., Maayani, S., Zhuang, Z.P., 1994. A potential 5-HT_{1A} receptor antagonist: *p*-MPPI. Life Sci. 55, 1459–1462.

Kung, M.P., Frederick, D., Mu, M., Zhuang, Z.P., Kung, H.F., 1995. 4-(2′-Methoxy-phenyl)-1-[2′-(*n*-2″-pyridinyl)-*p*-iodobenzamido]-ethylpiperazine ([125I]*p*-MPPI) as a new selective radioligand of serotonin-1A sites in rat brain: in vitro binding and autoradiographic studies. J. Pharmacol. Exp. Ther. 272, 429–437.

Luscombe, G.P., Martin, K.F., Hutchins, L.J., Gosden, J., Heal, D.J., 1993. Mediation of the antidepressant-like effect of 8-OH-DPAT in mice by postsynaptic 5-HT_{1A} receptors. Br. J. Pharmacol. 108, 669–677.

Machado, D.G., Kaster, M.P., Binfare, R.W., Dias, M., Santos, A.R., Pizzolatti, M.G., Brighente, I.M., Rodrigues, A.L., 2007. Antidepressant-like effect of the extract from leaves of *Schinus molle* L. in mice: evidence for the involvement of the monoaminergic system. Prog. Neuro-psychopharmacol. Biol. Psychiatry 31, 421–428.

Martin, K.F., Hannon, S., Phillips, I., Heal, D.J., 1992. Opposing roles for 5-HT_{1B} and 5-HT_3 receptors in the control of 5-HT release in rat hippocampus in vivo. Br. J. Pharmacol. 106, 139–142.

Martin, L.P., Jackson, D.M., Wallsten, C., Waszczak, B.L., 1999. Electrophysiological comparison of 5-hydroxytryptamine1A receptor antagonists on dorsal raphe cell firing. J. Pharmacol. Exp. Ther. 288, 820–826.

Mayorga, A.J., Dalvi, A., Page, M.E., Zimov-Levinson, S., Hen, R., Lucki, I., 2001. Antidepressant-like behavioral effects in 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B) receptor mutant mice. J. Pharmacol. Exp. Ther. 298, 1101–1107.

Nemeroff, C.B., 2007. The burden of severe depression: A review of diagnostic challenges and treatment alternatives. J. Psychiatri. Res. 41, 189–206.

O'Neill, M.F., Fernandez, A.G., Palacios, J.M., 1996. GR127935 blocks the locomotor and antidepressant-like effects of RU24969 and the action of antidepressants in the mouse tail suspension test. Pharmacol. Biochem. Behav. 53, 535–539.

O'Neill, M.F., Conway, M.W., 2001. Role of 5-HT_{1A} and 5-HT_{1B} receptors in the mediation of behavior in the forced swim test in mice. Neuropsychopharmacology 24, 391–398.

Page, M.E., Detke, M.J., Dalvi, A., Kirby, L.G., Lucki, I., 1999. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology 147, 162–167.

Pineyro, G., Blier, P., 1999. Autoregulation of serotonin neurons: role in antidepressant drug action. Pharamcol. Rev. 51, 534–591.

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327–336.

Redrobe, J.P., Bourin, M., 1997. Partial role of 5-HT_2 and 5-HT_3 receptors in the activity of antidepressants in the mouse forced swimming test. Eur. J. Pharmacol. 325, 129–135.

Redrobe, J.P., Bourin, M., 1998. Clonidine potentiates the effects of 5-HT_{1A}, 5-HT_{1B} and 5-$HT_{2A/2C}$ receptor antagonists and 8-OH-DPAT in the mouse forced swimming test. Eur. Neuropsychopharm. 8, 169–173.

Redrobe, J.P., Bourin, M., 1999. Evidence of the activity of lithium on 5-HT_{1B} receptors in the mouse forced swimming test: comparison with carbamazepine and sodium valproate. Psychopharmacology 141, 370–377.

Redrobe, J.P., MacSweeney, C.P., Bourin, M., 1996. The role of 5-HT_{1A} and 5-HT_{1B} receptors in antidepressant drug actions in the mouse forced swimming test. Eur. J. Pharmacol. 318, 213–220.

Rosenzweig-Lipson, S., Beyer, C.E., Hughes, Z.A., Khawaja, X., Rajarao, S.J., Malberg, J.E., Rahman, Z., Ring, R.H., Schechter, L.E., 2007. Differentiating antidepressants of the future: Efficacy and safety. Pharmacol. Ther. 113, 134–153.

Thachil, A.F., Mohan, R., Bhugra, D., 2007. The evidence base of complementary and alternative therapies in depression. J. Affect. Disord. 97, 23–35.

Thielen, R.J., Fangon, N.B., Frazer, A., 1996. 4-(2′-Methoxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-iodobenzamido]ethyl] piperazine and 4-(2′-methoxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]-piperazine, two new antagonists at pre-and post-synaptic serotonin-1A receptors. J. Pharmacol. Exp. Ther. 277, 661–670.

Toth, M., 2003. 5-HT_{1A} receptor knockout mouse as a genetic model of anxiety. Eur. J. Pharmacol. 463, 177–184.

Viana, A.F., do Rego, J.C., von Poser, G., Ferraz, A., Heckler, A.P., Costentin, J., Kuze Rates, S.M., 2005. The antidepressant-like effect of *Hypericum caprifoliatum* Cham & Schlecht (Guttiferae) on forced swimming test results from an inhibition of neuronal monoamine uptake. Neuropharmacology 49, 1042–1052.

Xu, Y., Ku, B.S., Yao, H.Y., Lin, Y.H., Ma, X., Zhang, Y.H., Li, X.J., 2005a. The effects of curcumin on depressive-like behaviors in mice. Eur. J. Pharmacol. 518, 40–46.

Xu, Y., Ku, B.S., Yao, H.Y., Lin, Y.H., Ma, X., Zhang, Y.H., Li, X.J., 2005b. Antidepressant effects of curcumin in the forced swim test and olfactory bulbectomy models of depression in rats. Pharmacol. Biochem. Behav. 82, 200–206.

Yalcin, I., Aksu, F., Belzung, C., 2005. Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. Eur. J. Pharmacol. 514, 165–174.