Antidepressant-like effect of trans-resveratrol: Involvement of serotonin and noradrenaline system

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trans-Resveratrol; Forced swimming; Tail suspension; Serotonin; Noradrenaline; MAO

\textbf{Abstract}
The antidepressant-like effect of trans-resveratrol, a phenolic compound present in polygonum cuspidatum, was evaluated through behavioral and neurochemical methods. trans-Resveratrol (20, 40 and 80 mg/kg, via gavage) significantly decreased the immobility time in mouse models of despair tests, but did not influence locomotor activity. Two behavioral models and neurochemical assays suggested that trans-resveratrol produced a significant increase in serotonin and noradrenaline levels at 40 or 80 mg/kg in brain regions. In addition, trans-resveratrol dose dependently inhibited MAO-A activity. These findings indicate that the antidepressant-like effect of trans-resveratrol might be related to serotonergic and noradrenergic activation.

\textbf{1. Introduction}
Affective disorders are a major cause of morbidity and mortality in children and adolescents, with an estimated prevalence rate in the United States of 8.3% (Skaer et al., 2009). Patients with major depression have symptoms that are reflected by changes in brain monoamine neurotransmitters, specifically noradrenaline (norepinephrine, NE) and 5-hydroxytryptamine (serotonin, 5-HT) (Blier and De Montigny, 1994; Dhingra and Sharma, 2006). The first-line option in the management of depressive illness is pharmacotherapy. At present, there are several types of classical antidepressants used in clinical practice, including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs) and serotonin and noradrenaline reuptake inhibitor (SNRI), that exert their...
antidepressant effects by increasing the levels of monoamines, such as serotonin and/or noradrenaline (Deniker, 1984). Many of these drugs can produce undesirable side effects and the mechanism of action has not been satisfactorily resolved (Xu et al., 2006); thus, identification of potent and safe therapeutic agents is still a significant need.

There are numerous herbal medicines that have been introduced into psychiatric practice because of greater compliance and milder side effects (Thachil et al., 2007). Polygonum cuspidatum is a plant used historically in Asia, known for its medicinal properties and traditionally used in the treatment of neuropsychiatric disorders, such as psychosocial stress, dementia and Parkinson’s disease (Tredici et al., 1999; Chen et al., 2007). The trans-isomer of resveratrol is the active ingredient of P. cuspidatum and is also found abundantly in the skin of red grapes and red wine (Bai et al., 2010). Researchers have suggested that trans-resveratrol demonstrates a variety of pharmacological activities including antioxidant, anti-inflammatory, neuroprotective properties and amelioration of learning and memory impairment (Tredici et al., 1999; Chen et al., 2007; Kumar et al., 2007; Ranney and Petro, 2009). Previous studies indicated that trans-resveratrol inhibits monoamine oxidase (MAO) isoform activity in C6 glial cells (Mazzio et al., 1998). MAOs are mitochondrial bound isoenzymes which catalyze the oxidative deamination of dietary amines and monoamine neurotransmitters, such as 5-HT, noradrenaline, dopamine and other trace amines. The development of MAO inhibitors has led to important breakthroughs in therapies for several neuropsychiatric disorders ranging from mood disorders to Parkinson’s disease (Bortolato et al., 2008). Recent studies showed that resveratrol is an inhibitor of noradrenaline and 5-HT uptake activity in rats (Yáñez et al., 2006a). However it remains unknown whether the antidepressant-like effects of trans-resveratrol are due to neurotransmitter changes and MAO inhibition.

In this study we examined the antidepressant-like effect of trans-resveratrol in mouse behavioral despair tasks. As the monoaminergic system is one of the most important targets in the pathophysiology and therapy of depression (Blier and De Montigny, 1994), we investigated the possible role of monoaminergics in the antidepressant-like effect of trans-resveratrol through various behavioral paradigms. In addition, the brain monoamine levels and MAO activity were also tested by neurochemical and biochemical assays to confirm the participation of monoamine transmitters in treatment involving trans-resveratrol.

2. Experimental procedures

2.1. Animals

Male ICR mice (20–22 g) were obtained from the Animal Center of Shanghai Branch, Chinese Academy of Sciences. Upon arrival, the mice were housed eight per cage and acclimatized to a colony room with controlled ambient temperature (22 ± 1 °C), humidity (50 ± 10%) and a 12 hour natural light/dark cycle. They were fed a standard diet, water was provided ad libitum and they acclimated 7 days before entry into the subsequent study. The experiments were performed with 10 mice per treatment group according to a randomized schedule. In behavioral tests, animals in every group were intermixed during the observation (10:00 h and 14:00 h) and the observers were unaware of the treatment conditions. All experiments were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), and approved by the Wenzhou Medical College Committee on Animal Care and Use.

2.2. Drugs and drug administration

trans-Resveratrol, imipramine hydrochloride, p-chlorophenylalanine HCl (PCPA, an inhibitor of serotonin synthesis), apomorphine hydrochloride, kynuramine dihydrobromide, 4-hydroxyquinoline, clorgyline, deprenyl, 5-hydroxytryptamine, noradrenaline, dopamine, 5-hydroxyindoleacetic acid (5-HIAA) and 4-dihydroxyphenylacetic acid (DOPAC) were purchased from Sigma Chemical Co. (USA). Moclomide hydrochloride and sodium carboxymethyl cellulose were provided by the Beijing Institute of Pharmacology and Toxicology (China). For oral administration (via gavage, i.g.), trans-resveratrol was dissolved in 0.5% sodium carboxymethyl cellulose and moclomide was dissolved in redistilled water on the day of testing. For intraperitoneal injection, imipramine and fluoxetine were dissolved in redistilled water.

The plasma and brain concentrations of trans-resveratrol peak at 20–30 min and maintain their levels up to 60 min after oral administration in mice (Sale et al., 2004). Accordingly, the behavioral and neurochemical tests were conducted 30 min after trans-resveratrol treatment. The effects of positive antidepressants such as moclobemide (20 mg/kg, i.g.), imipramine (10 mg/kg, i.p.) and fluoxetine (10 mg/kg, i.p.), were tested 1 h (moclomide) and 30 min (imipramine and fluoxetine) respectively, after administration of the drugs as previously described (Xu et al., 2005b; Wang et al., 2008).

2.3. Forced swimming test

The forced swimming test employed was similar to that described previously (Porsolt et al., 1977; Porsolt et al., 1978) with minor modification (Xu et al., 2005a). Briefly, mice performed a swimming-stress session for 15 min (pre-test), 24 h before being individually placed in glass cylinders (height: 25 cm; diameter: 10 cm; containing 10 cm of water at 24 ± 1 °C) for 6 min (test). A mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only small movements necessary to keep its head above water. The duration of observed immobility was recorded during the last 4 min of the 6-min testing period.

2.4. Tail suspension test

The tail suspension test was based on the method of Steru et al. (1985) as our previous work (Xu et al., 2005b). Animals were suspended 50 cm above the floor by means of an adhesive tape, placed approximately 1 cm from the tip of the tail. The time during which mice remained immobile was quantified during a test period of 6 min. Mice were considered immobile only when they hung passively and completely motionless.

2.5. Locomotor activity

The assessment of locomotor activity was carried out on mice using a slightly modified method (Xu et al., 2005b). Briefly, the locomotor activity of the mice was measured by an ambulometer with five activity chambers (JZZ98, Institute of Materia Medica, Chinese Academy of Medical Sciences, China). Mice were placed in the chambers and their paws contacted or disconnected the active bars producing random configurations that were converted into pulses. The pulses, which were proportional to the locomotor activity of the mice, were automatically recorded as the cumulative total counts of motor activity. Mice were placed in test chambers 5 min prior to the
evaluation for acclimatization and then locomotion counts were recorded for a period of 10 min.

2.6. Roles of the serotonergic and noradrenergic systems in the antidepressant-like effects of trans-resveratrol

2.6.1. The depletion of serotonin in the forced swimming and tail suspension tasks

To investigate whether the serotonergic system is involved in the antidepressant-like effect of trans-resveratrol, mice were pretreated with PCPA (300 mg/kg, i.p.) or vehicle (0.5% sodium carboxymethyl cellulose) once a day for three consecutive days before trans-resveratrol administration (Porsolt et al., 1978; Redrobe et al., 2005). On the fourth day, 30 min after trans-resveratrol administration the forced swimming and tail suspension tests were performed.

2.6.2. Antagonism of apomorphine-induced hypothermia and stereotypical behavior

To evaluate the possible involvement of trans-resveratrol on the noradrenergic system, different groups of mice received trans-resveratrol administration (10, 20, 40 and 80 mg/kg, i.g.). The animals were then injected with a high dose of apomorphine (16 mg/kg, s.c.) immediately after trans-resveratrol treatment. Rectal temperature and stereotypical behavior (rearing) were measured at three time points, 0 min (before any drugs administration, as an initial body temperature), 15 min and 30 min (15 and 30 min after the injection of apomorphine). The ambient room temperature was maintained at 22 ± 1 °C for the duration of the experiment (Puech et al., 1981).

2.7. Determination of monoamines and metabolites

Mice were decapitated and their brains were rapidly removed and frozen on dry ice. Various brain areas, including the frontal cortex, hippocampus and hypothalamus, were dissected on a cold plate (−16 °C) according to Franklin and Paxinos (1997). The tissue samples were weighed and stored at −80 °C until homogenization.

The contents of 5-HT, noradrenaline, dopamine and 5-HIAA were measured as described previously (Nitta et al., 1992) using high-performance liquid chromatography (HPLC) with electrochemical detection with minor modifications. Each frozen tissue sample was homogenized by ultrasonication in 200 μl of 0.4 M perchloric acid (solution A). The homogenate was kept on ice for 1 h and then centrifuged at 12,000 rpm (4 °C) for 20 min. The pellet was discarded. An aliquot of 160 μl of supernatant was added to 80 μl of solution B (containing 0.2 M potassium citrate, 0.3 M dipotassium hydrogen phosphate and 0.2 M EDTA). The mixture was kept on ice for 1 h and then centrifuged at 12,000 rpm (4 °C) for 20 min. Twenty μl of the resultant supernatant was directly injected into an ESA liquid chromatography system equipped with a reversed-phase C18 column (150 × 4.6 mm I.D., 5 μm) and an electrochemical detector (ESA CouloArray, Chelmstord, MA, USA.). The detector potential was set at 50, 100, 200, 300, 400, and 500 mV, respectively. The mobile phase consisted of 125 mM citric acid–sodium citrate (pH 4.3), 0.1 mM EDTA, 1.2 mM sodium octanesulfonate and 16% methanol. The flow rate was 1.0 ml/min. The tissue levels of monoamine were expressed in terms of nanograms per gram of tissue. Protein concentrations were determined by the method of Bradford (1976).

2.8. Measurements of monoamine oxidase activity

Mice were sacrificed and the brain tissues were rapidly frozen (−80 °C) until analyzed. Mouse brain monoamine oxidase activity was measured following the procedure described previously (Xu et al., 2005b) with a slight modification. Briefly, the brain tissues were homogenized with 4 ml of phosphate buffer (pH 7.4, 0.05 M). The activities of monoamine oxidase-A and -B in brain tissues were measured in the presence of either 1 μM deprenyl (type B inhibitor) or clorgylline (type A inhibitor). For lysis of the membranes, the tissue homogenate was treated with 0.4 ml of 20% Triton X-100, 2.5 ml of phosphate buffer (pH 7.4) was then mixed with 0.2 ml of the tissue homogenate. The mixture was preincubated at 37 °C for 15 min. Then 30 μl of 2.19 mM kynuramine dihydrobromide was added to the reaction mixture (final concentration 22 μM) as substrate. Samples were then incubated at 37 °C for 30 min again. After incubation, the reaction was terminated by adding 0.2 ml of 5 M perchloric acid. After cooling and centrifugation at 1500 × g for 10 min, an aliquot of 0.5 ml of the supernatant was added to 2.5 ml of 1 M NaOH. The fluorescence intensity was detected with excitation at 315 nm and emission at 380 nm using a fluorescence spectrometer. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve of 4-hydroxyquinoline. Monoamine oxidase activity was expressed as nmol of 4-hydroxyquinoline formed/30 min/mg protein.

2.9. Data analysis

Results were expressed as the mean ± standard error of the mean (S.E.M.). All data were analyzed statistically using one-way analysis of variance (ANOVA), followed by a post hoc Dunnett’s test. Differences with P < 0.05 were considered statistically significant.

3. Results

3.1. The effects of trans-resveratrol on the immobility time in the forced swimming and tail suspension tasks

Fig. 1 shows the effects of trans-resveratrol (10, 20, 40 and 80 mg/kg, i.g.) on the duration of immobility in two models of depression in mice. trans-Resveratrol, imipramine and fluoxetine induced a significant decrease in immobility time in the forced swimming [F (6, 63) = 5.79, P < 0.001, Fig. 1A] and tail suspension [F (6, 63) = 9.41, P < 0.01, Fig. 1B] tests. Post hoc analysis revealed that trans-resveratrol, at doses of 20, 40 and 80 mg/kg, led to a dose-dependent reduction in the immobility period as compared to the vehicle-treated control group, respectively (P < 0.05; P = 0.01; P = 0.001) in the forced swimming test. trans-Resveratrol at 40 and 80 mg/kg was also shown to decrease the immobility time in the tail suspension test (P < 0.05; P = 0.05). Two classical antidepressants, imipramine (10 mg/kg, i.p.) and fluoxetine (10 mg/kg, i.p.), induced similar effects of decreasing the immobility time in the forced swimming (P < 0.001; P < 0.001) and tail suspension (P < 0.01; P < 0.05) tests.

3.2. The effects of trans-resveratrol on the locomotor activity in mice

In order to determine whether trans-resveratrol actually possesses an antidepressant-like effect, we tested the locomotion counts to exclude the excitatory or inhibitory effects after administration of various drugs (Fig. 2). Neither trans-resveratrol nor positive antidepressants affected locomotor activity, at the same doses that significantly reduced immobility response in the forced swimming and tail suspension tasks [F (6, 63) = 0.45, P = 0.84].

3.3. The effects of trans-resveratrol on the immobility time of pre-treatment with PCPA in the forced swimming and tail suspension tasks

As shown in Fig. 3, trans-resveratrol (40 and 80 mg/kg, i.g.) or fluoxetine (10 mg/kg, i.p.), significantly decreased the immobi-
lity time in the forced swimming \( F(5, 54) = 2.84, P < 0.05 \), Fig. 3A] and tail suspension \( F(5, 54) = 2.57, P < 0.05 \), Fig. 3B] tests. However, pre-treatment with an inhibitor of serotonin synthesis, PCPA (300 mg/kg, i.p.) abolished the anti-immobility effect of both \textit{trans}-resveratrol and fluoxetine (10 mg/kg) before testing. \textit{Trans}-resveratrol administration alone did not affect the immobility time.

Results from these tests indicated that there was an indispensable involvement of the serotonergic system in the antidepressant-like effects of \textit{trans}-resveratrol.

### 3.4. The effects of \textit{trans}-resveratrol on apomorphine-induced hypothermia and rearing behavior

Table 1 shows that only marginal initial body temperature variation among groups was found at 0 min (before any drugs treatment) \( F(5, 54) = 0.91, P = 0.20 \). Hypothermia was not observed at 15 min after treatment with a high dose of apomorphine (16 mg/kg, s.c.) \( F(5, 54) = 1.42, P = 0.13 \). The maximal change in temperature was found at 30 min after apomorphine injection. \textit{Trans}-Resveratrol (20, 40 and 80 mg/kg) and the tricyclic antidepressant imipramine (10 mg/kg) significantly reversed the hypothermia response induced by apomorphine at this time point \( F(5, 54) = 93.43, P < 0.001 \). However, \textit{trans}-resveratrol administration alone did not induce any temperature change. Rearing behavior following apomorphine administration was unaffected by \textit{trans}-resveratrol at the doses administered (data not shown).

This result suggests that the noradrenergic system might be related to the antidepressant-like effects of \textit{trans}-resveratrol.

### 3.5. The effects of \textit{trans}-resveratrol on brain monoamines and their metabolite levels

The levels of monoamines and their metabolites detected in the frontal cortex are summarized in Table 2. Three monoamines 5-HT, noradrenaline and dopamine levels were significantly increased after \textit{trans}-resveratrol, imipramine (10 mg/kg) and fluoxetine (10 mg/kg) administration \( F(6, 63) = 16.37, P < 0.001 \), \( F(6, 63) = 5.55, P < 0.001 \), \( F(6, 63) = 6.04, P < 0.001 \). \textit{Trans}-Resveratrol also exhibited a tendency to decrease the measured 5-HIAA levels. Moreover, the ratio of 5-HIAA/5-HT was reduced when 80 mg/kg \textit{trans}-resveratrol was administered \( P < 0.05 \). Both imipramine (10 mg/kg) and fluoxetine (10 mg/kg) produced increases in 5-HT levels \( P < 0.05 \), but noradrenaline only increased after imipramine treatment \( P < 0.05 \).

In the hippocampus, an increase in 5-HT levels was found following administration of \textit{trans}-resveratrol (40 and 80 mg/kg), imipramine (10 mg/kg) or fluoxetine (10 mg/kg) \( F(6, 63) = 3.47, P < 0.05 \). The noradrenaline levels were also observed to increase after \textit{trans}-resveratrol (80 mg/kg) and imipramine (10 mg/kg) administration to this region \( P < 0.05 \), \( P < 0.05 \). We...
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The immobility time was then observed 20 min after (300 mg/kg, i.p.) and vehicle once a day for three consecutive tail suspension (B) tasks. Mice were pretreated with PCPA time of pre-treatment with PCPA in the forced swimming (A) and Figure 3: The effects of resveratrol administration on the fourth day. Values were the mean± S.E.M with 10 mice in each group. *P<0.05 and **P<0.01 vs. the vehicle-treated, control group.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Temperature (°C)</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 10</td>
<td>37.5±0.14</td>
<td>36.4±0.12</td>
<td>34.2±0.17</td>
<td></td>
</tr>
<tr>
<td>trans 20</td>
<td>37.3±0.08</td>
<td>36.5±0.11</td>
<td>35.2±0.17</td>
<td></td>
</tr>
<tr>
<td>Resveratrol 40</td>
<td>37.5±0.11</td>
<td>37.1±0.10</td>
<td>35.4±0.22**</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>37.6±0.16</td>
<td>37.3±0.11</td>
<td>37.3±0.10***</td>
<td></td>
</tr>
<tr>
<td>Imipramine 10</td>
<td>37.5±0.17</td>
<td>37.3±0.12</td>
<td>37.4±0.19***</td>
<td></td>
</tr>
</tbody>
</table>

3.6. The effects of trans-resveratrol on monoamine oxidase activity in the mouse brain

Table 5 summarizes the inhibition of type A and type B monoamine oxidase activities by trans-resveratrol and the positive antidepressants in mouse brain. Thirty minutes after administration of trans-resveratrol at doses of 10, 20, 40 and 80 mg/kg, the mouse brain monoamine oxidase-A (depenyl-treated) activity was inhibited by 9.1%, 13.2%, 16.1% and 25.4%, respectively (P<0.05; P<0.01; P<0.001). trans-Resveratrol at lower doses (10, 20 and 40 mg/kg) did not appear to inhibit monoamine oxidase-B (clorgyline-treated) activity; however, a higher dose of trans-resveratrol (80 mg/kg) was found to inhibit MAO-B activity in mouse brain (P<0.05). Moclobemide produced MAO-A inhibition of 40.1% (P<0.001), but did not affect MAO-B activity. Both imipramine and fluoxetine did not display any significant effects on MAO-A and MAO-B activities.

4. Discussion

It is suggested that behavioral study plays an important role in the evaluation of antidepressant drugs. Knowledge of the acute mechanisms underlying the action of antidepressant drugs led to the general belief that the effective antidepressant medications act by increasing the activity of the serotonergic or noradrenergic system (Berton and Nestler, 2006). In this study, the antidepressant-like effects of trans-resveratrol were evaluated through two mouse models of despair tasks, which are well established screening paradigms for antidepressants. In addition to the behavioral tests, this study provided neurochemical and biochemical evidence for the involvement of particular monoamines including serotonin, noradrenaline and MAO-A in the antidepressant-like effects of trans-resveratrol.

The results presented here show that trans-resveratrol produces a significant inhibition in the recorded immobility time of mice performing the forced swimming and tail suspension tests, with comparable profiles to that observed for the classical antidepressants imipramine and fluoxetine. Since psychostimulants are also shown to reduce immobility but in contrast to antidepressants they cause a marked motor stimulation. Locomotor activity was also observed post trans-resveratrol treatment. These results suggested that trans-resveratrol, at the same doses that produce an antidepressant-like effect, did not show significant locomotor stimulation.
These findings suggest that trans-resveratrol exerts selective antidepressant-like effects.

The forced swimming and tail suspension tasks are behavioral despair tests useful for probing the pathological mechanism of depression and for the evaluation of antidepressant drugs (Porsolt et al., 1978; Xu et al., 2005a). The present study showed that the duration of immobility in these two models was decreased significantly after administration (i.g.) of 40 and 80 mg/kg trans-resveratrol. However, this effect was prevented by pre-treatment with the irreversible tryptophan hydroxylase inhibitor PCPA. This inhibitory effect is in agreement with the data showing that PCPA treatment completely prevented the anti-immobility effect of the serotonin reuptake inhibitor fluoxetine (10 mg/kg, i.p.). It is reported that PCPA treatment at a dose of 300 mg/kg for 3 consecutive days, which was administered in the present study, induced a depletion of the endogenous storage of brain serotonin without affecting the noradrenaline and dopamine levels (Wang et al., 2008). Thus, the present findings suggest that the serotonergic system might be implicated in the antidepressant-like effect of trans-resveratrol.

Apomorphine acts on the pre-synaptic D2 receptors situated on noradrenergic nerve terminals to prevent the release of norepinephrine (Langer, 1977). Activation of central D2, and to a lesser extent D1 receptors, increases the release of norepinephrine in the hippocampus and hypothalamus (Egan et al., 1999).

### Table 2
The effects of trans-resveratrol on the concentrations of monoamines and their metabolites in the frontal cortex of mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Frontal cortex (ng/g)</th>
<th>5-HT</th>
<th>5-HIAA</th>
<th>5-HIAA/5-HT</th>
<th>Noradrenaline</th>
<th>Dopamine</th>
<th>DOPAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trans-Resveratrol</td>
<td>10</td>
<td>324.5± 8.5</td>
<td>138.5±1.9</td>
<td>0.43±0.02</td>
<td>835.1± 9.8</td>
<td>295.6± 4.5</td>
<td>17.2± 0.5</td>
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</tr>
<tr>
<td></td>
<td>20</td>
<td>307.4± 6.7</td>
<td>133.5±1.6</td>
<td>0.44±0.01</td>
<td>824.2± 4.7</td>
<td>286.3± 2.6</td>
<td>17.7± 0.4</td>
<td></td>
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<tr>
<td></td>
<td>40</td>
<td>326.8± 6.5</td>
<td>131.3±1.3</td>
<td>0.41±0.01</td>
<td>831.0± 6.4</td>
<td>293.7± 3.0</td>
<td>18.3± 0.3</td>
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</tr>
<tr>
<td></td>
<td>80</td>
<td>333.8± 5.6</td>
<td>133.9±1.2</td>
<td>0.40±0.01</td>
<td>854.4± 7.0</td>
<td>308.4± 3.0</td>
<td>17.7± 0.3</td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td>10</td>
<td>366.9± 8.2**</td>
<td>143.1±1.6</td>
<td>0.39±0.01</td>
<td>875.4±11.8*</td>
<td>302.1± 5.5</td>
<td>17.2± 0.4</td>
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<tr>
<td>Fluoxetine</td>
<td>10</td>
<td>385.0± 6.1***</td>
<td>153.0± 2.4</td>
<td>0.40±0.01</td>
<td>846.0± 8.0</td>
<td>310.2± 3.8</td>
<td>17.6± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

Table values are expressed as mean ± S.E.M. with units of ng/g for 10 mice in each group. Data analysis was performed using Dunnett’s t-test. *P<0.05, **P<0.01, ***P<0.001, compared with the vehicle-treated, control group.

### Table 3
The effects of trans-resveratrol on the concentrations of monoamines and their metabolites in the hippocampus of mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Hippocampus (ng/g)</th>
<th>5-HT</th>
<th>5-HIAA</th>
<th>5-HIAA/5-HT</th>
<th>Noradrenaline</th>
<th>Dopamine</th>
<th>DOPAC</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>330.3± 8.5</td>
<td>82.7± 0.8</td>
<td>0.25±0.01</td>
<td>730.2± 7.4</td>
<td>109.2± 3.4</td>
<td>13.5± 0.5</td>
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<tr>
<td>trans-Resveratrol</td>
<td>10</td>
<td>335.9± 4.2</td>
<td>84.5± 0.8</td>
<td>0.25±0.01</td>
<td>710.9± 4.5</td>
<td>111.7± 3.8</td>
<td>12.3± 0.6</td>
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<tr>
<td></td>
<td>20</td>
<td>344.0± 3.5</td>
<td>83.3± 0.9</td>
<td>0.24±0.01</td>
<td>711.9± 5.3</td>
<td>106.9± 3.7</td>
<td>11.6± 0.6</td>
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<tr>
<td></td>
<td>40</td>
<td>349.5± 4.1*</td>
<td>83.4± 0.8</td>
<td>0.24±0.01</td>
<td>741.1± 5.5</td>
<td>107.3± 3.6</td>
<td>13.1± 0.6</td>
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<tr>
<td></td>
<td>80</td>
<td>355.3± 3.6**</td>
<td>82.1± 1.4</td>
<td>0.23±0.01*</td>
<td>752.6± 3.7*</td>
<td>114.2± 3.9</td>
<td>13.4± 0.5</td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td>10</td>
<td>358.5± 3.1**</td>
<td>85.0± 0.7</td>
<td>0.24±0.01</td>
<td>754.3± 6.9*</td>
<td>113.9± 4.4</td>
<td>14.8± 0.4</td>
<td></td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>10</td>
<td>358.6± 4.8**</td>
<td>85.2± 1.5</td>
<td>0.24±0.01</td>
<td>737.1± 4.2</td>
<td>110.9± 4.7</td>
<td>14.1± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

Table values are expressed as mean ± S.E.M. with units of ng/g for 10 mice in each group. Data analysis was performed using Dunnett’s t-test. *P<0.05 and **P<0.01, compared with the vehicle-treated, control group.

### Table 4
The effects of trans-resveratrol on the concentrations of monoamines and their metabolites in the hypothalamus of mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Hypothalamus (ng/g)</th>
<th>5-HT</th>
<th>5-HIAA</th>
<th>5-HIAA/5-HT</th>
<th>Noradrenaline</th>
<th>Dopamine</th>
<th>DOPAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>342.5± 7.8</td>
<td>144.4±1.3</td>
<td>0.42±0.01</td>
<td>360.0± 8.1</td>
<td>35.3± 0.9</td>
<td>26.1± 0.6</td>
<td></td>
</tr>
<tr>
<td>trans-Resveratrol</td>
<td>10</td>
<td>332.3± 4.2</td>
<td>140.8±0.8</td>
<td>0.42±0.01</td>
<td>339.4± 5.2</td>
<td>33.2± 1.4</td>
<td>25.7± 0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>324.5± 7.4</td>
<td>140.4±1.6</td>
<td>0.43±0.01</td>
<td>367.5± 8.8</td>
<td>39.5± 1.2</td>
<td>27.9± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>349.7± 10.2</td>
<td>140.6±1.3</td>
<td>0.40±0.01</td>
<td>354.9± 9.4</td>
<td>34.0± 1.5</td>
<td>25.9± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>370.8± 6.3*</td>
<td>141.4±1.3</td>
<td>0.38±0.01*</td>
<td>362.2± 7.8</td>
<td>37.4± 1.4</td>
<td>27.5± 0.6</td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td>10</td>
<td>370.1± 2.8*</td>
<td>149.1±1.4</td>
<td>0.40±0.01</td>
<td>374.5± 5.8</td>
<td>37.3± 1.4</td>
<td>26.3± 0.7</td>
<td></td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>10</td>
<td>374.8± 4.3**</td>
<td>148.7±2.3</td>
<td>0.40±0.01</td>
<td>357.5± 5.4</td>
<td>35.0± 0.8</td>
<td>26.6± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

Table values are expressed as mean ± S.E.M. with units of ng/g for 10 mice in each group. Data analysis was performed using Dunnett’s t-test. *P<0.05 and **P<0.01, compared with the vehicle-treated, control group.
a lesser extent D1, receptors by apomorphine leads to a characteristic increase in stereotypic behavior (e.g., rearing). Drugs that antagonize this effect possess anti-dopaminergic activity (Redrobe et al., 1998). However, antagonism of high dose apomorphine- (16 mg/kg) induced hypothermia is proposed as an improved screening test for antidepressants inhibiting norepinephrine reuptake, such as imipramine, amoxapine and viloxazine (Puech et al., 1981; Viana et al., 2005). The results obtained in this test may exclude the dopaminergic system's involvement in the activity of trans-resveratrol as it failed to antagonize stereotypic rearing behavior at all doses tested. Therefore, the observed antagonism of the hypothermia induced by apomorphine indicates that the noradrenergic system might be involved in the response of trans-resveratrol to depressive-like behavior.

Intensive research into the neurobiology of depression suggests that an increase in the level of monoamines at the synapse is believed to be the first step in a complex cascade of events that ultimately results in antidepressant activity (Pineyro and Blier, 1999). Accordingly, most antidepressants like the tricyclic antidepressants (imipramine) and monoamine oxidase inhibitors (moclobemide) exert their action by increasing synaptic monoamine concentrations (Deniker, 1984; Kulkarni et al., 2008; Xu et al., 2005b). In our study, we detected the brain monoamine levels by HPLC after trans-resveratrol treatment. Three brain regions were studied: the frontal cortex, the hippocampus and the hypothalamus, which are involved in important behavioral functions, such as emotion, motivation and learning and memory (Xu et al., 2005a; Butterweck et al., 2002). Abnormal monoamine levels in these brain regions may be relevant to clinical investigations of the depressed state. Our results show that trans-resveratrol (40 or 80 mg/kg) increases the 5-HT levels in a dose-dependent manner in all of the three brain regions. These effects were similar to those observed for the positive drugs imipramine and fluoxetine. Moreover, the ratio of 5-HIAA:5-HT, an indicator of serotonergic activity, decreased in three brain regions after administration of 80 mg/kg trans-resveratrol. This result suggests that trans-resveratrol administration leads to serotonergic activation in all three brain regions, which is consistent with the behavioral changes exhibited in the pharmacological interaction model (pretreatment with PCPA in tail suspension test).

In general, the most widely accepted hypothesis for the biological basis of depression implicates norepinephrine or serotonin system dysfunction (Blier and De Montigny, 1994; Xu et al., 2005b). Norepinephrine projections innervate the limbic system, suggesting the involvement of norepinephrine in the regulation of emotions and cognition. Various antidepressant drugs, such as norepinephrine reuptake inhibitors, are effective in the treatment of affective disorders. However, the depletion of norepinephrine causes a resurgence of depressive symptoms after successful treatment with antidepressant drugs (Dremencov et al., 2009). In the present study, measurement of noradrenaline after trans-resveratrol administration (80 mg/kg) showed that it produced an increase in central noradrenaline concentrations in both the frontal cortex and hippocampus. Based on the results from both the behavioral model and HPLC detection, the antidepressant-like effects of trans-resveratrol might be partly due to its influence on the function of adrenergic system through the regulation of adrenergic receptors and/or the metabolism of noradrenaline.

Although serotonin and noradrenaline are major targets for currently available antidepressant drugs, recent evidence suggests that dopamine (DA) is also involved in the pathophysiology and treatment of depression (Nitta et al., 1992). It is well established that the increase in central dopaminergic transmission regulates the neuronal activity of 5-HT and noradrenaline (Guiard et al., 2009). The HPLC assay showed that a significant increase in dopamine in the frontal cortex was observed after 80 mg/kg trans-resveratrol treatment. The results from behavior and HPLC assay were inconsistent possibly because behavioral changes are not significantly sensitive to small changes in dopamine levels in the brain.

Preservation of monoamine neurotransmitters can be achieved either by inhibiting their reuptake or through the monoamine oxidase mechanism (Deniker, 1984; Bhutani et al., 2009). In order to clarify whether the increase in monoamines resulted from the inhibition of monoamine oxidase activity, we determined mouse brain monoamine oxidase activity after trans-resveratrol administration. MAO exists in two subtypes: A and B. The A form of MAO preferentially metabolizes 5-HT and noradrenaline, which are the monoamines most closely linked to depression. The B form preferentially metabolizes trace amines including phenylethylamine. Both MAO-A and MAO-

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### Table 5 The inhibitory effects of trans-resveratrol on type A and type B monoamine oxidase activities in mouse brain.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Monoamine oxidase-A activity (nmol/30 min/mg protein)</th>
<th>Monoamine oxidase-B activity (nmol/30 min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>60.4 ± 1.7</td>
<td>182.3 ± 2.8</td>
</tr>
<tr>
<td>trans-Resveratrol</td>
<td>10</td>
<td>55.5 ± 1.7</td>
<td>175.6 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>52.7 ± 2.0*</td>
<td>173.5 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>51.1 ± 1.5**</td>
<td>172.6 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>45.3 ± 1.2***</td>
<td>167.9 ± 2.4***</td>
</tr>
<tr>
<td>Moclobemide</td>
<td>20</td>
<td>36.6 ± 1.7***</td>
<td>179.0 ± 2.8</td>
</tr>
<tr>
<td>Imipramine</td>
<td>10</td>
<td>59.8 ± 1.9</td>
<td>177.3 ± 2.9</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>10</td>
<td>61.0 ± 1.8</td>
<td>179.4 ± 2.2</td>
</tr>
</tbody>
</table>

Monoamine oxidase-A or monoamine oxidase-B activity was determined fluorimetrically using kynuramine as a substrate in the presence of 1 μM deprenyl or clorgyline, respectively. Table values are expressed as the mean ± S.E.M. with units of nmol/30 min/mg protein for 10 mice in each group. Data analysis was performed using Dunnett's t-test. *P < 0.05, **P < 0.01 and ***P < 0.001, compared with the vehicle-treated, control group.
B are involved in metabolizing dopamine and tyramine (Stahl and Felker, 2008). The MAO inhibitors isocarboxazid and moclobemide are used pharmaceutically for the treatment of some neuropsychiatric disorders including depression and schizophrenia (Deniker, 1984). The present study showed that trans-resveratrol inhibited MAO-A activity dose dependently; whereas, MAO-B inhibitory activity was observed only at higher doses. Thus, the enhanced activity of the three monoamines following the trans-resveratrol administration might be related to the inhibition of MAO enzyme. These observations were consistent with the previous studies, which suggested that trans-resveratrol concentration-dependently inhibited the enzymatic activity of commercial (human recombinant) MAO activity (Yáñez et al., 2006a; 2006b).

However, previously published studies did not demonstrate an inverse relationship between antidepressant-like action and MAO activity after trans-resveratrol administration.

Recent studies indicate that free oxygen radicals and nitric oxide may be involved in depression due to the actions of these molecules on cell function and the relatively high vulnerability of the central nervous system to oxidative stress (Herken et al., 2007; Eren et al., 2007). Some studies have reported a positive correlation between oxidative stress and depression; furthermore, normalization of oxidative stress markers with antidepressant treatment has been also demonstrated (Herken et al., 2007). Drugs with potential antioxidant action could be an attractive target for treatment of depressive disorders. The beneficial effects of moderate red wine consumption on oxidative stress and cognition have been attributed to the presence of antioxidant components (Tredici et al., 1999). trans-Resveratrol, which is present in wine and grapes exerts numerous biological activities as a naturally occurring antioxidant polyphenol. Recently trans-resveratrol has received attention for its important protective effects on the nervous system, such as delayed axonal degeneration after injury and protection from brain ischemia in rodents (Dasgupta and Milbrandt, 2007). It is currently being evaluated in clinical trials of patients with Alzheimer's disease due to its promising neuroprotective effects. High concentrations of trans-resveratrol have been recorded in the brain following oral administration, which suggests that it is capable of crossing the blood–brain barrier following oral administration (Vitrac et al., 2003; Sale et al., 2004). Our preliminary data indicated that the drug concentrations were 0.0125–0.1141 nmol/ml and 0.3204–1.9120 nmol/ml in the brain and plasma after trans-resveratrol administration (10, 20, 40, and 80 mg/kg, i.g., 30 min) (data now shown). These data were nearly identical to previous studies in C57BL/6J mice, which showed that the concentration of trans-resveratrol in the brain was nearly 1 nmol/ml when the mice received a single dose of trans-resveratrol up to 240 mg/kg (i.g., 20–30 min) (Sale et al., 2004). Therefore, the observed beneficial roles of trans-resveratrol against antidepressant-like behaviors may have resulted from its actions on the monoamine neurotransmitters and MAO activity in mice brain.

In conclusion, this study describes for the first time how trans-resveratrol exerts antidepressant-like effects in behavioral despair tests. Our investigations also indicate that the underlying mechanism is primarily involved in serotonergic and noradrenergic activation through the behavioral models: the depletion of serotonin in mouse models of despair tasks and high dose of apomorphine-induced hypothermia. The neurochemical and biochemical assays described herein confirm that trans-resveratrol influences, at least in part, the metabolism of the monoamines, particularly 5-HT and noradrenaline, to restore normal monoaminergic function. Further studies are conducted in our laboratory to elucidate the exact mechanism of trans-resveratrol in depressive-like behaviors.

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This work was supported by an Ellison Medical Foundation New Scholar Award to William O. Ogle, National Natural Science Foundation of China (No. 30973892) and Zhejiang Province Extremely Key Subject Building Fund “Pharmacology and Biochemical Pharmaceutics 2008”.

Contributors

Author (1) designed the study and wrote the first draft of the manuscript. Authors (2) and (3) conducted the study, performed the statistical analysis and revised the manuscript. Authors (4), (5), (7) and (9) took part in the analysis of the behavioral data and contributed in literature searches. Authors (6) and (8) participated in the preparation of the manuscript and literatures searches. Authors (10) and (11) designed and supervised the study including editing of the manuscript, from the first draft to the final version. Dr. Y. Xu, Z.C. Wang and W.T. You contributed equally to this work.

Conflict of interest

The authors do not have financial or personal conflicts of interest associated with this work.

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References


Antidepressant-like effect of trans-resveratrol: Involvement of serotonin and noradrenaline system


