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Vanillin-induced amelioration of depression-like behaviors in rats by modulating monoamine neurotransmitters in the brain

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ABSTRACT

Olfaction plays an important role in emotions in our daily life. Pleasant odors are known to evoke positive emotions, inducing relaxation and calmness. The beneficial effects of vanillin on depressive model rats were investigated using a combination of behavioral assessments and neurotransmitter measurements. Before and after chronic stress condition (or olfactory bulbectomy), and at the end of vanillin or fluoxetine treatment, body weight, immobility time on the forced swimming test and sucrose consumption in the sucrose consumption test were measured. Changes in these assessments revealed the characteristic phenotypes of depression in rats. Neurotransmitters were measured using ultrahigh-performance liquid chromatography. Our results indicated that vanillin could alleviate depressive symptoms in the rat model of chronic depression via the olfactory pathway. Preliminary analysis of the monoamine neurotransmitters revealed that vanillin elevated both serotonin and dopamine levels in brain tissue. These results provide important mechanistic insights into the protective effect of vanillin against chronic depressive disorder via olfactory pathway. This suggests that vanillin may be a potential pharmacological agent for the treatment of major depressive disorder.

1. Introduction

Major depressive disorder (MDD) is a neuropsychiatric disorder characterized by persistent despondent state accompanied by low self-esteem and a loss of interest or pleasure in normally enjoyable activities (anhedonia). It is a highly prevalent, multifactorial disorder with high disability and mortality rates (Zalsman et al., 2006). The psychosocial difficulties lead to serious physical, mental and socioeconomic consequences, such as impairments in social communication and vocational function. Many sufferers do not live independently. Half of patients attempt suicide at least once and up to 20% ultimately commit suicide (Pompili et al., 2009). Almost all of them need long-term medication and so research into pharmacotherapy of MDD has evolved over the centuries. Some powerful antidepressants have made indelible contributions, such as selective serotonin reuptake inhibitor (SSRIs), tricyclic antidepressant (TCA), and monoamine oxidase (MAO) inhibitors. However, some of them are of variable effectiveness and some exert undesirable side effects (Xu et al., 2005). Therefore, the development of alternative antidepressants is still the ideal aim pursued by researchers. Olfaction is an important function for animals, playing roles in food hunting, sexual behavior, aggression, territorial defense, identification, and among others (Thiessen and Rice, 1976). The olfactory centers include the prepyriform cortex, amygdala, hypothalamus, hippocampus and other limbic system structures (Benignus and Prah, 1982). Interestingly, a number of these regions also play important roles in emotion processing and this overlap explains the high level of functional connectivity between odor and emotions. This has made olfactory stimulation a promising method for mood induction (Zald and Pardo, 1997; Rolls, 2004; Royet et al., 2003). Vanillin, a single molecule, extracted from vanilla beans, is a popular odor used widely in perfume, food and medicine (Ho et al., 2009, 2011). It has even been used by injection, at an effective dose amount, to calm or sedate patients (Abraham et al., 1997). As an odorant, vanillin is generally rated as pleasant and correspondingly evokes

Abbreviations: MDD, major depressive disorder; CUMS, chronic unpredictable mild stress; FST, forced swimming test; SCT, sucrose consumption test; ANOVA, analysis of variance; UPLC, ultrahigh-performance liquid chromatographic technique; 5-HT, serotonin; DA, dopamine; NE, noradrenaline; HPA, hypothalamic–pituitary–adrenal axis

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positive moods (Seubert et al., 2009). This makes vanillin a promising aroma for modulating emotions.

On the basis of the close anatomic and physiological link between olfaction and emotions, we hypothesized that odorants with strong hedonic qualities, such as vanillin, could evoke happiness and modulate emotions in MDD. The aim of the present study was to assess the influence of vanillin on depression-like behaviors in rats. Additionally, to elucidate the possible underlying mechanisms, a quantitative analysis of monoamine neurotransmitters in the brain was performed using ultrahigh-performance liquid chromatography (UPLC).

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (200–250 g) were obtained from the Department of Laboratory Animal Science, Anhui Medical University (Hefei, China). The animals were housed individually under standard colony conditions, with a 12 h light/dark cycle and ad libitum food and water. They were allowed to acclimatize to the colony for at least 7 days before any experimentation. All experimental manipulations were carried out during the light phase of the light/dark cycle.

All experimental procedures were performed in compliance with the Animal Scientific Procedures Act of revised directive of 2010/63/EU on the protection of animals used for scientific purpose and received local ethics committee approval (number: LLSC2013006). All efforts were made to minimize the number of animals used and their suffering.

2.2. Animal model

Two models of depressive disorder were used to assess the effects of vanillin in rats. The chronic depressive disorder model was induced by chronic unpredictable mild stress (CUMS), and the acute depressive disorder model was induced by olfactory bulbectomy which disrupted the olfactory pathway (Jesberger and Richardson, 1988). The experimental protocol is shown in Fig. 1. Here, pre-model is defined as the parameters measured before modeling (CUMS or olfactory bulbectomy) and post-model is defined as the parameters observed after modeling. By analogy, the principle is also applied to pre-CUMS, post-CUMS, pre-operation and post-operation.

2.2.1. CUMS procedure

Stressors were administered once daily between 08:30 and 10:30 h, with the exception of the 24 h duration stressors. The CUMS procedure was revised from the reference (Wu et al., 2007). Stressors consisted of (1) 5 min warm swim at 42 °C; (2) 24 h wet litter;(3) 24 h food deprivation; (4) 90 s tail pinch; (5) 24 h water deprivation; (6) 5 min cold swim at 4 °C, after which they were toweled dry; (7) 24 h cage tilt (cages were tilted to 45° from the horizontal). The stressors were distributed randomly with intervals of at least 7 days. All stressors were administered four times within 4 weeks.

After the procedure, the animals were divided into three groups with 8–10 rats per group: the stress+fluoxetine group; the stress+vanillin aromatherapy group and the stress (untreated) group.

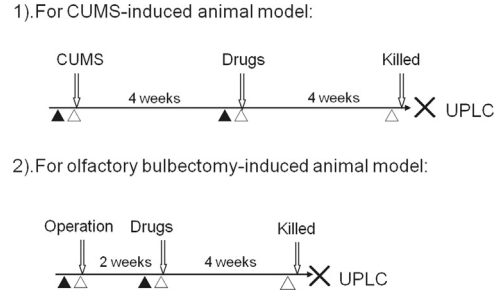

Fig. 1. Overall flow of methodology used for studying the effects of vanillin on: (1) CUMS induced animal model; and (2) olfactory bulbectomy-induced animal model. ▲: Determination of serum corticosterone from tail blood, 1 day ahead of behavioral assessments; △: Assessments of depression-like behaviors at different time points.

2.2.2. Olfactory bulbectomy

In order to investigate the pathway by which vanillin worked, another two groups (10 rats per group) were added: the bulbectomy+vanillin group and the control group (sham). Olfactory bulbectomy was performed as described previously (Jaako-Movits et al., 2006). Animals were deeply anesthetized with 10% chloral hydrate (330 μl/100 g body weight, i.p.). The head hair was shaved and swabbed with antiseptic, after which the animal was placed under a stereotaxic instrument and a midline frontal incision was made in the scalp, with the skin being retracted bilaterally. The surgical procedure involved drilling two burr holes on either side 1 mm from the midline of the frontal bone coving the olfactory bulbs. The bulbs were aspirated. The cavity was packed with surgical foam and the skin was closed with surgical sutures. The animals were allowed to recover by warming to maintain body temperature. Rats in the sham surgery condition received burr holes only. The completeness of olfactory bulb removal was verified upon sacrifice. After surgery the animals were housed individually for 2 weeks of recovery. Before operation and after recovery, the animals underwent stress hormone determination and behavioral assessments to confirm the validity of olfactory bulbectomy-induced depressive disorder model. The bulbectomy+vanillin group was treated as the stress+vanillin group and the control group was treated as the stress group.

2.3. Pharmacological treatment

For the stress+fluoxetine group, the animals were administered a daily oral dose (10 mg/kg/d, diluted in distilled water) of the SSRI fluoxetine (Eli Lilly &Co., Souzhou, China) each morning. For the stress+vanillin group and the bulbectomy+vanillin group, vanillin (Sangon Biotech, Shanghai co., Ltd, China) was administrated in a Plexiglas cylinder 50 cm tall and 35 cm diameter with two layers separated by a porous Plexiglas board. The rat still in its cage was gently placed on the upper layer, and 5 ml of 600 mg/l vanillin (in distilled water) sprayed on to the floor of the lower layer (Atanasova et al., 2012). The odor of vanillin would pervade to the upper layer through the porous board where the rat received vanillin aromatherapy for 1/2h at 8 hourly intervals. Rats in the stress and the control groups received similar handling to the stress+vanillin group, but without any odor administrated.

2.4. Body weight

The effect of the stress and treatment on body weight was measured by electronic balance and recorded at fixed time points. This is a diagnostic criterion for depressive disorder with accompanying weight loss or weight gain (Nestler et al., 2002).

2.5. Tail Blood corticosterone determination

Corticosterone is an important stress hormone in animals and has significant activities as glucocorticoid involved in MDD. When elevated it reveals chronic stress and so provides an endocrinological diagnostic criterion for depressive disorder in animal models. For CUMS-induced depressive disorder model, serum corticosterone was determined before the CUMS procedure and next day after the CUMS procedure. For olfactory bulbectomy-induced depressive disorder model, serum corticosterone was determined before the operation and 14 days after the operation. In both models, blood was drawn from the tail vein of anesthetized rats at 9:30 -11:00 a.m. of the day, which was 1 day ahead of other behavioral assessments. The blood was centrifuged at 3000 rpm for 10 min at 4 °C (Eppendorf, 5810R).The clear serum was extracted(about 0.5 ml) and sent to the endocrinology center of the First Affiliated Hospital of Anhui Medical University where serum corticosterone concentration was determined by chemiluminescence detection (ADVIA centaur immunoassay system, Siemens, German).

2.6. Behavioral assessments

Before and after CUMS (or operation), and at the end of administration, immobility time in the forced swimming test (FST) and the sucrose consumption in the sucrose consumption test (SCT) were measured. Changes in these parameters reflected the behavioral characteristic phenotypes of depression in rats.

2.6.1. FST

The FST is a procedure in which rats are forced to swim for 6 min. This parameter was performed as described previously (Porsolt et al., 1978). The rat was placed individually in a Plexiglas cylinder 50 cm tall, 20 cm diameter filled to 40 ± 1.5 cm with 24 ± 0.5 °C water from which escape was impossible. Fifteen minutes later, the rat was removed and dried before being returned to its home cage. Fresh water was used for each rat and the cylinder was also cleaned between trials. After 24 h, the animals were returned to the cylinders and the procedure was repeated for another 6 min. Immobility time was recorded for 2–6 min by video camera through the side of the cylinder, which was illuminated from the opposite side. An experimenter monitored the video camera unaware of the treatment