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Original Article

Guanosine ameliorates positive symptoms of schizophrenia *via* modulating 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors

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Abstract: Schizophrenia is a serious mental disorder characterized by hallucinations, delusions, and extremely disordered thinking and behavior. There are several hypotheses of pathogenesis in schizophrenia: dopaminergic, glutamatergic, or serotonergic hyperfunction. Guanosine reportedly protects the central nervous system by modulating the glutamatergic system. Thus, we assumed that guanosine may exert a positive effect on the pathophysiology of schizophrenia. Herein, we demonstrated that guanosine significantly reduced MK-801-induced hyperlocomotion and stereotyped behaviors, but showed no effect on hyperlocomotion induced by d-amphetamine, indicating that guanosine may directly affect the glutamatergic system. Guanosine dose-dependently reduced 5-HTP-induced wet dog shakes (WDS) and other serotonin syndromes (SS) behaviors, indicating that it might block serotonin 5-HT$_{1A}$ or 5-HT$_{2A}$ receptors. Finally, we confirm that that guanosine modulates serotonin 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors and it might be anti-schizophrenic partly through pertussis toxin-sensitive $G_{i/o}$-coupled PI3K/Akt signaling. Collectively, this study provides possible compounds and mechanisms for therapeutic effects on schizophrenia.

Keywords: Guanosine, schizophrenia, 5-HT$_{1A}$ receptor, 5-HT$_{2A}$ receptor, PI3K/Akt

Introduction

Schizophrenia is a degenerative neuropsychiatric disorder that is caused by genetic and environmental factors. The clinical features of schizophrenia are commonly classified into distinct clusters of positive, negative, and cognitive symptoms [1]. There are several hypotheses of pathogenesis: dopaminergic, glutamatergic, or serotonergic hyperfunction [2]. Two generations of anti-schizophrenic drugs have been developed. However, serious side effects, such as extrapyramidal reactions and autonomic dysfunction, are still unavoidable [3, 4]. Depression is believed to be caused by the chronic deficiency of monoamines [5]. Drugs that target synaptic monoamines, such as serotonin, dopamine, and norepinephrine, have been used for more than 50 year [6]. The antidepressant effects of N-methyl D-aspartate (NMDA) receptor antagonists, including ketamine, MK-801, dizocilpine, and CGP 37849, have been reported in recent years [7]. Nevertheless, depressive episodes are receiving increasing attention because it is highly relevant with a higher risk of suicide, poorer quality of life and decreased adherence to treatment [8]. The current serious situation of schizophrenia and depression suggests that the deep mechanism of these two diseases is still not been well understood.

5-HT, a monoamine neurotransmitter widely distributed in the brain, is essential for almost all of the central nervous system integrative functions [9]. The second-generation antipsychotic theory considers serotonin (5-HT) receptors [10, 11]. The second-generation antipsy-

chotics are also known as atypical antipsychotics, which have a strong affinity for dopamine receptors and 5-HT_{2A} receptors. They are mainly used to treat the positive symptoms of schizophrenia. Early studies have shown that this kind of drugs have potential therapeutic effects for the negative symptoms of schizophrenia. However, these effects have not been proved [12]. 5-HT_{1A} receptor is considered to be an ideal target for the treatment of schizophrenia, because stimulation of 5-HT_{1A} receptor can improve cognitive impairment in patients with schizophrenia [13]. 5-Hydroxytryptophan (5-HTP) is a precursor of 5-HT. Wet dog shakes (WDS) are a signal for studying the function of 5-HT_{2A} receptor activity [14]. 5-HT_{1A} receptor activation is thought to mainly contribute to serotonin syndromes (SS) behaviors, such as forepaw treading, flat body posture, and hind limb abduction [15]. 5-HT_4 receptor is related to neurocognitive disorders in the symptoms of in schizophrenia, and activation of 5-HT4 receptors alleviates the cognitive impairments [16]. Based on the above research, in this study, we used the animal models of SS behaviors, WSD and the HEK293 cell models to measure the effects of guanosine on 5-HT_{1A}, 5-HT_{2A} and 5-HT_4 receptors, respectively, and to explore the potential role of guanosine in the treatment of schizophrenia. The serotonin system contains 14 different serotonin receptors. All of the serotonin receptors are G-protein coupled (GPC), except the 5-HT_3 receptors gating a cation-permeable ion channel. Dysfunction in the serotonin system is related to a variety of neuropsychiatric disorders, including depression and schizophrenia [9]. In animal models, the 5-HT_{2C} receptor agonist WAY-163909 produces antidepressant-like effects [17]. Besides, another investigation found that 5-HT_{2A} receptor antagonists can block MK-801-induced stereotypies and hyperlocomotion [18].

In the central nervous system (CNS), dopamine is associated with controlling locomotion, cognition, affect and neuroendocrine secretion [19]. These actions of dopamine are mediated by five different receptor subtypes, which are pharmacologically classified as D_1- or D_2-like [20]. Schizophrenia is associated with changes in dopamine neurotransmission during adolescence and adulthood, leading to deficits in motivation, cognition and sensory functions [21]. The hypothesis that the dopaminergic system is overactive in schizophrenia was confirmed by the following facts: psychostimulants, such as amphetamine increase dopaminergic transmission mainly by increasing the release of dopamine, thereby inducing similar activities to the positive symptoms of schizophrenia (euphoria, auditory hallucinations, and akathisia or the inability to remain inactive). Besides, antipsychotic drugs used to successfully treat certain symptoms of schizophrenia can selectively block dopamine receptors [22]. Another example is mice that have been genetically modified to overexpress dopamine D_2 receptors in the striatum, which also exhibit multiple schizophrenia-like behaviors [23]. Similarly, the genetically transfer of tyrosine hydroxylase and guanosine triphosphate (GTP) cyclohydrase 1 into the substantia nigra during early puberty increases dopamine synthesis, and is associated with schizophrenia-like behavioral phenotype [24].

Guanosine is a purine nucleoside that mostly accumulates under certain physiological conditions [25]. It is an extracellular signaling molecule released by astrocytes in ischemic conditions [26-28]. Recently, guanine-based purines, including the nucleotides GTP, GDP, and GMP, and the nucleoside guanosine have also been proven to exert extracellular effects. The studies could be subdivided into 3 approaches: (i) inhibitory effects on the activity of the glutamatergic system in physiological and pathological conditions; (ii) effects on memory and behavior; (iii) trophic effects on neural cells [29]. It has been shown that guanosine has protective effects on models of central nervous system diseases like Alzheimer's disease, Parkinson's disease, ischemic stroke, and depression [30-33]. Guanosine is also protective against glutamate-induced excitotoxicity [34] and oxygen/glucose deprivation in hippocampal slices [35]. Guanosine was found to be an anti-depressant in a forced swimming test (FST) through the modulation of NMDA receptors and phosphatidylinositol-3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathways [36]. Also, most neuroprotection effects of nucleotides (mainly GMP) seemed to be associated with its conversion to guanosine [37]. Based on the above research, we assume that the guanosine may play a role in modulating the dopaminergic, glutamatergic or serotonergic system to improve schizophrenia and depression.

Pharmacological animal models, which are important preclinical tools to develop more effective drugs [38], were used in this study to test the effects of guanosine on experimental schizophrenia induced by MK-801 and d-amphetamine, forced swimming-induced depression-like psychotic disorders, and 5-HTP-induced serotonin syndrome. We demonstrated that guanosine decreased hyperlocomotion induced by MK-801, but showed no effect in hyperlocomotion induced by d-amphetamine. Besides, guanosine reduced the serotonin syndrome induced by 5-HTP. Guanosine appeared to modulate 5-HT_{1A} and 5-HT_{2A} receptors instead of dopamine D_1 or D_2 receptors and might be anti-schizophrenic partly through PTX-sensitive $G_{i/o}$-coupled PI3K/Akt signaling.

Materials and methods

Animals

Male ICR mice (25-35 g, aged 8 weeks) and male Sprague Dawley (SD) rats (200-300 g, 8 weeks) were purchased from Shanghai Sipper-bk Laboratory Animal Co., Ltd. (Shanghai, China) and housed in plastic cages with food and water available *ad libitum* and maintained at 22 ± 1°C with a 12-h light-dark cycle from 6 am to 6 pm. All of the procedures were conducted according to the guidelines of the Care and Use of Laboratory Animals of China, and designed to minimize suffering and reduce the number of animals used in the experiments.

Hyperlocomotion induced by MK-801

Hyperlocomotion is considered a positive symptom of schizophrenia. ICR mice were divided into control, model (MK-801 0.5 mg/kg), guanosine (0.5 and 2.5 mg/kg), and clozapine (2 mg/kg short-term and long-term treatments) with 10 mice per group. Clozapine was used as the positive control and saline was used as the negative control. Two doses of guanosine were administered once per day for 15 days. As the positive control, clozapine was treated once per day for 15 days or once on day 15. MK-801 (0.5 mg/kg, i.p.) was injected 1 h later after guanosine and clozapine administration. After MK-801 injection, mice were maintained individually in a plastic cage (49 × 49 × 40 cm), and the locomotion was recorded for 20 min by a video tracking system (Anilab Software and Instruments Co., Ltd, Zhejiang, China).

Stereotypies induced by MK-801

Stereotypic behavior is a typical symptom of schizophrenia. ICR mice were divided into five groups (10 mice/group): Control, Model (MK-801 0.5 mg/kg), Clozapine 2 mg/kg, and Guanosine (0.5, 2.5 mg/kg). Clozapine was used as the positive control. Control and model animals were given saline instead of guanosine. Guanosine was administered once a day for 15 days. On day 15, MK-801 (0.5 mg/kg, i.p.) was intraperitoneally injected 1 hour after guanosine and clozapine administration. MK-801-induced stereotypies (rearing, circling behavior, sniffing, licking, biting, gnawing, and grooming) were recorded every 5 min for 1.5 h. The intensity of stereotypy was recorded using a modified ranked intensity scale where 0 = absent, 1 = equivocal, 2 = present, 3 = intense, and 4 = intense and continuous [18].

Locomotor activities induced by d-amphetamine

SD rats were divided into four groups (10 rats/group): (1) Control, (2) Model (d-amphetamine, 1 mg/kg), (3) Haloperidol (0.3 mg/kg), and (4) Guanosine (2.5 mg/kg). Haloperidol was used as the positive control. Control and Model rats were given saline instead of guanosine. Rats were maintained individually to get familiarized with the environment for 30 min. Haloperidol (0.3 mg/kg) and guanosine (2.5 mg/kg) were subcutaneously injected and oral administered, respectively, 30 min before d-amphetamine (1 mg/kg, i.p.) treatment. After d-amphetamine injection, rats were placed individually in a plastic cage (43 × 43 × 35 cm). Locomotor activity was measured for 90 min using the ANY-maze video tracking system (Stoelting Co., Wood Dale, IL, USA).

Forced swimming test (FST)

ICR mice were randomly assigned to the following five groups (8 mice each): Saline, Desipramine (positive medicine) 20 mg/kg, Guanosine 0.5, 1, and 5 mg/kg. All of the compounds were orally administered once a day for 7 days. The FST was performed in all mice with different treatments. Mice were forced to swim for a 15-min session on day 6 followed by a 6-min session on day 7. After acclimatization, 1 hour after drug administration, mice were individu-

ally forced to swim in a Plexiglas cylinder (40 cm high, 40 cm diameter) containing 30-cm deep water (25 ± 1°C) under dim light conditions between 8:30 a.m. and 11:30 a.m. The duration of immobility (stopped struggling, became immobile, kept floating) within the last 6 min was recorded. A decrease in the duration of immobility indicated antidepressant-like effects.

5-HTP induces serotonin syndrome

Male SD rats were divided into five groups of 10 rats each: treatments of 5-HTP 320 mg/kg (Model), Cyproheptadine 4.8 mg/kg, Guanosine 0.75, 2.5, and 7.5 mg/kg. Cyproheptadine hydrochloride sesquihydrate was used as the positive control. The rats in the Model group were treated with saline. Guanosine (0.75, 2.5, 7.5 mg/kg) was orally administered 1 h prior to 5-HTP treatment (320 mg/kg, i.p.). The behavior of rats, including head shaking, forepaw treading, head weaving, hind limb abduction, tremor, hyperlocomotion, and WDS, was video recorded for 1 h and blindly rated. Zero-point for no behavior, one-point for unclear behavior, two-point for obvious behavior, three-point for persistent behavior, and four-point for intensive behavior.

Cell culture and transfection

When the dopamine receptor was activated, it activated Gα16 protein and then activated phospholipase C to produce inositol triphosphate (IP3) and 1,2-diacylglycerol. IP3 can bind to the IP3 receptor on the endoplasmic reticulum and mitochondria of the cell, causing the release of intracellular calcium. HEK293 cells were co-transfected with a plasmid encoding D_1/Gα16 or D_2/Gα16 cDNA. Stably expressing cells were seeded in 96-well plates and continuously cultured overnight. The medium was replaced with Fluo-4/AM (40 μL/well), a fluorescent dye for calcium, and incubated in a 37°C incubator for 40 min. Guanosine (10^{-11}-10^{-4} M, 50 μL), diluted with calcium buffer, was added after washing. The fluorescence value was read at 525 nm by FlexStation II, which automatically added 25 μL dopamine (10^{-12}-10^{-5} M) diluted with calcium buffer. To analyze the effect of guanosine on 5-HT_4R, HEK293 cells were co-transfected with a plasmid encoding 5-HT_4R cDNA. Then, cells were seeded 5 μL/well in a 384-well plate at a density of 4×10^5/mL. In the agonist model, cells were incubated with 5 μL agonist (5-hydroxytryptamine, 5-HT) for 30 min at room temperature (control with DMSO at the same concentration). In antagonist model, cells were firstly incubated with 2.5 μL antagonist for 30 min at room temperature, and then 2.5 μL agonist was added. Different concentrations of guanosine from 10^{-11} to 10^{-4} M were tested. By measuring changes in intracellular cAMP concentration, the activity of guanosine on 5-HT_4R can be determined. To analyze the effect of guanosine on 5-HT_{1A} receptor, SH-SY5Y cells were collected and incubated with 5 μL agonist (5-HT) for 30 min at room temperature. In the antagonist model, cells were first incubated with 2.5 μL antagonist for 30 min at room temperature, and then 2.5 μL agonist was added. By measuring changes in intracellular cAMP concentration, the activity of guanosine on 5-HT_{1A} receptor could be determined.

Western blot analysis

SH-SY5Y cells were seeded in 6-well microplates at a density of 3×10^5 cells/well in Dulbecco's Modified Eagle Medium/F12 (1:1) containing 10% fetal bovine serum for 24 h. To evaluate the PI3K/protein kinase B (Akt) pathway, cells were pretreated with a PI3K inhibitor LY294002 (10 μM; AbMole Bioscience, Houston, TX, USA) for 30 min, and then incubated with guanosine (0, 5, 50 μM) for 60 min. To investigate the involvement of $G_{i/o}$ protein-coupled receptors, cells were pretreated with the $G_{i/o}$ inhibitor pertussis toxin (PTX) (100 ng/mL; Merck, Darmstadt, Germany) for 12 h, and then incubated with guanosine (0, 5, 50 μM) for 60 min. The treated cells were washed with ice-cold D-Hanks and incubated with lysis tissue protein extraction buffer for 30 min on ice.

SD rats were treated with guanosine (5 mg/kg) once a day for 7 days and then sacrificed by decapitation. Brain tissues were expeditiously removed and cortices were divided on ice. Cortices were homogenized in lysis buffer (2 mM EDTA, 50 mM Tris HCl pH = 7.5, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.25% sodium deoxycholate) containing phosphatase inhibitor complex III (Sangon Biotech, Shanghai, China) and phosphatase inhibitor cocktails (Sangon Biotech). Tissue and cells lysates were centri-

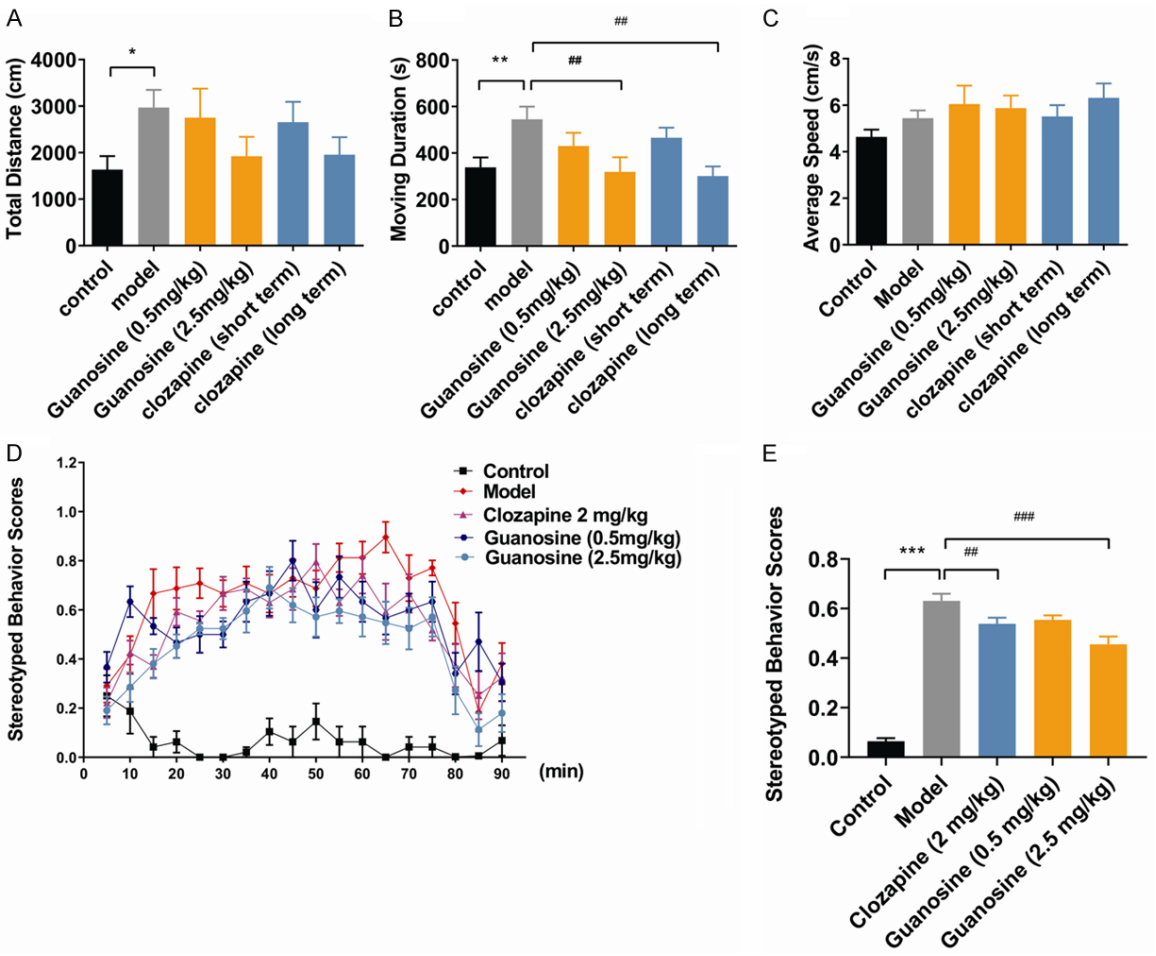

Figure 1. Guanosine attenuated MK-801-induced hyperlocomotion and stereotyped behaviors. Hyperlocomotion and stereotyped were stimulated by intraperitoneal injection of MK-801 (0.3 mg/kg). (A) Total distance (cm) and (B) moving duration (C) average speed of mice were recorded in 20 min after MK-801 injection. (D) Average stereotyped behavior scores of mice evaluated every 5 min for a total of 90 min after injection. Each group tested is represented by one symbol: Control (black squares), Model (pink diamonds), Clozapine (2 mg/kg) (purple triangles), Guanosine (0.5 mg/kg) (blue hexagons), Guanosine (2.5 mg/kg) (indigo circles). (E) Average stereotyped behavior scores of mice in 90 min. ICR mice were divided into five groups: Control, Model (MK-801 0.5 mg/kg), Clozapine 2 mg/kg, and 0.5 mg/kg or 2.5 mg/kg Guanosine. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ $^{***}P < 0.001$ vs. Control group, $^{\#\#}P < 0.01$, $^{\#\#\#}P<0.001$ vs. Model group.

fuged at 5000 rpm at 4°C for 10 min. The protein levels in the supernatant were determined using the Bradford assay. The supernatant was diluted loading buffer (0.25 mM Tris pH = 6.8, 8% sodium dodecyl sulfate, 40% glycerol, 0.4 M DL-dithiothreitol, 0.04% Bromophenol Blue), denatured at 100°C for 5 min. Protein samples (25 μg/lane) were separated by electrophoresis using a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to polyvinylidene difluoride membrane. After blocking in 5% BSA at room temperature for 2 h, the membranes were incubated with one of following primary antibodies: rabbit anti-phospho-Akt (Ser473), rabbit anti-Akt, and anti-GADPH (1:1500; Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. Membranes were incubated with horseradish peroxidase-labeled secondary antibody (Santa Cruz, Dallas, TX, USA) for 2 h at room temperature. Blots were visualized with enhanced chemiluminescence (ECL) (Millipore, Billerica, MA, USA) and integrated optical densities of protein bands were measured using ImageJ 1.46 software.

Statistical analysis

All of the data are reported as the mean ± standard error of the mean (SEM) and analyzed with SPSS 17.0 software. Statistical significance

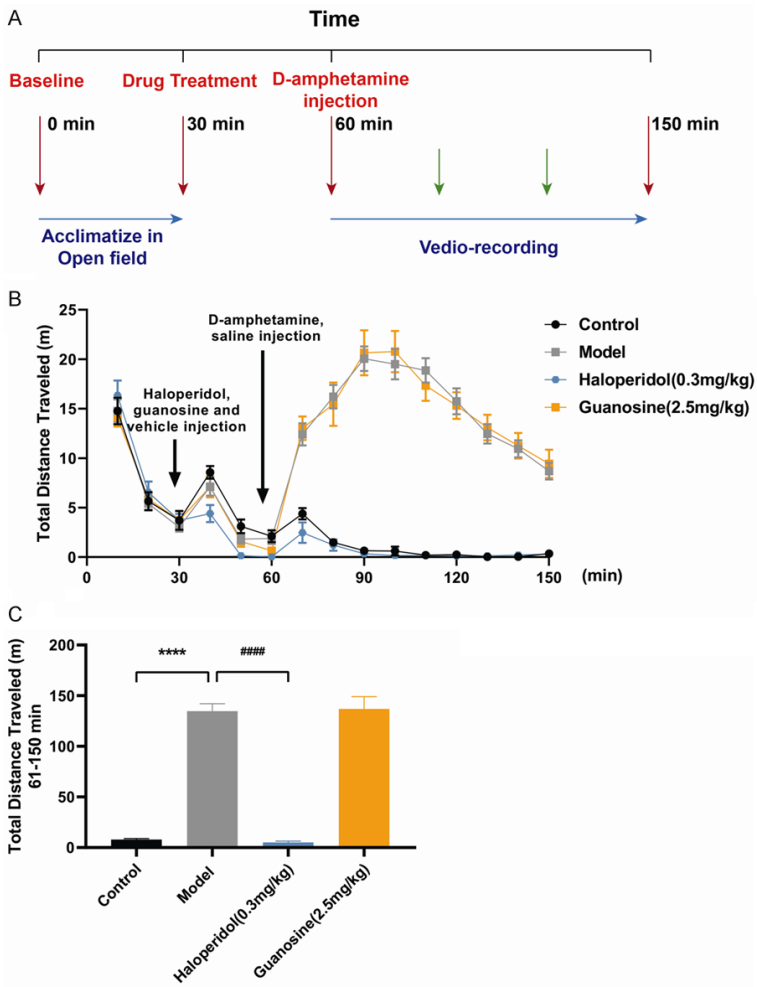

Figure 2. Effects of guanosine on D-amphetamine-induced hyperlocomotion. Hyperlocomotion was induced by intraperitoneal injection of d-amphetamine (1 mg/kg). A. Experimental protocol diagram of detection locomotor activities induced by D-amphetamine. B. The average distance (m) of each group recorded every 10 minutes for a total of 150 minutes. C. The total distance (m) traveled by each rat from 61 to 150 minutes. Data are expressed as mean ± SEM. $^{****}P < 0.0001$ vs. Control group, $^{####}P < 0.0001$ vs. Model group.

was assessed with one-way analysis of variance followed by multi-comparison analysis (least significant difference, Student-Newman-Keuls). $P < 0.05$ was considered to be statistically significant.

Results

Guanosine attenuates MK-801-induced hyperlocomotion and stereotyped behaviors

MK-801 can induce negative, positive and cognitive deficits of schizophrenia. Among them, hyperlocomotion is considered a positive symptom. And stereotypic behavior is also a typical symptom of schizophrenia. The effects of guanosine (0.5 mg/kg and 2.5 mg/kg) on MK-801 (0.5 mg/kg)-induced hyperlocomotion and stereotyped behaviors were tested. MK-801 significantly increased the total moving distance and duration in the open field test (**Figure 1A**, **1B**). No significant difference in average speed was observed in the different groups (**Figure 1C**). A 15-day administration of both clozapine and guanosine dose dependently attenuated moving duration, with statistical differences at higher doses (**Figure 1B**). Stereotyped behaviors including rearing, circling behavior, sniffing, licking, biting, gnawing, and grooming were graded every 5 min for 1.5 h, and the average scores (The sum of the six behavior scores divided by 6.) are shown in **Figure 1D** and **1E**. MK-801 (0.5 mg/kg) significantly induced stereotyped behaviors between 15 and 75 min (**Figure 1D**). Clozapine (2 mg/kg) and guanosine (2.5 mg/kg) attenuated stereotyped behaviors compared with MK-801-treated mice. However, no significant effect was observed in mice treated with 0.5 mg/kg guanosine compared with the model group (**Figure 1E**).

Guanosine cannot block hyperlocomotion induced by D-amphetamine

To investigate whether the dopaminergic system is involved in the anti-Schizophrenia effects of guanosine, the experiments of guanosine (2.5 mg/kg) on D-amphetamine (1 mg/kg)-induced hyperlocomotion was performed. The dopamine activity in the midbrain of the animals was increased in this model, which simulated the positive symptoms of schizophrenia,

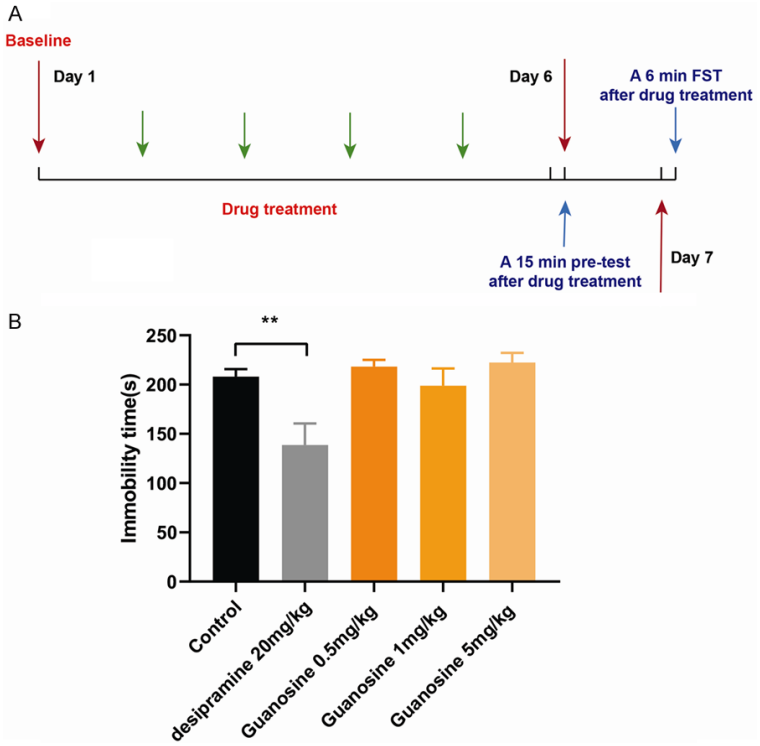

Figure 3. Guanosine did not influence the immobility time of the Forced Swimming Test (FST). A. Experimental protocol diagram of the FST. FST was conducted 60 min after drug administration on the seventh day. B. The duration of immobility (stopped struggling, became immobile, kept floating) within the last 6 min of the rats in different group. Data are expressed as mean ± SEM. $^{**}P < 0.01$ vs. Control group.

such as auditory hallucinations and persecuted delusions. Moving distance of every 10 min from 0 to 150 min are shown in **Figure 2A**. The distance traveled by each group was recorded every 10 minutes for a total of 150 minutes, which is shown in **Figure 2B**. **Figure 2C** displayed the total distances traveled by each rat in the different groups from 61 minutes to 150 minutes. D-amphetamine (1 mg/kg) induced hyperlocomotion immediately after injection ($P < 0.0001$). Haloperidol, as a positive control, significantly decreased hyperlocomotion induced by D-amphetamine ($P < 0.0001$). However, guanosine (2.5 mg/kg) could not block D-amphetamine-induced hyperlocomotion.

Guanosine has no effect on the forced swimming test

Previous evidence suggested that the dysfunction of glutamate-mediated neurotransmission is associated with a range of neuropsychiatric disorders, including schizophrenia and depression. The forced swimming test (FST), also known as the behavioral despair test, is a recognized and reliable depression model for predicting the therapeutic potential of compounds. So, we evaluated the effect of guanosine (0.5, 1, 5 mg/kg) on the FST. The FST was performed in a Plexiglas cylinder, and the duration of immobility (stopped struggling, became immobile, kept floating) within the last 6 min was recorded. A decrease in the duration of immobility indicated antidepressant-like effects. Compared with the FST control, desipramine (5 mg/kg) significantly reduced the immobility time ($P < 0.01$), while guanosine (0.5, 1, 5 mg/kg) did not show an effect on relieving depressive behaviors in the FST (**Figure 3**).

Guanosine reduced 5-HTP induced rats' intensity of serotonin syndrome

Dysfunction of the 5-HT system is associated with a variety of neuropsychiatric diseases, including schizophrenia. 5-HT2A receptor antagonists can increase dopaminergic transmission in the substantia nigra striatum and prefrontal cortex, thereby reducing the risk of EPS, and improve negative symptoms and cognitive impairment by increasing the release of dopamine and acetylcholine in the prefrontal cortex. Based on the previous work, we have already known that guanosine can play an anti-schizophrenic effect by modulating the glutamatergic system. However, it remains unaware that guanosine affect the glutamatergic system alone or jointly regulate the serotoninergic system. So, we further investigated the effect of guanosine on serotonin system and related receptors. 5-HTP (320 mg/kg) induced intense serotonin syndromes (SS) in rats. The videos were watched by an unsuspecting observer, and the rats' behaviors were evaluated blindly: head shaking, forepaw treading, head weaving, hind limb abduction, tremor, and hyperlocomotion were rated, and wet dog-like shaking (WDS)

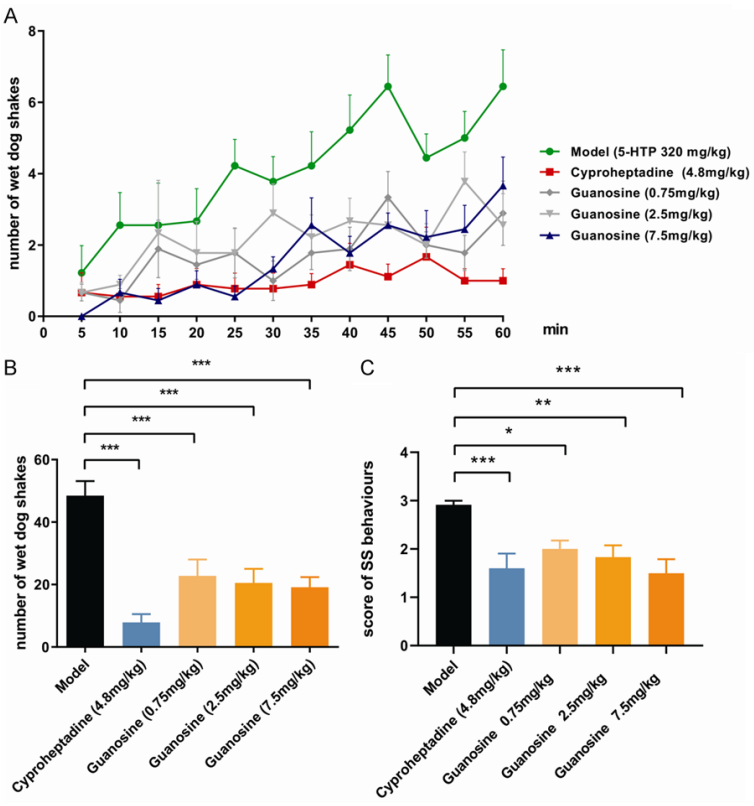

Figure 4. Guanosine reduced 5-HTP induced intense serotonin syndrome. Effects of guanosine on 5-HTP-induced WDS. A. Number of WDS of rats recorded every 5 min for a total of 60 min. Each group tested is represented by one symbol: Model (green circles), Cyproheptadine (red squares), 0.75 mg/kg (grey diamonds), 2.5 mg/kg (grey triangles) or 7.5 mg/kg (blue triangles) Guanosine. B. Total number of WDS recorded after injection (60 min). C. Other SS behaviors including head shaking, forepaw treading, head weaving, hind limb abduction, tremor, and hyperlocomotion induced by 5-HT_{1A} receptor were rated. Zero-point for no behavior, one-point for unclear behavior, two-point for obvious behavior, three-point for persistent behavior, and four-point for intensive behavior. Data are expressed as mean ± SEM. $^{***}P < 0.001$ vs. Model group.

behavior was counted every 5 min for 1 h. Guanosine (0.75, 2.5, 7.5 mg/kg) dose dependently attenuated WDS and other SS behaviors (**Figure 4A**, **4B**). Cyproheptadine (4.8 mg/kg), as a positive control, also significantly decreased WDS and other SS behaviors (**Figure 4C**) induced by 5-HTP.

Guanosine could not antagonize D_1 or D_2 receptors

We have demonstrated that guanosine (2.5 mg/kg) could not block D-amphetamine-induced hyperlocomotion. To rule out the contingency of animal experiments, we constructed HEK293 cells with stable expression of D_1/Gα16 or D_2/Gα16. Normally, the stimulation of dopamine receptors leads to the activation of Gα16 receptors, which in turn activates PLC. PLC activation will increase IP3 and DAG, and then IP3 binds to the IP3 receptors on the endoplasmic reticulum and mitochondria, resulting in intracellular calcium release. Fluorescent probe Fluo-4/AM was used to detect calcium flux. If dopamine receptors were antagonized, a decrease in calcium will be detected. D_1 receptors and D_2 receptors were activated by dopamine (10^{-12}-10^{-5} M), and the EC_{50} was 1.325×10^{-8} M (**Figure 5A**) and 2.964×10^{-8} M (**Figure 5B**). Guanosine (10^{-11}-10^{-4} M) did not change the calcium release in HEK293 cells stably expressing D_1 receptors (**Figure 5C**) or D_2 receptors (**Figure 5D**) activated by dopamine at a dose of 50 nM.

Effect of guanosine on 5-HT_4 receptors and 5-HT_{1A} receptors

To investigate the mechanism by which guanosine improves 5-HTP induced intense serotonin syndrome, HEK293 cells with stable expression of 5-HT_4 receptors were constructed. 5-HT_4 receptors are Gs-coupled. When the receptor binds to an agonist, adenylate cyclase is activated, which results in an increase of cAMP. However, when they bind to antagonists, the activity of adenylate cyclase is inhibited and decreases cAMP. Even with the addition of agonist stimulation, the intracellular cAMP concentration will not increase. 5-HT (10^{-12}-10^{-5} M, 5-HT_4R agonist) activated 5-HT_4R, which led to an increase in cAMP (EC_{50} = 3.252×10^{-10} M, **Figure 6A**). In contrast, guanosine (10^{-11}-10^{-4} M) did not activate 5-HT_4R (**Figure 6B**). On the other hand, cells were pretreated with guanosine (10^{-11}-10^{-4} M) and then treated with 5-HT. 5-HT still stimulated 5-HT_4R in HEK293 cells, which led to an increase in cAMP (EC_{50} = 2.885×10^{-10} M, **Figure 6C**). The results showed that guanosine had no antagonistic effect on 5-TH_4R (**Figure 6D**). 5-HT_{1A} receptor belongs to G protein-coupled receptors, which can be coupled with $G_{i/o}$

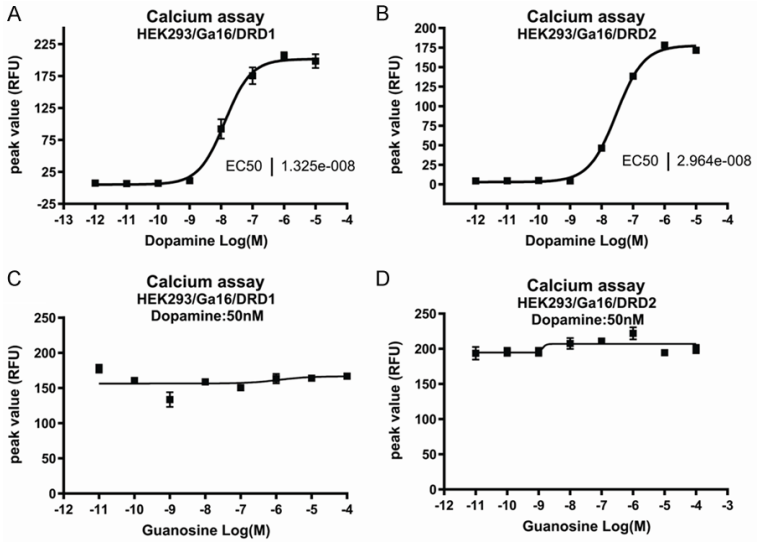

Figure 5. Calcium release induced by dopamine or guanosine in HEK293 cells expressing D_1 or D_2 receptors. A. Dopamine stimulated D_1R in HEK293 cells expressing Gα16 and D_1 receptors. B. Dopamine stimulated D_2R in HEK293 cells expressing Gα16 and D_2 receptors. C. Guanosine did not antagonize D_1R since it is activated by 50 nM dopamine. D. Guanosine did not antagonize D_2R stimulated by 50 nM dopamine. Data are expressed as mean ± SEM.

protein after activation to inhibit adenylate cyclase activity. The 5-HT (10^{-14}-10^{-5} M, 5-HT_{1A}R agonist) did not lead to the decrease of cAMP in SH-SY5Y (**Figure 6E**), after pretreatment with guanosine, 5-HT led to the increase of cAMP in SH-SY5Y (**Figure 6F**). The results showed that guanosine had modulating effects on 5-HT_{1A}R.

Guanosine activates PTX-sensitive $G_{i/o}$-coupled PI3K/Akt signaling

5-HT receptors are G-protein coupling to a variety of intracellular signaling cascades, including cAMP, arachidonic acid accumulation, and the activation/inhibition of PI3K/Akt and mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinase (ERK) [39-41]. To investigate the effect of PI3K/Akt in guanosine's anti-psychotic effects, we first detected the protein expression rate of p-Akt/total Akt in the cortex of rats. The phosphorylation of Akt was significantly increased in the cortex after 7-day administration of guanosine compared with untreated rats (**Figure 7A**). Next, SH-SY5Y cells were treated with the PI3K inhibitor LY294002 (20 μM, 30 min) before guanosine (0, 5, 50 μM, 30 min) treatment. Similar to *in vivo* treatment, guanosine (50 μM) significantly increased the phosphorylation of Akt compared with control (0 μM). The activation of Akt (p-Akt) induced by guanosine was inhibited by LY294002 (**Figure 7B**), indicating that downstream $G_{i/o}$-coupled signaling was involved in the activity of guanosine. We further investigated whether guanosine acts on PTX-sensitive $G_{i/o}$ protein, the upstream of 5-TH coupled receptors. PTX (a $G_{i/o}$ inhibitor) was used as a probe. The results showed that PTX significantly decreased guanosine-induced phosphorylation of Akt, suggesting that guanosine works through $G_{i/o}$-linked signaling (**Figure 7C**).

Discussion

NMDAR antagonists, such as MK-801, can cause glutamatergic failure, and therefore, induce both negative and positive symptoms. Dopamine agonists, including amphetamine, may induce positive symptoms. This study confirmed the anti-psychotic effect of guanosine (2.5 mg/kg, *in vivo*) by showing that it reduced hyperlocomotion and stereotyped behaviors induced by MK-801. In addition, guanosine showed no effect on D-amphetamine-induced hyperlocomotion. Low dosage of guanosine (0.5 mg/kg) slightly inhibited the positive behaviors induced by MK-801, but the differences were not significant, most likely because 0.5 mg/kg was too low to be effective. These results are in accordance with a previous study [42], indicating that guanosine may exert anti-schizophrenic effects through modulating activity. The glutamatergic system also plays an important role in depressive disorders [43]. In anti-depressant tests, guanosine showed no effects in FST, which is inconsistent with the research of Bettio *et al.* [36, 44]. Because the slight difference in experimental devices and animal strain may cause different results, further tests are needed to verify its anti-depressant effect.

Based on the behavioral experiments above, it is unclear whether guanosine affects the glutamatergic system alone or in combination with the serotonergic system. The 5-HT_4 receptor is

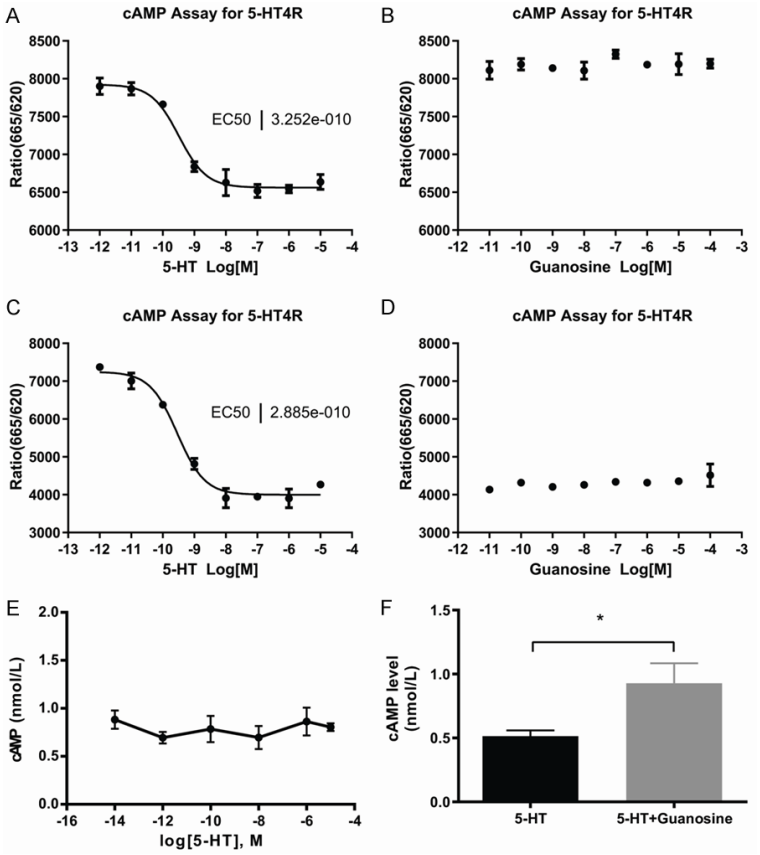

Figure 6. Effect of guanosine on 5-HT_4 receptors and 5-HT_{1A} receptors. A. 5-HT stimulated 5-HT_4R in HEK293 cells which led to the increase of cAMP. B. Guanosine did not activate 5-HT_4R in HEK293 cells. C. After pretreatment with guanosine, 5-HT stimulated 5-HT_4R in HEK293 cells. (EC50 = 2.885 × 10^{-10} M). D. Guanosine did not antagonize 5-HT_4R. E. 5-HT did not lead to the decrease of cAMP in SH-SY5Y. F. After pretreatment with guanosine, 5-HT led to the increase of cAMP in SH-SY5Y. Data are expressed as mean ± SEM.

coupled to the Gs protein. Activation of this receptor increases the activity of cerebral cortex neurons and the release of Ach in hippocampus. Previous study has demonstrated that the serotonin 5-HT_4 receptor is related to cognitive function, of which the disorder is considered to be one of the core disorders of schizophrenia. And the variations in the 5-HT_4 receptor gene (HTR4) change the genetic susceptibility to schizophrenia [45]. In order to determine the specific serotonin receptors acted on by guanosine, HEK293 cells with 5-HT_4/Gs expression were used. **Figure 6** indicates that guanosine did not antagonize 5-HT_4/Gs receptors. A previous study showed that most SS behavioral responses are mediated by 5-HT_1 receptors, which are $G_{i/o}$-coupled [40]. Consistent with the behavioral test, guanosine increased the cAMP level, indicating that the 5-HT_{1A} receptor is involved in the modulation of 5-HT receptors.

The effect of guanosine on serotonin syndrome induced by 5-HTP was also investigated. 5-HTP induces SS behaviors such as forepaw treading, head weaving, WDS, and rearing [46-49]. 5-HT_{1A} receptor mediates most behavioral responses in rats. For example, buspirone, a partial 5-HT_{1A} receptor agonist, induces a narrower spectrum of behaviors compared with 8-OH-DPAT, which is a 5-HT_{1A} receptor agonist. Other behaviors, including WDS and head weaving, are mediated by 5-HT_{2A} receptors [50, 51], and there are strong interaction effects between 5-HT_1 and 5-HT_2 receptors. **Figure 4** demonstrates that guanosine decreased WDS in a dose-dependent manner, indicating that it also blocked the serotoninergic receptor 5-HT_{2A}.

Typical antipsychotics, also regarded as first-generation antipsychotic agents, mainly work through blocking D_2 receptors [52, 53]. First, combined with the results showing anti-psychotic effects, we investigated the involvement of dopamine D_1 or D_2 receptors. HEK293 cells with D_1/Gα16 and D_2/Gα16 expression were used. **Figure 5** indicated that guanosine did not change the calcium release in HEK293 cells, indicating that guanosine does not antagonize D_1 or D_2 receptors. Previous in vitro studies have shown that D_2R have two mutual affinity states for endogenous dopamine, which called G-protein coupled high affinity ($D2^{High}$) and G-protein uncoupled low affinity ($D2^{Low}$) states [54, 55]. $D2^{High}$ is the active state of D_2R function. There is also in vivo clinical evidence that the modulation ratio of $D2^{High}$ is related to the molecular etiology of schizophrenia [56]. Overall, $D2^{High}$ may be one of the common mechanisms of schizophrenia. Besides, in the pathophysiological examination of schizophre-

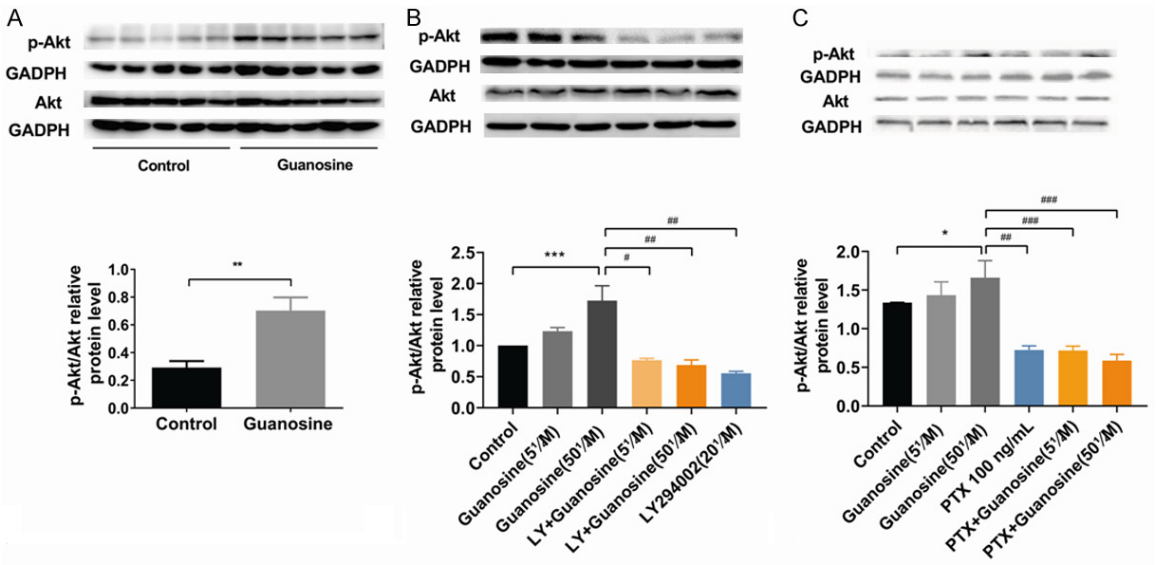

Figure 7. Expression of p-Akt/Akt in brain tissues and SH-SY5Y cells. A. Relative expression of p-Akt/Akt in cortex treated with guanosine (5 mg/kg) for 7 days. B. Relative expression of p-Akt/Akt in cells pretreated with LY294002 for 30 min and then 5 μM, 50 μM guanosine for 60 min. C. Relative expression of p-Akt/Akt in SH-SY5Y cells. Cells were pretreated with PTX for 12 h and then 5 μM, 50 μM guanosine was added to the cultures for 60 min. Data are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. Control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ vs. relative guanosine concentration group.

nia, it was found that the prefrontal D_1 receptor density in was reduced [57]. Therefore, we believe that guanosine may exert an anti-schizophrenic effect from the above-mentioned mechanisms instead of directly antagonizing D_1 or D_2 receptors, and further investigation is needed.

GPC receptor proteins can form active homomers and heteromers with different GPC receptors or even tyrosine kinase receptors to induce cellular signaling [58, 59]. It has been indicated that Akt and glycogen synthase kinase-3 are involved in the regulation of behavior by the monoamine neurotransmitters dopamine and 5-HT [60]. Several studies have shown that the anti-apoptotic effect and neuroprotection of guanosine were mediated by the activation of PI3K/Akt and MAPK/ERK [59, 61]. To investigate the participant of PI3K/Akt in guanosine's anti-psychotic effects, we first detected the protein expression rate of p-Akt/total Akt in the cortex of rats. The phosphorylation of Akt was significantly increased in the cortex of rats after 7-day administration of guanosine compared with the control group (**Figure 7A**). In addition, SH-SY5Y cells were first treated with LY294002 and guanosine. Guanosine significantly increased the phosphorylation of Akt compared with the control. P-Akt significantly decreased in cells pretreated with LY294002 compared with relevant guanosine groups (**Figure 7B**). PTX (a $G_{i/o}$ inhibitor) was used to investigate whether $G_{i/o}$ coupled receptors were involved in the process. PTX significantly decreased the phosphorylation of Akt compared with relevant guanosine groups (**Figure 7C**), indicating the participation of $G_{i/o}$ proteins. However, both PI3K inhibitor and inhibitor of $G_{i/o}$-coupled receptors decreased the phosphorylation of Akt in SH-SY5Y cells. These results indicated that guanosine activated PTX-sensitive $G_{i/o}$-coupled PI3K/Akt signaling. The possible pathways for guanosine to exert anti-schizophrenic effects are shown in **Figure 8**.

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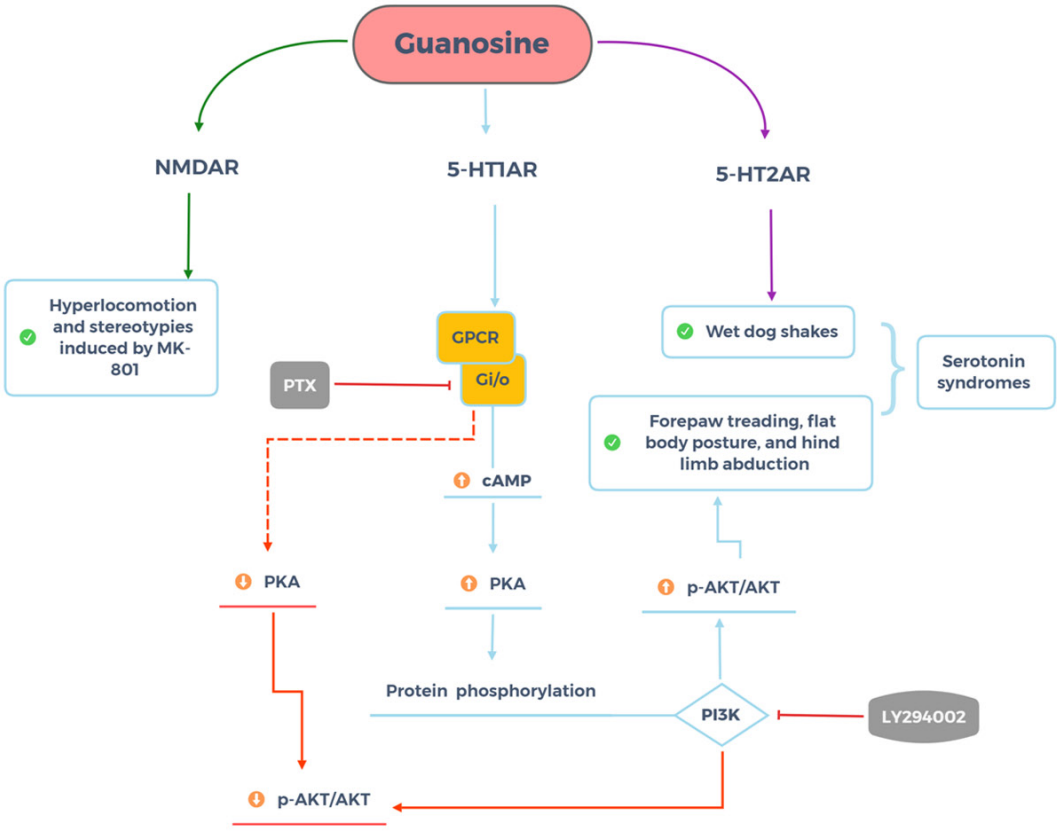
Disclosure of conflict of interest

None.


Guanosine
NMDAR
5-HT1AR
5-HT2AR
Hyperlocomotion and stereotypies induced by MK-801
GPCR
Gi/o
PTX
cAMP
PKA
PKA
Wet dog shakes
Serotonin syndromes
Forepaw treading, flat body posture, and hind limb abduction
p-AKT/AKT
Protein phosphorylation
PI3K
LY294002
p-AKT/AKT

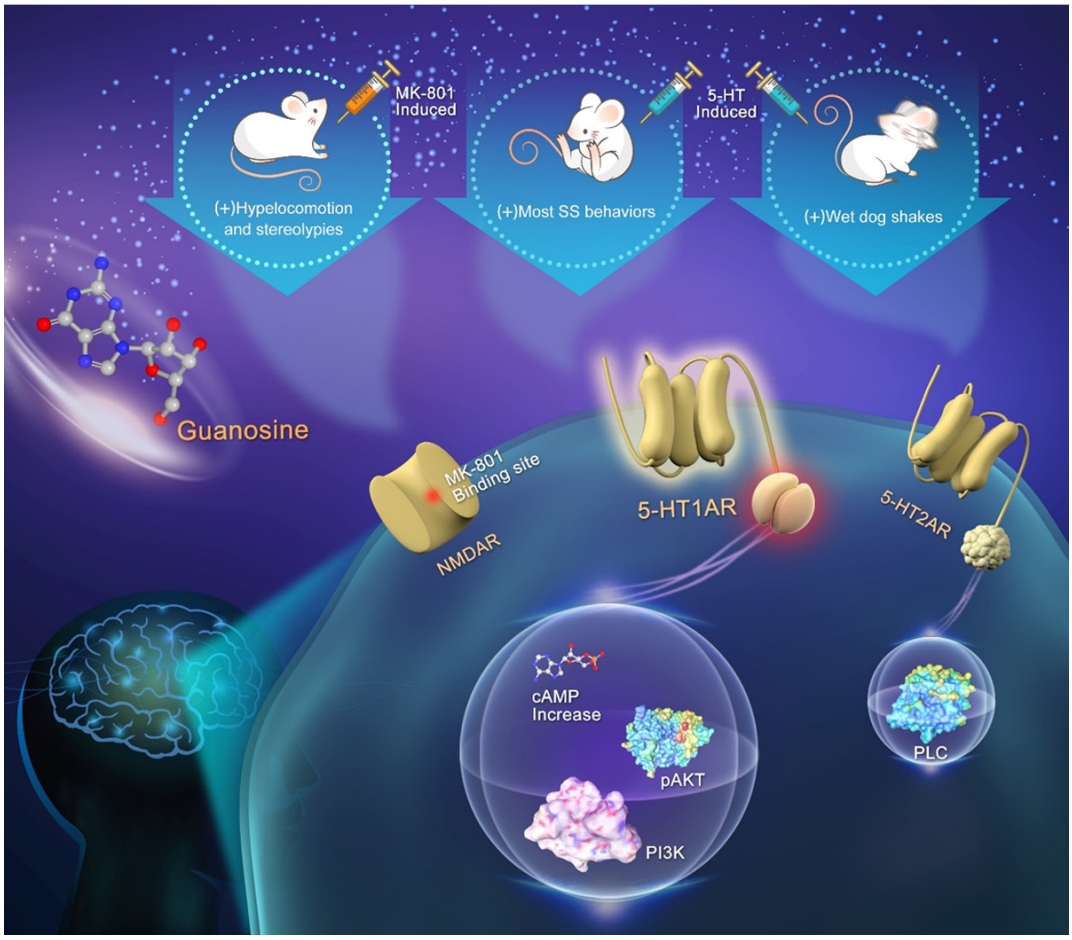

MK-801 Induced
5-HT Induced
(+)Hypelocomotion and stereolypies
(+)Most SS behaviors
(+)Wet dog shakes
Guanosine
MK-801 Binding site
NMDAR
5-HT1AR
5-HT2AR
cAMP Increase
pAKT
PI3K
PLC

Figure 8. The possible pathways for guanosine to exert anti-schizophrenic effects. NMDAR antagonists MK-801 could cause glutamatergic failure, inducing both negative and positive symptoms. Guanosine (2.5 mg/kg, in vivo) could reduce hyperlocomotion and stereotyped behaviors induced by MK-801. In the serotonin system, 5-HTP induces serotonin syndromes (SS) behaviors such as forepaw treading, flat body posture, hind limb abduction, head weaving, rearing, and wet dog shakes. Guanosine could improve the wet dog shakes induced by 5-HTP, indicating that guanosine blocked the serotoninergic receptor 5-HT_{2A}R. Besides, guanosine also modulated 5-HT_{1A}R, which led to an increase in cAMP. The phosphorylation of Akt was then significantly increased in the cortex after administration of guanosine. Furthermore, the increase of p-Akt/Akt caused by Guanosine was reversed by the $G_{i/o}$ inhibitor PTX and the PI3K inhibitor LY294002. The results above suggested that guanosine may significantly decrease the most SS behaviors induced by 5-HTP through activating PTX-sensitive $G_{i/o}$-coupled PI3K/Akt signaling.

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References

[1] Schultz SK and Andreasen NC. Schizophrenia. Lancet 1999; 353: 1425-1430.

[2] Seshadri S, Klaus A, Winkowski DE, Kanold PO and Plenz D. Altered avalanche dynamics in a developmental NMDAR hypofunction model of cognitive impairment. Transl Psychiatry 2018; 8: 3.

[3] Urban-Kowalczyk M, Pigonska J and Smigielski J. Pain perception in schizophrenia: influence of neuropeptides, cognitive disorders, and negative symptoms. Neuropsychiatr Dis Treat 2015; 11: 2023-2031.

[4] Buoli M, Kahn RS, Serati M, Altamura AC and Cahn W. Haloperidol versus second-generation antipsychotics in the long-term treatment of schizophrenia. Hum Psychopharmacol 2016; 31: 325-331.

[5] Seki K, Yoshida S and Jaiswal MK. Molecular mechanism of noradrenaline during the stress-induced major depressive disorder. Neural Regen Res 2018; 13: 1159-1169.

[6] Abdallah CG, Sanacora G, Duman RS and Krystal JH. The neurobiology of depression, ketamine and rapid-acting antidepressants: is it glutamate inhibition or activation? Pharmacol Ther 2018; 190: 148-158.

[7] Juengst SB, Kumar RG and Wagner AK. A narrative literature review of depression following traumatic brain injury: prevalence, impact, and management challenges. Psychol Res Behav Manag 2017; 10: 175-186.

[8] van Rooijen G, Vermeulen JM, Ruhé HG and de Haan L. Treating depressive episodes or symptoms in patients with schizophrenia. CNS Spectrums 2019; 24: 239-248.

[9] Juza R, Vlcek P, Mezeiova E, Musilek K, Soukup O and Korabecny J. Recent advances with 5-HT3 modulators for neuropsychiatric and gastrointestinal disorders. Med Res Rev 2020; 40: 1593-1678.

[10] Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW and Lieberman JA. Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatry 2012; 17: 1206-1227.

[11] Kapur S, Zipursky RB and Remington G. Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. Am J Psychiatry 1999; 156: 286-293.

[12] Jones MT, Strassnig MT and Harvey PD. Emerging 5-HT receptor antagonists for the treatment of Schizophrenia. Expert Opin Emerg Drugs 2020; 25: 189-200.

[13] Wang Y, Yang X, Song X, Zhao L, Wei J, Wang J, Tian H, Zheng C, Wei M, Wang Q, Guo W, Deng W, Li T and Ma X. Co-treatment of buspirone with atypical antipsychotic drugs (AAPDs) improved neurocognitive function in chronic schizophrenia. Schizophr Res 2019; 209: 135-140.

[14] Tran HQ, Shin EJ, Hoai Nguyen BC, Phan DH, Kang MJ, Jang CG, Jeong JH, Nah SY, Mouri A, Saito K, Nabeshima T and Kim HC. 5-HT1A receptor agonist 8-OH-DPAT induces serotonergic behaviors in mice via interaction between PKCdelta and p47phox. Food Chem Toxicol 2019; 123: 125-141.

[15] Tran HQ, Lee Y, Shin EJ, Jang CG, Jeong JH, Mouri A, Saito K, Nabeshima T and Kim HC. PKCdelta knockout mice are protected from dextromethorphan-induced serotonergic behaviors in mice: involvements of downregulation of 5-HT1A receptor and upregulation of Nrf2-dependent GSH synthesis. Mol Neurobiol 2018; 55: 7802-7821.

[16] Shimizu S, Mizuguchi Y and Ohno Y. Improving the treatment of schizophrenia: role of 5-HT receptors in modulating cognitive and extrapyramidal motor functions. CNS Neurol Disord Drug Targets 2013; 12: 861-869.

[17] Odabas-Geldiay M, Shields H, Berro LF, Rice KC and Howell LL. Effects of acute treatments with the serotonin 2A antagonist M100907 alone or in combination with the serotonin 2C

agonist WAY163909 on methamphetamine self-administration in rhesus monkeys. Drug Alcohol Depend 2019; 194: 252-256.
[18] Ninan I and Kulkarni SK. 5-HT2A receptor antagonists block MK-801-induced stereotypy and hyperlocomotion. Eur J Pharmacol 1998; 358: 111-116.
[19] Jaber M, Robinson SW, Missale C and Caron MG. Dopamine receptors and brain function. Neuropharmacology 1996; 35: 1503-1519.
[20] Martel JC and Gatti McArthur S. Dopamine receptor subtypes, physiology and pharmacology: new ligands and concepts in schizophrenia. Front Pharmacol 2020; 11: 1003.
[21] Simpson EH and Kellendonk C. Insights about striatal circuit function and schizophrenia from a mouse model of dopamine D2 Receptor upregulation. Biol Psychiatry 2017; 81: 21-30.
[22] Sigmundson HK. Pharmacotherapy of schizophrenia: a review. Can J Psychiatry 1994; 39: S70-75.
[23] Kellendonk C, Simpson EH, Polan HJ, Malleret G, Vronskaya S, Winiger V, Moore H and Kandel ER. Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. Neuron 2006; 49: 603-615.
[24] Petty A, Cui X, Tesiram Y, Kirik D, Howes O and Eyles D. Enhanced dopamine in prodromal schizophrenia (EDiPS): a new animal model of relevance to schizophrenia. NPJ Schizophr 2019; 5: 6.
[25] da Silva JS, Nonose Y, Rohden F, Lukasewicz Ferreira PC, Fontella FU, Rocha A, Brochier AW, Apel RV, de Lima TM, Seminotti B, Amaral AU, Galina A and Souza DO. Guanosine Neuroprotection of Presynaptic mitochondrial calcium homeostasis in a mouse study with amyloid-beta oligomers. Mol Neurobiol 2020; 57: 4790-4809.
[26] Ciccarelli R, Ballerini P, Sabatino G, Rathbone MP, D'Onofrio M, Caciagli F and Di Iorio P. Involvement of astrocytes in purine-mediated reparative processes in the brain. Int J Dev Neurosci 2001; 19: 395-414.
[27] Zanella CA, Tasca CI, Henley JM, Wilkinson KA and Cimarosti HI. Guanosine modulates SUMO2/3-ylation in neurons and astrocytes via adenosine receptors. Purinergic Signal 2020; 16: 439-450.
[28] Dal-Cim T, Poluceno GG, Lanznaster D, de Oliveira KA, Nedel CB and Tasca CI. Guanosine prevents oxidative damage and glutamate uptake impairment induced by oxygen/glucose deprivation in cortical astrocyte cultures: involvement of A1 and A2A adenosine receptors and PI3K, MEK, and PKC pathways. Purinergic Signal 2019; 15: 465-476.
[29] Schmidt AP, Lara DR and Souza DO. Proposal of a guanine-based purinergic system in the mammalian central nervous system. Pharmacol Ther 2007; 116: 401-416.
[30] Bettio LE, Gil-Mohapel J and Rodrigues AL. Guanosine and its role in neuropathologies. Purinergic Signal 2016; 12: 411-426.
[31] Lanznaster D, Dal-Cim T, Piermartiri TC and Tasca CI. Guanosine: a Neuromodulator with therapeutic potential in brain disorders. Aging Dis 2016; 7: 657-679.
[32] Ribeiro FF, Xapelli S, Miranda-Lourenco C, Tanqueiro SR, Fonseca-Gomes J, Diogenes MJ, Ribeiro JA and Sebastiao AM. Purine nucleosides in neuroregeneration and neuroprotection. Neuropharmacology 2016; 104: 226-242.
[33] Tasca CI, Lanznaster D, Oliveira KA, Fernandez-Duenas V and Ciruela F. Neuromodulatory effects of guanine-based purines in health and disease. Front Cell Neurosci 2018; 12: 376.
[34] Dalla Corte CL, Bastos LL, Dobrachinski F, Rocha JB and Soares FA. The combination of organoselenium compounds and guanosine prevents glutamate-induced oxidative stress in different regions of rat brains. Brain Res 2012; 1430: 101-111.
[35] Thomaz DT, Dal-Cim TA, Martins WC, Cunha MP, Lanznaster D, de Bem AF and Tasca CI. Guanosine prevents nitroxidative stress and recovers mitochondrial membrane potential disruption in hippocampal slices subjected to oxygen/glucose deprivation. Purinergic Signal 2016; 12: 707-718.
[36] Bettio LEB, Cunha MP, Budni J, Pazini FL, Oliveira A, Colla AR and Rodrigues ALS. Guanosine produces an antidepressant-like effect through the modulation of NMDA receptors, nitric oxide-cGMP and PI3K/mTOR pathways. Behav Brain Res 2012; 234: 137-148.
[37] Decker H, Piermartiri TCB, Nedel CB, Romao LF, Francisco SS, Dal-Cim T, Boeck CR, Moura-Neto V and Tasca CI. Guanosine and GMP increase the number of granular cerebellar neurons in culture: dependence on adenosine A2A and ionotropic glutamate receptors. Purinergic Signal 2019; 15: 439-450.
[38] Jones CA, Watson DJ and Fone KC. Animal models of schizophrenia. Br J Pharmacol 2011; 164: 1162-1194.
[39] Nakano N, Matsuda S, Ichimura M, Minami A, Ogino M, Murai T and Kitagishi Y. PI3K/AKT signaling mediated by G proteincoupled receptors is involved in neurodegenerative Parkinson's disease (Review). Int J Mol Med 2017; 39: 253-260.
[40] Albert PR and Vahid-Ansari F. The 5-HT1A receptor: signaling to behavior. Biochimie 2019; 161: 34-45.

[41] Santos-Otte P, Leysen H, van Gastel J, Hendrickx JO, Martin B and Maudsley S. G protein-coupled receptor systems and their role in cellular senescence. Comput Struct Biotechnol J 2019; 17: 1265-1277.
[42] Deutsch SI, Rosse RB, Long KD, Gaskins BL and Mastropaolo J. Guanosine possesses specific modulatory effects on NMDA receptor-mediated neurotransmission in intact mice. Eur Neuropsychopharmacol 2008; 18: 299-302.
[43] Ostadhadi S, Imran Khan M, Norouzi-Javidan A and Dehpour AR. Antidepressant effect of pramipexole in mice forced swimming test: a cross talk between dopamine receptor and NMDA/nitric oxide/cGMP pathway. Biomed Pharmacother 2016; 81: 295-304.
[44] Bettio LE, Freitas AE, Neis VB, Santos DB, Ribeiro CM, Rosa PB, Farina M and Rodrigues AL. Guanosine prevents behavioral alterations in the forced swimming test and hippocampal oxidative damage induced by acute restraint stress. Pharmacol Biochem Behav 2014; 127: 7-14.
[45] Suzuki T, Iwata N, Kitamura Y, Kitajima T, Yamanouchi Y, Ikeda M, Nishiyama T, Kamatani N and Ozaki N. Association of a haplotype in the serotonin 5-HT4 receptor gene (HTR4) with Japanese schizophrenia. Am J Med Genet B Neuropsychiatr Genet 2003; 121B: 7-13.
[46] Jurek L, Nourredine M, Megarbane B, d'Amato T, Dorey JM and Rolland B. The serotonin syndrome: an updated literature review. Rev Med Interne 2019; 40: 98-104.
[47] Mestre TA, Zurowski M and Fox SH. 5-Hydroxytryptamine 2A receptor antagonists as potential treatment for psychiatric disorders. Expert Opin Investig Drugs 2013; 22: 411-421.
[48] Jastrzebska-Wiesek M, Partyka A, Rychtyk J, Sniecikowska J, Kolaczkowski M, Wesolowska A, Varney MA and Newman-Tancredi A. Activity of serotonin 5-HT1A receptor biased agonists in rat: anxiolytic and antidepressant-like properties. ACS Chem Neurosci 2018; 9: 1040-1050.
[49] Matsumoto K, Morishige R, Murakami Y, Tohda M, Takayama H, Sakakibara I and Watanabe H. Suppressive effects of isorhynchophylline on 5-HT2A receptor function in the brain: behavioural and electrophysiological studies. Eur J Pharmacol 2005; 517: 191-199.
[50] Elmore JS and Baumann MH. Repeated exposure to the "Spice" cannabinoid JWH-018 induces tolerance and enhances responsiveness to 5-HT1A receptor stimulation in male rats. Front Psychiatry 2018; 9: 55.
[51] Ma Z, Rudacille M, Prentice HM and Tao R. Characterization of electroencephalographic and biochemical responses at 5-HT promoting drug-induced onset of serotonin syndrome in rats. J Neurochem 2013; 125: 774-789.
[52] Blazer DG. The American Psychiatric Press textbook of psychopharmacology, 2nd edition. J Psychosom Res 2001; 51: 615-615.
[53] Miyamoto S, Mailman RB, Lieberman JA and Duncan GE. Blunted brain metabolic response to ketamine in mice lacking D-1A dopamine receptors. Brain Res 2001; 894: 167-180.
[54] Richfield EK, Penney JB and Young AB. Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience 1989; 30: 767-777.
[55] George SR, Watanabe M, Di Paolo T, Falardeau P, Labrie F and Seeman P. The functional state of the dopamine receptor in the anterior pituitary is in the high affinity form. Endocrinology 1985; 117: 690-697.
[56] Kubota M, Nagashima T, Takano H, Kodaka F, Fujiwara H, Takahata K, Moriguchi S, Kimura Y, Higuchi M, Okubo Y, Takahashi H, Ito H and Suhara T. Affinity states of striatal dopamine D2 receptors in antipsychotic-free patients with schizophrenia. Int J Neuropsychopharmacol 2017; 20: 928-935.
[57] Stenkrona P, Matheson GJ, Halldin C, Cervenka S and Farde L. D1-dopamine receptor availability in first-episode neuroleptic naive psychosis patients. Int J Neuropsychopharmacol 2019; 22: 415-425.
[58] Sleno R and Hebert TE. The dynamics of GPCR oligomerization and their functional consequences. Int Rev Cell Mol Biol 2018; 338: 141-171.
[59] Pyne NJ and Pyne S. Receptor tyrosine kinase-G-protein-coupled receptor signalling platforms: out of the shadow? Trends Pharmacol Sci 2011; 32: 443-450.
[60] Beaulieu JM. A role for Akt and glycogen synthase kinase-3 as integrators of dopamine and serotonin neurotransmission in mental health. J Psychiatry Neurosci 2012; 37: 7-16.
[61] Pettifer KM, Kleywegt S, Bau CJ, Ramsbottom JD, Vertes E, Ciccarelli R, Caciagli F, Werstiuk ES and Rathbone MP. Guanosine protects SH-SY5Y cells against beta-amyloid-induced apoptosis. Neuroreport 2004; 15: 833-836.