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

Yu Mao$^{1*}$, Yao Xing$^{1*}$, Jie Li$^{1*}$, Dong Dong$^{1}$, Shoude Zhang$^{1,3}$, Zhenjiang Zhao$^{1}$, Jingli Xie$^{2}$, Rui Wang$^{1}$, Honglin Li$^{1}$

$^{1}$Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science & Technology, Shanghai 200237, China; $^{2}$State Key Laboratory of Bioreactor Engineering, College of Bioengineering, East China University of Science & Technology, Shanghai 200237, China; $^{3}$State Key Laboratory of Plateau Ecology and Agriculture, Department of Pharmacy, Medical College of Qinghai University, Qinghai University, Qinghai 810016, China. $^{*}$Equal contributors.

Received December 22, 2020; Accepted February 28, 2021; Epub May 15, 2021; Published May 30, 2021

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. 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 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 anti-depressant 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-
Guanosine attenuate schizophrenia-like symptoms

Psychostimulants 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$_{3}$ 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, antipsychotics 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) cyclohydrazse 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.
Guanosine attenuate schizophrenia-like symptoms

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 D1/Gα16 or D2/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-HT₄R, HEK293 cells were co-transfected with a plasmid encoding 5-HT₄R cDNA. Then, cells were seeded 5 μL/well in a 384-well plate at a density of 4 × 10⁵/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-HT₄R can be determined. To analyze the effect of guanosine on 5-HT₁A 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-HT₁A receptor could be determined.

Western blot analysis

SH-SY5Y cells were seeded in 6-well microplates at a density of 3 × 10⁵ 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 Gᵢ/o protein-coupled receptors, cells were pretreated with the Gᵢ/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-
Guanosine attenuate schizophrenia-like symptoms

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
Guanosine attenuate schizophrenia-like symptoms

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 symp-

tom. 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,
Guanosine attenuate schizophrenia-like symptoms

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 groups. Data are expressed as mean ± SEM. **P < 0.01 vs. Control group.

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 serotonergic system. So, we further investigated the effect of guanosine on serotonin system and related receptors. 5-HP (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)
Guanosine attenuate schizophrenia-like symptoms

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-HT₁₆ 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.

Guanosine could not antagonize D₁ or D₂ 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 D₁/Goα16 or D₂/Goα16. Normally, the stimulation of dopamine receptors leads to the activation of Goα16 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. D₁ receptors and D₂ receptors were activated by dopamine (10⁻¹²-10⁻⁸ M), and the EC₅₀ 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 D₁ receptors (Figure 5C) or D₂ receptors (Figure 5D) activated by dopamine at a dose of 50 nM.

Effect of guanosine on 5-HT₄ receptors and 5-HT₁₆ receptors

To investigate the mechanism by which guanosine improves 5-HTP induced intense serotonin syndrome, HEK293 cells with stable expression of 5-HT₄ receptors were constructed. 5-HT₄ 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-HT₄ agonist) activated 5-HT₄R, which led to an increase in cAMP (EC₅₀ = 3.252 × 10⁻¹⁰ M, Figure 6A). In contrast, guanosine (10⁻¹¹-10⁻⁴ M) did not activate 5-HT₄R (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-HT₄R in HEK293 cells, which led to an increase in cAMP (EC₅₀ = 2.885 × 10⁻¹⁰ M, Figure 6C). The results showed that guanosine had no antagonistic effect on 5-HT₄R (Figure 6D). 5-HT₁₆ receptor belongs to G protein-coupled receptors, which can be coupled with Gα₁₆...
Guanosine attenuate schizophrenia-like symptoms

Figure 5. Calcium release induced by dopamine or guanosine in HEK293 cells expressing D_{1} or D_{2} receptors. A. Dopamine stimulated D_{1}R in HEK293 cells expressing G_{ol} and D_{1} receptors. B. Dopamine stimulated D_{2}R in HEK293 cells expressing G_{ol} and D_{2} receptors. C. Guanosine did not antagonize D_{1}R since it is activated by 50 nM dopamine. D. Guanosine did not antagonize D_{2}R stimulated by 50 nM dopamine. Data are expressed as mean ± SEM.

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 compared with control (0 μM). The activation of Akt (p-Akt) induced by guanosine was inhibited by LY294002 (Figure 7B), indicating that downstream G_{ol}-coupled signaling was involved in the activity of guanosine. We further investigated whether guanosine acts on PTX-sensitive G_{ol} protein, the upstream of 5-HT coupled receptors. PTX (a G_{ol} inhibitor) was used as a probe. The results showed that PTX significantly decreased guanosine-induced phosphorylation of Akt, suggesting that guanosine works through G_{ol}-linked signaling (Figure 7C).

Guanosine activates PTX-sensitive G_{ol}-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).
Guanosine attenuate schizophrenia-like symptoms

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$_1$/G$\alpha$16 and D$_2$/G$\alpha$16 expression were used. Figure 5 indicated that guanosine did not change the calcium release in HEK293 cells, indicating that guanosine does not antagonize D$_1$ or D$_2$ receptors. Previous in vitro studies have shown that D$_1$R 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$_1$R 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-
Guanosine attenuate schizophrenia-like symptoms

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.

Acknowledgements

The research is supported in part by the National Key Research and Development Program (Grant 2016YFA0502304), the National Natural Science Foundation of China (grant 81825020). Honglin Li is also sponsored by National Program for Special Supports of Eminent Professionals and National Program for Support of Top-Notch Young Professionals.

Disclosure of conflict of interest

None.
Guanosine attenuate schizophrenia-like symptoms

Guanosine

NMDAR

5-HT1AR

5-HT2AR

Hyperlocomotion and stereotypes induced by MK-801

GPCR

Cl(β)

Wet dog shakes

Serotonin syndromes

Forepaw treading, flat body posture, and hind limb abduction

cAMP

PKA

p-AKT/AKT

Protein phosphorylation

PI3K

LY294002

MK-801 induced

5-HT induced

(-) Hyperlocomotion and stereotypes

(+/-) Most SS behaviors

(+/-) Wet dog shakes

Guanosine

MK-801 binding site

NMDAR

5-HT1AR

5-HT2AR

cAMP increase

PAKT

PI3K

PLC
Guanosine attenuate schizophrenia-like symptoms

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-HT2A. Besides, guanosine also modulated 5-HT2C, 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}-coupled PTX.

Address correspondence to: Drs. Rui Wang and Honglin Li, Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China. Tel: +86-21-64250823; Fax: +86-21-6425-0823; E-mail: ruiwang@ecust.edu.cn (RW); Tel: +86-21-64250213; Fax: +86-21-64250213; E-mail: hlli@ecust.edu.cn (HLL)

References

[17] Odasab-Gellday 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
Guanosine attenuate schizophrenia-like symptoms


Guanosine attenuate schizophrenia-like symptoms


[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.


