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Research report

# Guanosine selectively inhibits locomotor stimulation induced by the NMDA antagonist dizocilpine

Adriano B.L. Tort[a,*], Carlos E. Mantese[a], Gabriel M. dos Anjos[a], Marcelo O. Dietrich[a], Oscar P. Dall'Igna[a], Diogo O. Souza[a], Diogo R. Lara[a,b]

[a] *Departamento de Bioquímica, ICBS, Universidade Federal do Grande do Sul (UFRGS), Rua Ramiro Barcellos 2600, anexo, 90035003 Porto Alegre, Brazil*
[b] *Faculdade de Biociências, PUCRS, Porto Alegre, Brazil*

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**Abstract**

Guanosine has been shown to modulate glutamate system by stimulating astrocytic glutamate uptake. Recent evidence suggest that the locomotor effects of NMDA receptor antagonists, an animal model of schizophrenia, is associated with activation of non-NMDA glutamatergic receptors caused by increased glutamate release. The present work was undertaken to evaluate whether guanosine could have influence on the hyperlocomotion induced in mice by dizocilpine (MK-801), a NMDA antagonist. We also evaluated the effect of guanosine on the hyperlocomotion induced by the indirect dopamine agonist amphetamine, and by the non-selective adenosine receptor antagonist caffeine. Guanosine (7.5 mg/kg) produced an attenuation of about 60% on the hyperlocomotion induced by dizocilpine (0.25 mg/kg), whereas it did not affect the hyperlocomotion induced by amphetamine (5 mg/kg) or caffeine (30 mg/kg). Guanosine pre-treatment did not affect total spontaneous locomotion in all experiments. To test neuronal pathway selectivity, we evaluated MK-801 against guanosine in a working memory paradigm (spontaneous alternation task). Guanosine did not reverted the impairment caused by MK-801 in the spontaneous alternation test, and when administered alone also presented an amnesic effect. The results are discussed based on the current hypothesis of locomotor activation induced by the psychoactive drugs studied. Further studies are necessary to evaluate if guanosine could have clinical utility for the treatment of schizophrenia.


*Keywords:* Guanosine; Amphetamine; Caffeine; MK-801; Locomotion; Schizophrenia

## 1. Introduction

Extracellular guanine-based purines (GBPs), namely the nucleotides GTP, GDP, and GMP and the nucleoside guanosine, have been shown to exert effects not directly related to the modulation of G-proteins. GBPs (including GMP and guanosine) have been studied in several in vivo and in vitro approaches, producing inhibition of binding of glutamate and analogs [6,23,24], neuroprotective effects to excitotoxic conditions [11,19], anticonvulsant action against seizures induced by glutamatergic agents [17,27,33], as well as an amnesic effect [26,33]. In line with these antiglutmatergic effects, we have recently shown that guanosine stimulates astrocytic glutamate uptake [10,12], which is the main mechanism of glutamate removal from the synaptic cleft [5,9].

In the last years, locomotor stimulation induced in rodents by psychoactive drugs has been used as a model with predictive validity for identification of novel antipsychotics. Among them, glutamate NMDA receptor antagonists, such as phencyclidine (PCP) and dizocilpine, have been regarded as the best pharmacological model for schizophrenia [1,2]. Recent evidence suggest that NMDA receptor antagonism is also associated with glutamatergic activation in non-NMDA receptors induced by increased glutamate release, which appears to be closely related to the behavioral alterations observed [2,3,20,21,31].

Based on such glutamatergic dependence of NMDA antagonists action, the present work was undertaken to evaluate whether guanosine, by its antiglutamatergic properties, could have influence on the locomotor stimulation induced by dizocilpine.

* Corresponding author. Tel.: +55-51-33165557/8; fax: +55-51-33165540/35.
*E-mail address:* adrianotort@hotmail.com (A.B.L. Tort).

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Since Loeffler et al. [18] have shown that guanosine decreases dopamine synthesis in cultured rat pheochromocytoma PC12 cells, we also evaluated the effect of guanosine on the hyperlocomotion induced by the indirect dopamine agonist amphetamine (Amph), a classical model of psychosis in rodents. Finally, as some in vitro effects of guanosine seemed to be mediated by adenosine release [25], we also investigated the role of adenosine in the mechanism of action of guanosine by studying the hyperlocomotion induced by the non-selective adenosine receptor antagonist caffeine.

## 2. Material and methods

### 2.1. Animals

Experiments were performed with male adult albino mice (CF1) purchased from Fundação Estadual de Pesquisa em Saúde (FEPS) and maintained in our own animal facilities under controlled environment (23 ± 2 °C, 12-h light:12-h dark cycle, free access to standard food and water) until 3–4 months old (35–45 g). All behavioral experiments were performed between 10:00 and 14:00 h, in accordance with the Guidelines for Animal Care of our university. Different groups of animals were used in the distinct experiments.

### 2.2. Locomotor activity assessment

To assess locomotor activity, mice were randomly allocated to individual triangular boxes (50 cm × 30 cm × 30 cm, 50 cm high) with rounded corners, placed on the floor of a soundproof and diffusely illuminated room. Locomotor activities of eight mice were recorded simultaneously by a video-computerized system, with image analysis at four frames per second. The software (programmed by ABL Tort) tracked the animals by distinguishing their white color from the black background of the floor, registering $X$ and $Y$ horizontal coordinates. The method was set to examine horizontal locomotor activity, ignoring small movements, such as breathing, head and tail actions, and tremors. In all experiments, animals had not been previously habituated to the boxes. The data on locomotor activity is divided in 10 min blocks and presented as a function of time.

### 2.3. Experimental design

#### 2.3.1. Dizocilpine experiment

Mice were treated with i.p. injection of guanosine at three different doses (0.75, 2.5, and 7.5 mg/kg) or saline and immediately had their locomotor activity recorded for 30 min, followed by i.p. injection of dizocilpine (0.25 mg/kg) and further recording for 3 h. Two control groups consisted of i.p. injection at time 0 of guanosine (7.5 mg/kg) or saline followed by a saline i.p. injection after 30 min.

#### 2.3.2. Amphetamine experiment

Mice were treated with i.p. injection of guanosine at 7.5 mg/kg or saline and immediately had their locomotor activity recorded for 30 min, followed by i.p. injection of amphetamine (5 mg/kg) and further recording for 3 h. Two control groups consisted of i.p. injection at time 0 of guanosine (7.5 mg/kg) or saline followed by a saline i.p. injection after 30 min.

#### 2.3.3. Caffeine experiment

Mice had their spontaneous locomotor activity recorded for 30 min; afterwards they were treated with i.p. injection of guanosine at 7.5 mg/kg or saline and had their locomotor activity recorded for more 30 min, followed by i.p. injection of caffeine (30 mg/kg) and further recording for 2 h. Two control groups consisted of i.p. injection at time 30 min of guanosine (7.5 mg/kg) or saline followed by a saline i.p. injection after 30 min.

### 2.4. Spontaneous alternation

Spontaneous alternation performance was assessed in the Y-maze. Each arm was 30 cm long, 20 cm high and 6 cm wide, and converged to an equal angle. Each mouse was placed at the end of one arm and allowed to freely move through the maze during 5 min. The series of arm entries was recorded visually. An alternation was defined as entries in all three arms on consecutive occasions. The percentage of alternation was calculated as (total of alternation/total arm entries − 2). Treatments were administered 30 min prior to test, and four groups of mice were studied: saline, guanosine (7.5 mg/kg), dizocilpine (0.25 mg/kg), and guanosine (7.5 mg/kg) + dizocilpine (0.25 mg/kg).

### 2.5. Drugs

Dizocilpine, amphetamine, guanosine, and caffeine were purchased from Sigma (St. Louis, MO, USA) and were dissolved in distilled water for acute administrations. For all injections, a volume of 10 ml/kg was administered.

### 2.6. Statistical analysis

The total locomotor activity in each experiment was quantified by calculating the area under the curve (of the function of locomotor activity versus time) obtained after the injection of different treatments. Comparisons of total locomotor activities and of spatial alternation scores among groups were performed with one-way ANOVA, followed by Duncan's post-hoc to determine differences among specific groups. A value of $P < 0.05$ was considered to be statistically significant.

## 3. Results

Guanosine treatment did not affect total locomotor activity of mice during the habituation period of 30 min after the first injection compared to the saline group (Figs. 1–3). Guanosine at the doses of 0.75 and 2.5 mg/kg did not interfere with the hyperlocomotion induced by dizocilpine (data not shown). However, at the dose of 7.5 mg/kg, guanosine produced a statistically significant attenuation of about 60% (in relation to baseline activity) on the locomotor stimulation induced by dizocilpine (Fig. 1).

Guanosine at 7.5 mg/kg failed to affect the hyperlocomotion induced by amphetamine and caffeine, as shown in Figs. 2 and 3, respectively.

Guanosine at 7.5 mg/kg did not reverted the impairment caused by dizocilpine in the spontaneous alternation task, and, when administered alone, also caused an impairment in the task (Fig. 4).

## 4. Discussion

The present study demonstrated a selective effect of guanosine in counteracting the locomotor stimulatory effect of the NMDA receptor antagonist dizocilpine without affecting spontaneous locomotor activity, whereas it presented no effect on the locomotor activation induced by the indirect dopamine agonist amphetamine and by the adenosine receptor antagonist caffeine.

In the last years, the antiglutamatergic effects of the GBPs have been intensively studied [3,10–12,17,19,23,24,26,27,33]. We have shown that systemic administration of guanosine and GMP prevent seizures induced by compounds that overstimulate the glutamatergic system (quinolic acid, alpha-dendrotoxin), but not by the GABAergic antagonist picrotoxin [17,27,33]. We also reported that GMP is neuroprotective against intrastriatal quinolinic acid lesion [19], and, in vitro, guanosine protected brain slices exposed to

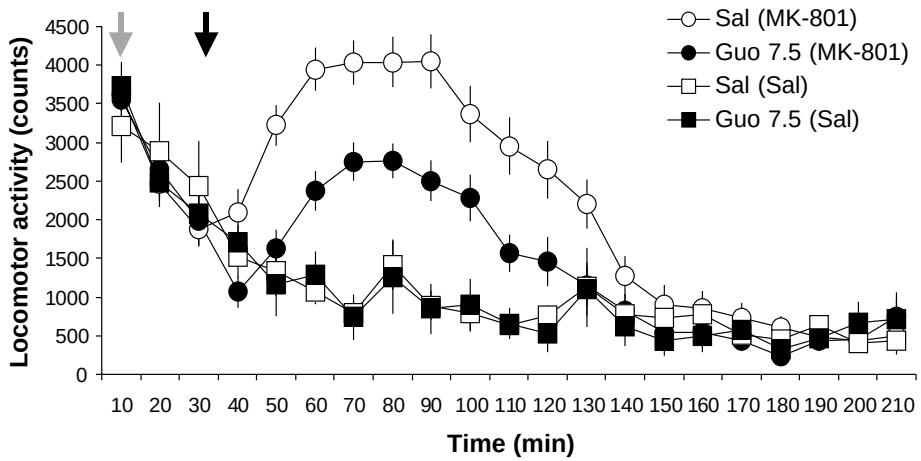


Fig. 1. Locomotor stimulatory effect induced by dizocilpine (MK-801) was inhibited by guanosine pre-treatment ($P < 0.05$). Grey arrow denotes first injection (guanosine 7.5 mg/kg (black symbols) or saline (white symbols)), and black arrow denotes second injection (MK-801 0.25 mg/kg (circle symbols) or saline (square symbols)). $N = 10$ in MK-801 treated groups, and $N = 4$ in control groups. Error bars represent standard error of the mean.

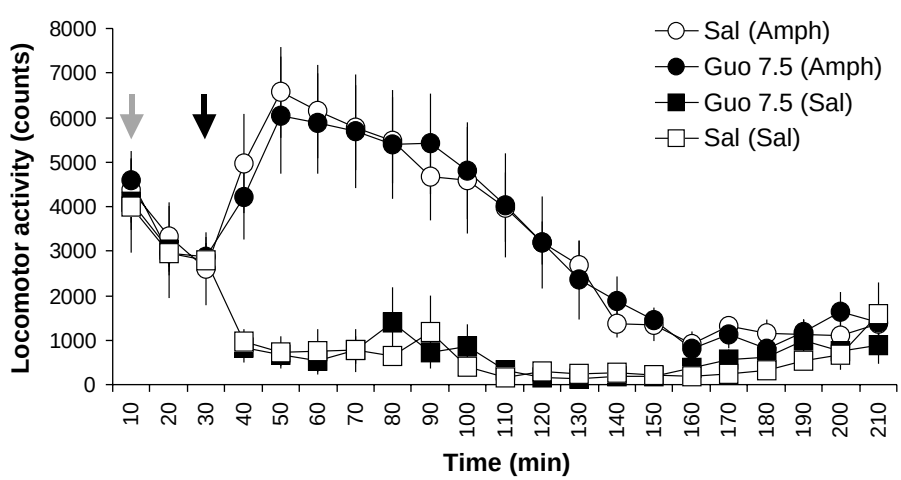


Fig. 2. Locomotor stimulatory effect induced by amphetamine was not affected by guanosine pre-treatment. Grey arrow denotes first injection (guanosine 7.5 mg/kg (black symbols) or saline (white symbols)), and black arrow denotes second injection (amphetamine 5 mg/kg (circle symbols) or saline (square symbols)). $N = 6$ in amphetamine treated groups, and $N = 4$ in control groups. Error bars represent standard error of the mean.

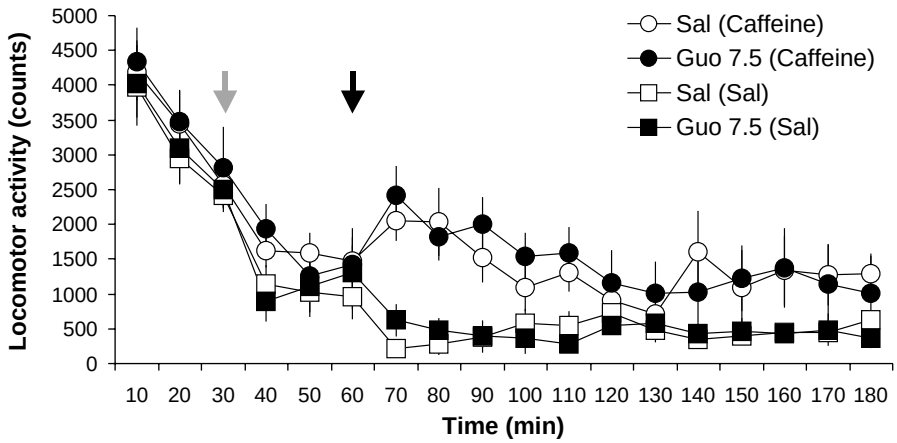


Fig. 3. Locomotor stimulatory effect induced by caffeine was not affected by guanosine pre-treatment. Grey arrow denotes first injection (guanosine 7.5 mg/kg (black symbols) or saline (white symbols)), and black arrow denotes second injection (caffeine 30 mg/kg (circle symbols) or saline (square symbols)). $N = 6$ in caffeine treated groups, and $N = 4$ in control groups. Error bars represent standard error of the mean.

hypoxia/hypoglycemia [29]. Guanosine also impaired inhibitory avoidance performance in rats [26,33], a model that also reveals amnesic effect of classical glutamatergic antagonists [14]. Regarding the mechanism of action of guanosine, a direct antagonistic action on glutamatergic receptors is unlikely, since guanosine is a poor displacer of glutamate ligands [28]. However, we showed that this antiglutamatergic effect could be mediated by astrocytes, as guanosine potently enhanced glutamate uptake in rat astrocytic cultures in a concentration-dependent manner [10,11]. More recently, we showed that the astrocytic glutamate uptake induced by guanine nucleotides depends on their conversion to guanosine [12]. Of note, astrocytic glutamate removal is know to play a major role in maintaining extracellular glutamate concentrations below neurotoxic levels [5,9].

The present results can be explained based on the different neurochemical mechanisms involved in the hyperlocomotion induced by each psychoactive drug studied. Moghaddam and coworkers have characterized the neurochemical and behavioral effects of NMDA antagonists [1–3,20,21,30,31], demonstrating that these compounds promote an increase in the efflux of both glutamate and dopamine in prefrontal cortex (PFC) and nucleus accumbens (NAc) [2,20,21,31], whereas they have only minor effects on striatal dopamine levels [4]. Thus, despite of reducing glutamate neurotransmission at NMDA receptors, dizocilpine promotes an increased stimulation of non-NMDA receptor [2,20,21,31], which may be due to disinhibition of GABAergic or other inhibitory inputs to glutamatergic neurons [31]. This non-NMDA receptor activation could then lead to the subsequent observed increase in dopamine extracelullar level, once it has been shown that AMPA and kainate glutamatergic receptors agonists could promote an increase in dopamine efflux in PFC [15], whereas the AMPA/kainate receptor antagonist LY293558 diminish dopamine levels in PFC [30]. In this context, non-NMDA receptor antagonists as well as inhibitors of glutamate release have been shown to counteract the behavioral and neurochemical effects of NMDA antagonist compounds [7,8,13]. Of functional anatomic importance, besides NMDA antagonists lead to subsequent increase in dopamine efflux in both PFC and NAc (probably via non-NMDA receptors activation), in recent works it was demonstrated that the locomotor activity induced by the NMDA antagonist PCP is closely related to

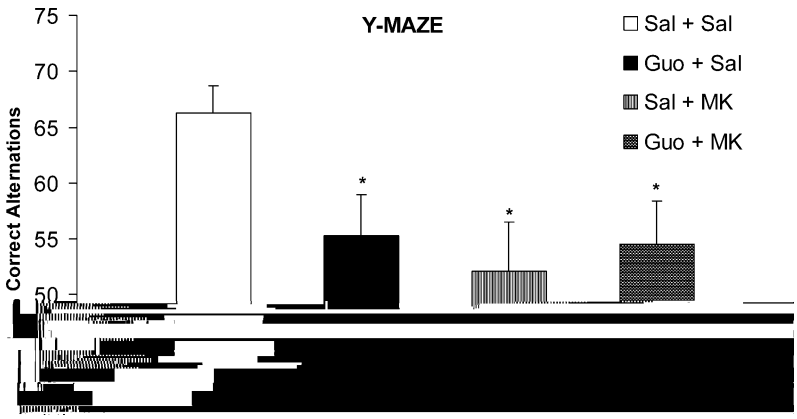


Fig. 4. Spontaneous alternation in the Y-maze. MK-801 (0.25 mg/kg) and guanosine (7.5 mg/kg) caused a significant impairment on the task when each drug was administered alone or combined. $N = 10$ in each group. Error bars represent standard error of the mean.

increased dopaminergic activity in the PFC but not in the NAc [16,21].

Altogether, we can thus hypothesize that the attenuation effect of the hyperlocomotion induced by dizocilpine observed in the present work could be due to an increase of glutamate uptake by astrocytes promoted by guanosine, reducing the neurotransmitter levels at the synaptic cleft, leading to less activation of non-NMDA receptors, with subsequent less increase in the efflux of dopamine in PFC. Guanosine did not affect the hyperlocomotion induced by amphetamine most probably due to the fact that the step of locomotor activation induced by dopamine is posterior in the neuronal circuitry to the action of glutamate in non-NMDA receptors, and hence is not interfered by antiglutamatergic compounds as guanosine. In agreement, as is known, selective D2 receptors blockers counteract the hyperlocomotion induced by NMDA antagonists, although usually at doses that also inhibit spontaneous locomotor activity [22].

We performed a paradigm of working memory with the same drug dosages studied in locomotion experiments to investigate the selectivity of the dizocilpine counter-regulatory effect of guanosine to motor activation pathways. However, in this behavioral task both guanosine and dizocilpine were amnesic when administered alone, which is in line with previous results showing an amnesic effect of both compounds in inhibitory avoidance task [26]. This effect may be related to an inhibition by guanosine of a physiological role of glutamate in learning and memory. Moreover, this cognitive impairment by guanosine may be a drawback in terms of developing new pharmacological treatments increasing guanosine activity, unless distinct receptor types for guanosine, not yet described, mediate the effect on locomotion and cognition.

In a previous work, Loeffler et al. [18] observed that guanosine at high concentration decreases dopamine synthesis in cultured rat pheochromocytoma PC12 cells. However, these results in vitro were not related to our results in vivo, since guanosine presented no effect in the hyperlocomotion induced by amphetamine. Similarly, an in vivo role of adenosine on the effect of guanosine is unlikely, since it failed to inhibit caffeine-induced hyperlocomotion.

Finally, despite of no effect on amphetamine induced hyperlocomotion, the present result point to a potential antipsychotic property of guanosine, once it was shown that NMDA antagonists model of schizophrenia could evaluate compounds that target psychotic symptoms that are not generally treated with typical antipsychotics [3]. Moreover, the neuroprotective and neurotrophic effects of guanosine may also be advantageous for the treatment of schizophrenia, which is associated with inadequate neurodevelopment and increased brain loss after onset of the disorder [32].

## 5. Conclusions

In conclusion, the present study shows that guanosine selectively counteracts the locomotor activation induced by the NMDA antagonist dizocilpine. This result is in agreement with the known antiglutamatergic effects of guanosine together with the nowadays accepted theory of motor activation induced by NMDA antagonists. Further studies could evaluate if glutamate and dopamine levels in the PFC are indeed inhibited by guanosine administration as well as explore its potential clinical utility for the treatment of schizophrenia.

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