Purinergic Signalling (2020) 16:379–387
https://doi.org/10.1007/s11302-020-09716-z

ORIGINAL ARTICLE

Involvement of adenosine A_1 and A_{2A} receptors on guanosine-mediated anti-tremor effects in reserpinized mice

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Received: 24 April 2020 / Accepted: 16 July 2020 / Published online: 28 July 2020

Abstract

Parkinson's disease (PD) signs and symptoms regularly include tremor. Interestingly, the nucleoside guanosine (GUO) has already proven to be effective in reducing reserpine-induced tremulous jaw movements (TJMs) in rodent models, thus becoming a promising antiparkinsonian drug. Here, we aimed at revealing the mechanism behind GUO antiparkinsonian efficacy by assessing the role of adenosine A_1 and A_{2A} receptors (A_1R and $A_{2A}R$) on GUO-mediated anti-tremor effects in the reserpinized mouse model of PD. Reserpinized mice showed elevated reactive oxygen species (ROS) production and cellular membrane damage in striatal slices assessed ex vivo and GUO treatment reversed ROS production. Interestingly, while the simultaneous administration of sub-effective doses of GUO (5 mg/kg) and SCH58261 (0.01 mg/kg), an $A_{2A}R$ antagonist, precluded reserpine-induced TJMs, these were ineffective on reverting ROS production in ex vivo experiments. Importantly, GUO was able to reduce TJM and ROS production in reserpinized mouse lacking the $A_{2A}R$, thus suggesting an $A_{2A}R$-independent mechanism of GUO-mediated effects. Conversely, the administration of DPCPX (0.75 mg/kg), an A_1R antagonist, completely abolished both GUO-mediated anti-tremor effects and blockade of ROS production. Overall, these results indicated that GUO anti-tremor and antioxidant effects in reserpinized mice were A_1R dependent but $A_{2A}R$ independent, thus suggesting a differential participation of adenosine receptors in GUO-mediated effects.

Keywords Guanosine · Tremor · Reserpine · Adenosine receptors

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide. It is mainly characterized by the progressive loss of dopaminergic neurons within the nigrostriatal pathway, which leads to a debilitating motor dysfunction [1]. The cardinal motor symptoms of Parkinsonism include akinesia, bradykinesia, rigidity, and a resting tremor [1]. Tremor can be defined as "a rhythmic and involuntary oscillation of a body part, caused by reciprocal innervations of a muscle, which leads to repetitive, stereotyped contractions with regular frequency and amplitude" [2]. The tremulous jaw movement (TJM) behavior is an extensively validated rodent model of tremor [3]. TJM is characterized by rapid vertical deflections of the lower jaw that resemble chewing but are not directed at any particular stimulus [4]. TJMs are induced by conditions that also lead to parkinsonism in humans (i.e., striatal dopamine depletion, dopamine antagonism, and cholinomimetic activity) [5]. Among them, reserpine, an inhibitor of vesicular monoamine transporter (VMAT-2) that causes monoamine neurotransmitters depletion, induces motor disturbances as hypolocomotion, muscle rigidity, and TJM. Therefore, reserpine administration can be used as a model for screening drugs with potential antiparkinsonian effect [6].

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The purine nucleoside guanosine (GUO), which is able to cross the blood-brain-barrier [7], is an important extracellular signaling molecule at the central nervous system [8]. Accordingly, GUO has been shown to display trophic effects in neural cells and significant neuroprotective effects [9]. Nonetheless, GUO also exerts some behavioral effects in rodents. In line with this, it has been reported that GUO can display anticonvulsive [10], antinociceptive [11], anxiolytic-like [12], and antidepressant-like effects [13]. For these reasons, we have investigated the potential effect of GUO in animal models of parkinsonism. Interestingly, in unilaterally 6-hydroxidopamine-(6-OHDA)-lesioned rats, GUO increased L-DOPA sub-maximal response and decreased L-DOPA-induced dyskinesia (LID). Similarly, GUO also reversed reserpine-induced TJM and catalepsy in mice [14], showing it may be effective for reversing parkinsonian motor impairments. Besides that, GUO also showed protective effects against in vitro cellular models of PD [15–17].

Although the antiparkinsonian-like effects of GUO have been already evaluated, the mechanism of action of this molecule is still unknown. Based on some data reporting anti-ischemic effects of GUO in hippocampal slices and cortical astrocytes, a possible role for adenosine receptors has been suggested [18, 19]. In fact, adenosinergic transmission has been pointed out as a promising therapeutic strategy for motor symptoms of PD [20, 21]. This therapeutic potential is mainly due to the fact that adenosine A_1 and A_{2A} receptors (A_1R and A_{2A}R) are largely expressed in the striatum and have a key role in modulating dopaminergic neurotransmission [22–28].

Here, we aimed to investigate the potential role of A_1R and A_{2A}R mediating GUO effects in the reserpinized mice by evaluating the behavioral and biochemical effects of GUO in the presence of selective A_1R and A_{2A}R antagonists.

Materials and methods

Animals

Male Swiss mice (central animal facility of Federal University of Santa Catarina) and A_{2A}R knock-out ($A_{2A}R^{-/-}$) mice developed in a CD-1 genetic background (animal facility of University of Barcelona) (30–50 g) were used. Animals were housed and tested in compliance with the guidelines described in the Guide for the Care and Use of Laboratory Animals [29] and following the European Union directives (2010/63/EU), FELASA and ARRIVE guidelines. The animals were conventionally housed in groups of 4 or 5 in a temperature-controlled (22 °C) and humidity-controlled (66%) environment under a 12-h/12-h light/dark cycle, where food and water intake was ad libitum. The study protocol was approved by the Ethical Committee on Animal Use and Care of the University of Barcelona (CEEA/UB) and Federal University of Santa Catarina (CEUA/UFSC, Protocol PP00955).

Drugs

Reserpine, guanosine (GUO), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) - A_1R antagonist, 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo(4,3-e)-1,2,4-triazolo(1,5-c)pyrimidine (SCH58261) - A_{2A}R antagonist, were from Sigma Chemical, St. Louis, MO.

Reserpine treatment

To induce the TJM behavior, we use a previous standardized protocol [30, 31], where mice were injected twice (every other day) with reserpine (1.0 mg/kg) subcutaneously (s.c.). Reserpine was dissolved in glacial acetic acid and then diluted to a final concentration of 0.1% acetic acid with saline (NaCl 0.9%). Controls were injected with a saline in 0.1% acetic acid solution.

Pharmacological treatment

Guanosine treatment

GUO was dissolved in saline (NaCl 0.9%) and administered in effective or sub-effective doses (7.5 or 5 mg/kg, respectively; [14]) by oral route (p.o.) 20 min prior the behavioral tests and 24 h after the last injection of reserpine. GUO doses were selected from our own group experience [14]. Controls were treated with saline (p.o.).

A_{2A}R experiments

To evaluate the involvement of A_{2A}R on GUO-induced antidyskinetic effect, a dose response of SCH58261 was initially performed. SCH58261 was dissolved in dimethylsulfoxide (DMSO) then in saline to the final desired concentrations, and the behavioral analysis was carried out after 30 min. To analyze a putative potentiation effect with GUO and SCH58261 treatment, they were administered in their sub-effective doses (5 mg/kg p.o. and 0.01 mg/kg i.p., respectively) with 10 min treatment interval. Behavioral tests were conducted 30 min after SCH58261 and 20 min after GUO treatments and 24 h after the last injection of reserpine.

In the A_{2A}R-KO mice protocol, mice were treated with GUO (5 or 7.5 mg/kg) 20 min prior the tests and 24 h after the last injection of reserpine.

A_1R experiments

To evaluate the involvement of A_1R on GUO-induced antidyskinetic effect, DPCPX (0.75 mg/kg; dissolved in

DMSO then in saline) was injected via intraperitoneal (i.p.) 30 min prior the GUO active dose administration (7.5 mg/kg, p.o.). The dose of DPCPX was selected on the basis of literature data on oral tremor [32].

Tremulous jaw movements

Tremulous jaw movements (TJMs) were defined as rapid vertical deflections of the lower jaw that resembled chewing but were not directed at any particular stimulus [4]. This protocol was initially standardized to rats [4, 33] and we adapted the protocol to mice based on previous published studies [30, 31]. To quantify the occurrence of this orofacial dyskinesia, mice were placed individually in a glass cylinder (13 cm diameter) and hand-operated counters were employed to score TJM frequencies. Mirrors were placed under the floor and behind the back wall of the cylinder to allow observation when the animal was faced away from the observer. If TJM occurred during a period of grooming, they were not taken into account. The incidence of these oral movements was measured continuously for 10 min.

Brain slices

Animals were euthanized by decapitation and brains were quickly removed and the cerebral cortex, hippocampus, and striatum were rapidly dissected in ice-cold KREBS ringer buffer (KRB) (122 mM NaCl, 3 mM KCl, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 0.4 mM KH_2PO_4, 25 mM $NaHCO_3$, and 10 mM D-glucose, bubbled with 95% O_2/5% CO_2 up to pH 7.4) [19]. For the biochemical assays, slices (0.4 mm) were prepared using a Mcllwain Tissue Chopper (The Mickle Laboratory Engineering Co. ltd., England) and separated in KRB at 4 °C. After sectioning, slices were incubated in KRB for 30 min, at 37 °C, for recovery.

ROS levels

ROS production was measured by using the molecular probe 2,7-dichlorofluorescein diacetate (H_2DCFDA, Sigma Chemical, St. Louis, MO.). H_2DCFDA diffuses through the cell membrane and is hydrolyzed by intracellular esterases to the non-fluorescent form 2′,7′-dichlorofluorescein (DCFH). DCFH reacts with intracellular ROS (such as H_2O_2) to form dichlorofluorescein (DCF), a green fluorescent dye. DCF fluorescence intensity is proportional to the amount of ROS. Brain slices were incubated with 80 μM of H_2DCFDA for 30 min at 37 °C and then washed in KRB. Fluorescence was read with the multifunctional microplate reader Infinite M200 (Tecan Group Ltd., Mannedorf, Switzerland), using excitation and emission wavelengths of 480 and 525 nm, respectively [19].

Membrane integrity evaluation

Membrane integrity was assessed by evaluating the uptake of the fluorescent exclusion dye, propidium iodide (PI, Sigma Aldrich, St Louis, MO, USA), which is a polar compound that enters only in cells with damaged membranes. Once inside the cells, PI complexes with DNA and emits an intense red fluorescence (630 nm) when excited by green light (495 nm) [34]. Slices were incubated with PI (7 μg/mL) for 30 min at 37 °C, and then washed with KRB for analysis on fluorescence microplate reader Infinite M200 from Tecan®.

Mitochondrial membrane potential

Mitochondrial membrane potential was measured by using the molecular probe tetramethylrhodamine ethyl ester (TMRE, Sigma Chemical, St. Louis, MO.) 100ηM for 30 min at 37 °C. Fluorescence was measured with the multifunctional microplate reader Infinite M200 from Tecan®, using wavelengths of excitation and emission of 550 and 590 ηm, respectively [35].

MTT reduction assay

Cellular viability in slices was quantified by measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical, St. Louis, MO.) to a dark violet formazan product by dehydrogenases. Slices were incubated with MTT (0.5 mg/mL) in KRB buffer for 20 min at 37 °C, the formazan produced was solubilized by replacing the medium with 200 μL of DMSO, resulting in a colored compound which was quantified spectrophotometrically at a wavelength of 550 ηm. Absorbance was measured with the multifunctional microplate reader Infinite M200 from Tecan®. The results are expressed and normalized as percentages relative to the control conditions.

Data analysis

Data are represented as means ± S.E.M. Normalized data from multiple experiments were averaged and statistical analysis was carried out as described in the figure legends. Data with two groups were analyzed by Student's *t* test, and other data used one-way or two-way ANOVA followed by Tukey's post hoc. Statistical difference was accepted when $P < 0.05$.

Results

Guanosine effects on the reserpinized mice

Initially, cortical, hippocampal, and striatal slices were used to biochemical evaluations, as cellular viability of

slices, ROS production, mitochondrial membrane potential, and cell membrane permeabilization. Reserpine administration caused no alteration in cortical or hippocampal slices (Fig. 1a–d). In striatal slices, reserpine administration did not alter cellular viability and mitochondrial membrane potential (Fig. 1a, c). However, striatal slices showed an increase of 50% in ROS production (P = 0.017) and an increase of 58% in PI incorporation (P = 0.046) by reserpine treatment as compared with control (Fig. 1b, d).

Accordingly, we obtained striatal slices and assessed GUO effects reversing reserpine-induced ROS production, and PI incorporation. The dose of 7.5 mg/kg of GUO was used based on a previous study, in which we described that GUO (7.5 mg/kg) decreased TJM frequency in reserpinized mice [14]. Interestingly, we could observe that GUO totally reversed reserpine-induced ROS production in striatal slices (P = 0.031), while it failed to block PI incorporation (P = 0.982) (Fig. 2a, b).

Involvement of adenosine A_{2A}R on guanosine-mediated TJM and ROS decrease

It is known that A_{2A}R antagonism has effects on motor disturbances related to PD [32, 36–38]. Therefore, we aimed to see if GUO effect of reducing TJM and ROS generation in the striatum could be related to antagonism of A_{2A}R. Firstly, a *dose-response curve* was performed with SCH58261 (Fig. 3a). The highest dose of SCH58261 (0.1 mg/kg) fully reversed the TJM by reserpine (P = 0.042) while the lowest dose (0.001 mg/kg) had no effect (P = 0.893). The 0.01 mg/kg dose was sub-effective (i.e., presented statistical difference either from control or to reserpine group). Then, we evaluated the effect of co-treatment of SCH58261 and GUO sub-effective doses. We previously showed that 5 mg/kg GUO presented a sub-effective effect on reserpine-induced TJM [14]. The co-administration of sub-effective doses of SCH58261 (0.01 mg/kg) and GUO

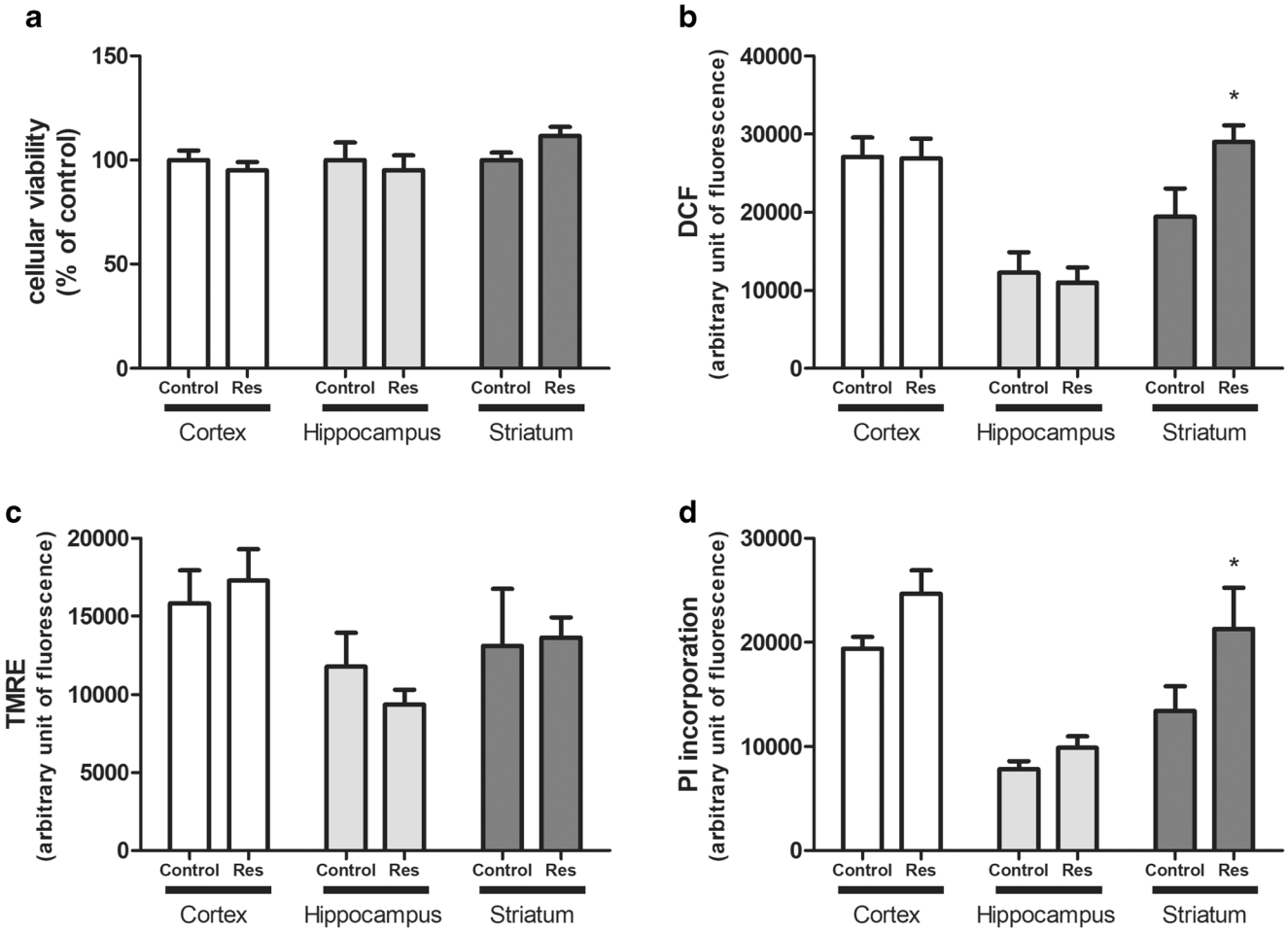

Fig. 1 Evaluation of reserpine (Res, 1 mg/kg) neurotoxicity in cortical, hippocampal, and striatal slices. **a** Cellular viability measured by MTT reduction, expressed as percentage of control. **b** ROS measurement through fluorescence of DCF dye. **c** Evaluation of mitochondrial membrane potential with TMRE fluorescent dye. **d** Membrane integrity evaluation due to PI incorporation. Fluorescence data are shown as arbitrary fluorescent unit. Results are presented as means ± SEM (*P < 0.05 vs control; Student's t test; n = 7)

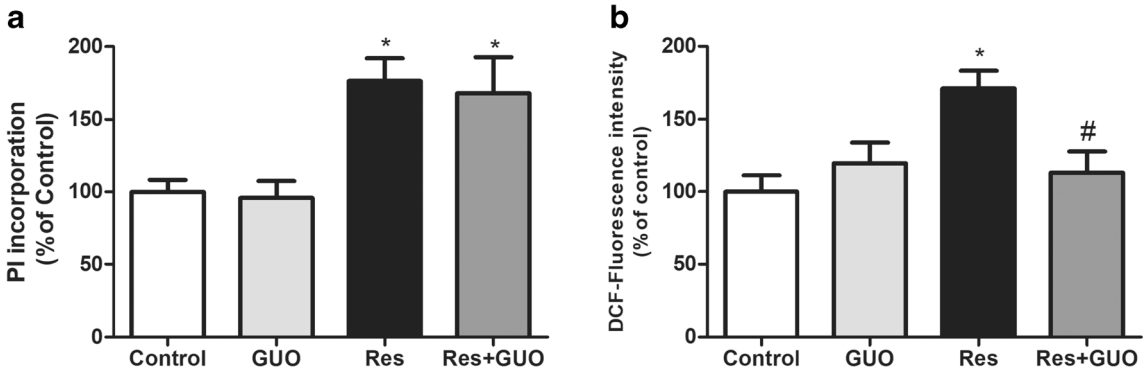

Fig. 2 Guanosine protective ex vivo effect in reserpinized mice. **a** GUO (7.5 mg/kg) effect on ROS production and **b** cellular membrane permeability through PI incorporation in striatal slices of mice treated with reserpine. Results are presented as means ± SEM.; $^{\#}P < 0.05$ vs reserpine (two-way ANOVA with Tukey's post hoc test; $n = 5$)

(5 mg/kg) completely reversed the reserpine-induced orofacial tremor ($P = 0.004$) (Fig. 3b). Regarding ROS generation, treatment with sub-effective dose of SCH58261 (0.01 mg/kg), or with sub-effective GUO dose (5 mg/kg), displayed no statistically significant effect of decreasing ROS production ($P = 0.111$ and 0.553, respectively). Co-administration of SCH58261 and GUO also showed no significant difference from reserpine-treated animals ($P = 0.287$) (Fig. 3c).

To clarify the role of $A_{2A}R$ on GUO effect in the reserpine-induced TJM and striatal ROS production, genetic-modified mice $A_{2A}R$ deficient ($A_{2A}R$-KO) were used. Animals were subjected to the same protocol of reserpine and GUO treatment. The sub-effective GUO dose (5 mg/kg) had no effect against reserpine in these animals, both in the TJM quantification ($P = 0.362$) and ROS measurement ($P = 0.807$) (Fig. 4a). On the other hand, GUO at 7.5 mg/kg dose presented an effect of reversing the reserpine induction of TJM ($P = 0.017$) and ROS increase ($P = 0.040$) (Fig. 4b), indicating that presence of $A_{2A}R$ is not necessary to GUO effect in reserpinized mice.

Involvement of adenosine A_1R on guanosine-mediated TJM and ROS decrease

To test the involvement of A_1R, 24 h after the last reserpine administration, mice were treated with the A_1R antagonist DPCPX (0.75 mg/kg i.p. [32], 30 min prior the GUO administration. DPCPX treatment did not alter reserpine-induced TJM ($P = 0.999$), but it completely blocked the effect of GUO on TJM frequency ($P = 0.0003$) (Fig. 5a).

As GUO (7.5 mg/kg) showed effect through reverting ROS increase by reserpine, we aimed to see if this effect is related to A_1R. Prior treatment with the A_1R antagonist DPCPX (0.75 mg/kg) did not significantly alter ROS increase induced by reserpine ($P = 0.383$) but it prevented the reversion of GUO ($P = 0.912$) (Fig. 5b). These data suggest a strong dependence of A_1R for GUO behavioral and biochemical effects.

Discussion

GUO treatment shows a promising effect on animal models of motor disorders. We already shown that in unilaterally 6-

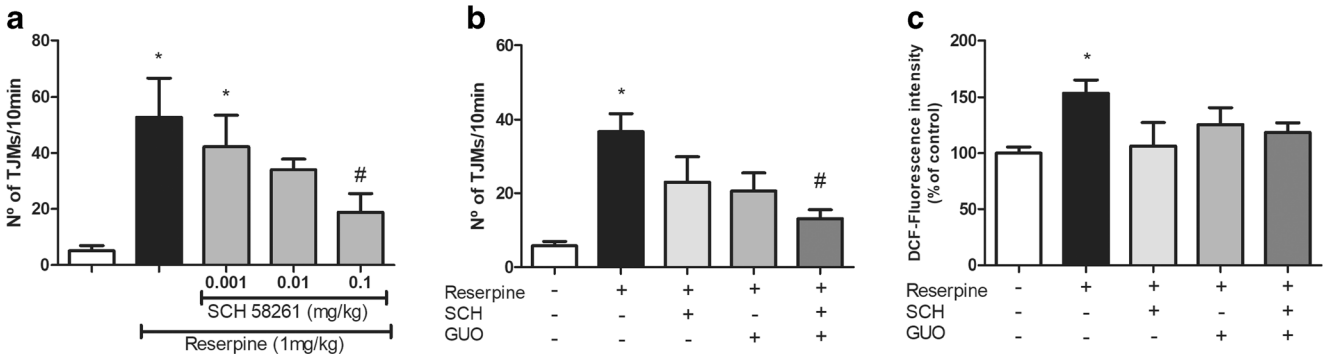

Fig. 3 Involvement of $A_{2A}R$ on reserpine-induced TJM and ROS production. **a** Dose-response curve of $A_{2A}R$ antagonist SCH58261 (0.001; 0.01 and 0.1 mg/kg) in reserpine-induced oral tremor (TJM). **b** SCH58261 (0.01 mg/kg) plus GUO (5 mg/kg) effect on reserpine-induced TJM. **c** SCH58261 (0.01 mg/kg) and GUO (5 mg/kg) effect on ROS increase in striatal slices of reserpinized mice. Results are presented as means ± SEM ($^{*}P < 0.05$ vs control; $^{\#}P < 0.05$ vs reserpine; one-way ANOVA with Tukey's post hoc test; $n = 8$–10)

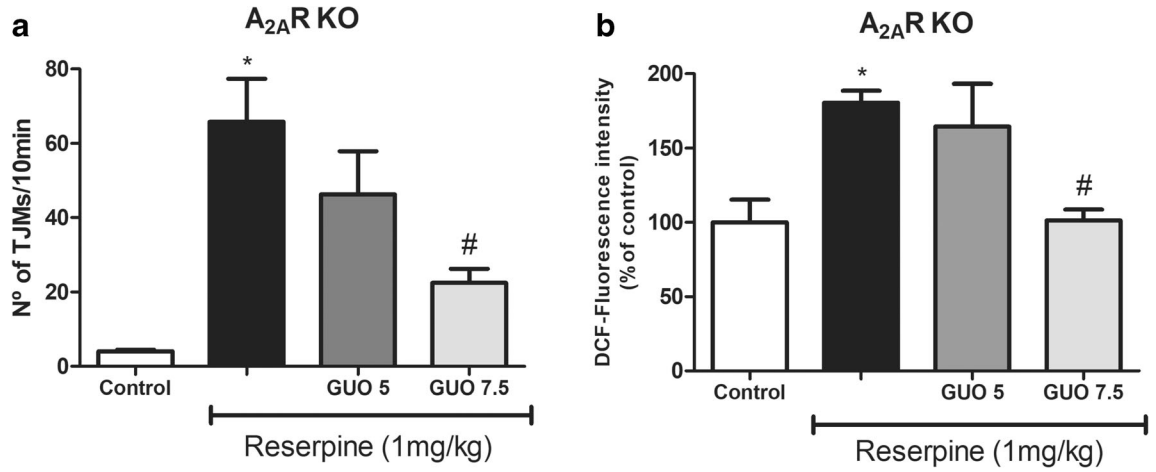

Fig. 4 Effect of guanosine on reserpine-induced TJM and ROS production in A_{2A}R-deficient (A_{2A}R-KO) mice. **a** TJM in mice treated with GUO sub-effective (5 mg/kg) or effective (7.5 mg/kg) doses ($n = 6$). **b** ROS production in striatal slices of mice treated GUO (5 mg/kg or 7.5 mg/kg, $n = 3$). Results are presented as means ±SEM (*$P < 0.05$ vs control; $^{\#}P < 0.05$ vs reserpine; one-way ANOVA with Tukey's post hoc test)

hydroxidopamine-(6-OHDA)-lesioned rats, GUO increased L-DOPA sub-maximal response and decreased LID. Also, GUO reversed reserpine-induced TJM and catalepsy in mice [14]. In this study, we investigated the mechanisms behind this GUO effect of reducing the orofacial tremor and the striatal oxidative damage evoked by reserpine, by assessing the possible involvement of adenosine receptors in the GUO effects.

The mechanism related to the induction of oral tremor is multifaceted, with multiple neurotransmitters, including GABA, serotonin, adenosine, and acetylcholine, interacting with dopamine in the regulation of basal ganglia motor functions [4, 39–43]. In this study, we focused in a possible therapeutic approach towards adenosinergic transmission.

A_{2A}R antagonists have emerged as a potential treatment of parkinsonian motor impairments as they can exert allosteric modulations upon D2R ligands [44]. Also, A_{2A}R is highly expressed in the striatum and it was shown that its antagonism reduces oral tremor in different rodent models [32, 36, 45–48]. Accordingly, SCH58261, the A_{2A}R antagonist tested in this study, exhibited effect in the reserpine-induced TJM in mice. Moreover, SCH58261 and GUO sub-effective doses potentiated each other's effect on TJM behavior. Despite the potentiated effect observed with SCH58261 and GUO co-treatment on reserpine-induced TJM, when GUO was tested in genetic-modified animals lacking adenosine A_{2A} receptors (A_{2A}R-KO), it was observed that this receptor is not essentially involved in the GUO antidyskinetic effect. Therefore, we decided to test the participation of A_1R on behavioral and biochemical GUO effects.

It is known that adenosine A_1R can antagonistically modulate D1R responses and that the stimulation of A_1R could inhibit the D1R stimulation [25, 49–51]. Interestingly, rats treated with reserpine (1 mg/kg; s.c.) for 5 days showed an

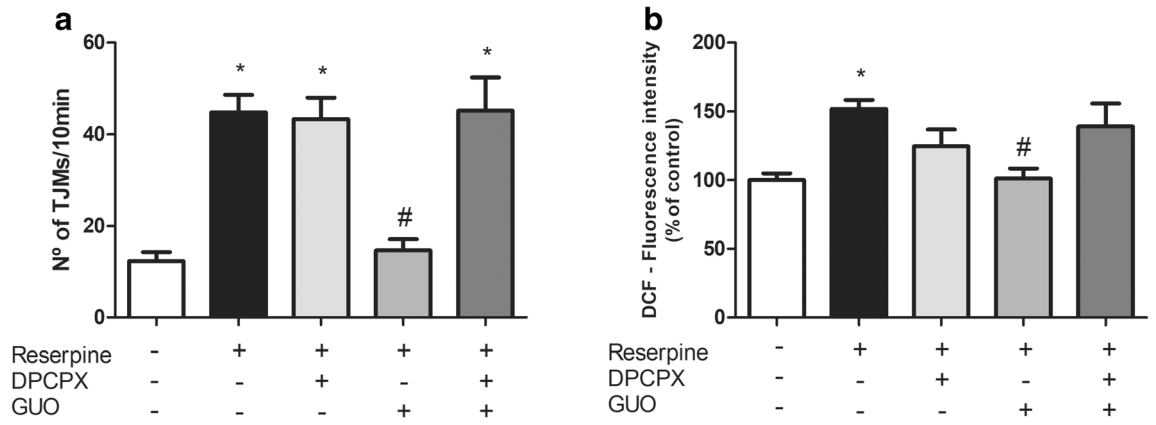

Fig. 5 Effect of A_1R blockade on reserpine-induced TJM and ROS production in mouse striatal slices. **a** Effect of A1R antagonist DPCPX (0.75 mg/kg i.p.) plus GUO (7.5 mg/kg) on TJM of mice. **b** Evaluation of GUO (7.5 mg/kg) plus DPCPX (0.75 mg/kg) effect on ROS increase in striatal slices of reserpinized mice. Results are presented as means ± SEM (*$P < 0.05$ vs control; $^{\#}P < 0.05$ vs reserpine; one-way ANOVA with Tukey's post hoc test; $n = 5$–6)

increase in the responsiveness of adenylate cyclase after D1R stimulation [52]. Also, rats treated with one single dose of reserpine (1 mg/kg; i.p.) showed upregulation of the transduction mechanism associated with D1R, without changing the activity of D2R [53]. Evidence from our results suggests that GUO motor effect could be through A_1R stimulation. In fact, as results with A_1R stimulation for oral tremor are lacking in the literature, we tested reserpinized mice with 2-chloro-N-6-cyclopentyladenosine (CCPA), an A_1R agonist, and a potent antidyskinetic effect was observed (in the doses 0.0125, 0.025, and 0.05 mg/kg). However, we also observed that CCPA promotes an important sedative response in these animals (data not shown) that precludes its use as a treatment against motor impairments. In this sense, A_1R stimulation promoted by GUO could be inhibiting the overstimulation of D1R in reserpinized mice, and then decreasing the oral tremor.

Besides the motor disturbance, we also investigated biochemical changes in reserpinized mice in different cerebral structures (i.e., cerebrocortex, hippocampus, and striatum). It is well known that the inhibition of dopamine vesicular storage leads to an increase in ROS; this occurs because dopamine metabolism intrinsically results in ROS formation [54]. The major area affected was the striatum, where it was seen an increase in ROS production and permeabilization of the cellular membrane. Thus, oxidative and cell membrane damage in the striatum might sum up to the monoamine depletion to impair motor performance. This increase in ROS nearby the cell membrane could cause its oxidation and lead to an injury in the membrane lipids as it was seen on incorporation of PI. In fact, some studies with the same reserpine protocol have already shown an increase on lipid peroxidation in striatum [30, 55]. This increase in ROS production and cell membrane permeabilization may reflect early events of toxicity by reserpine but surprisingly, we did not see alteration in the cell reductive capacity, assessed by MTT reduction method. Accordingly, the reserpine toxicity in this protocol does not affect the mitochondrial membrane potential. More important, GUO acutely administrated was able to reverse ROS increase induced by reserpine, and this effect was dependent on A_1R and not $A_{2A}R$.

To our knowledge, our previous study was the first to identify GUO treatment as an antiparkinsonian agent in a rodent model of orofacial tremor [14]. Although other studies have shown the protective effect of GUO in cellular models of PD [15, 17] or in vivo rodent models of PD [56], none of them has assessed the molecular targets related to GUO effects. Despite this, evidence from other brain disease models has pointed to GUO effect via adenosine receptor modulation. In an in vitro ischemia model, hippocampal slices subjected to oxygen/glucose deprivation presented increased ROS production prevented by GUO, but this effect is abolished by preincubation with DPCPX [19]. These data corroborate with the idea that GUO effect of preventing an oxidative damage is A_1R dependent. Notwithstanding, in the same ischemia protocol, not only DPCPX but also an $A_{2A}R$ agonist (CGS21680) blunted the protective effect of GUO in hippocampal slices [19] and in cortical astrocytes [18]. Likewise, the ischemia model in $A_{2A}R$-KO animals implies GUO-protective effects upon $A_{2A}R$ in the hippocampus [57]. As different results obtained with $A_{2A}R$-KO mice may be dependent on the cerebral area analyzed, there is still controversy regarding GUO effects via adenosine A_1R or $A_{2A}R$ interaction, and additionally, the possibility of GUO interaction with adenosine receptor heteromers. More important, a recent study from our group shed some light on this issue of GUO interaction on A_1R and/or $A_{2A}R$. We showed that GUO-induced effects may require both A_1R and $A_{2A}R$ co-expression in transfected HEK293 cells, indicating that GUO acts on adenosine receptors in an oligomeric conformation, i.e., the A_1R-$A_{2A}R$ heteromer [57]. Once GUO acts upon A_1R-$A_{2A}R$ heteromer formation, its effects on other receptor oligomeric organization of A_1R and/or $A_{2A}R$ are possible and were still not evaluated. Thus, it is feasible to speculate that in the striatum, GUO can interact with A_1R and then modulated D1R or A_1R-D1R heteromer interaction, and further investigations are necessary to clarify GUO mechanism on motor control.

In conclusion, our results strengthen the demonstration of extracellular actions of GUO and the dependence of adenosine A_1R activation to the motor-related effect of GUO. Considering the GUO-mediated motor improvement differs mechanistically from classic adenosine receptor modulators, it is important to understand the mechanisms behind GUO effects.

Funding information The research performed at the Universidade Federal de Santa Catarina was supported by the Brazilian funding agencies, CAPES (CAPES/PAJT), CNPq (INCT-EN for Excitotoxicity and Neuroprotection), and FAPESC (NENASC/PRONEX) to C.I.T. The research performed at the Universitat de Barcelona was supported by FEDER/Ministerio de Ciencia, Innovación y Universidades–Agencia Estatal de Investigación (SAF2017-87349-R) and ISCIII (PIE14/00034), the Catalan government (2017 SGR 1604), Fundació la Marató de TV3 (Grant 20152031), and FWO (SBO-140028) to F.C. Also, CAPES-PDSE (47/2017) provided doctoral fellowship to C.M.M. We thank LAMEB/UFSC team work for experimental support.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Ethical approval The study protocol was approved by the Ethical Committee on Animal Use and Care of the University of Barcelona (CEEA/UB) and Federal University of Santa Catarina (CEUA/UFSC, Protocol PP00955).

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