



# Guanosine, a guanine-based nucleoside relaxed isolated corpus cavernosum from mice through cGMP accumulation

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## Abstract

In corpus cavernosum (CC), guanosine triphosphate (GTP) is converted into cyclic guanosine monophosphate (cGMP) to induce erection. The action of cGMP is terminated by phosphodiesterases and efflux transporters, which pump cGMP out of the cell. The nucleotides, GTP, and cGMP were detected in the extracellular space, and their hydrolysis lead to the formation of intermediate products, among them guanosine. Therefore, our study aims to pharmacologically characterize the effect of guanosine in isolated CC from mice. The penis was isolated and functional and biochemical analyses were carried out. The guanine-based nucleotides GTP, guanosine diphosphate, guanosine monophosphate, and cGMP relaxed mice corpus cavernosum, but the relaxation ( $90.7 \pm 12.5\%$ ) induced by guanosine (0.000001–1 mM) was greater than that of the nucleotides ( $\sim 45\%$ ,  $P < 0.05$ ). Guanosine-induced relaxation was not altered in the presence of adenosine type 2A and 2B receptor antagonists. No augment was observed in the intracellular levels of cyclic adenosine monophosphate in tissues stimulated with guanosine. Inhibitors of nitric oxide synthase (L-NAME, 100  $\mu$ M) and soluble guanylate cyclase (ODQ, 10  $\mu$ M) produced a significant reduction in guanosine-induced relaxation in all concentrations studied, while in the presence of tadalafil (300 nM), a significant increase was observed. Pre-incubation of guanosine (100  $\mu$ M) produced a 6.6-leftward shift in tadalafil-induced relaxation. The intracellular levels of cGMP were greater when CC was stimulated with guanosine. Inhibitors of ecto-nucleotidases and xanthine oxidase did not interfere in the response induced by guanosine. In conclusion, our study shows that guanosine relaxes mice CC and opens the possibility to test its role in models of erectile dysfunction.

**Keywords** Guanosine · Corpus cavernosum · cGMP · Nitric oxide · Soluble guanylate cyclase

## Introduction

In the corpus cavernosum (CC), nitric oxide (NO) released from nitrergic fibers and endothelial layer activates its intracellular receptor, soluble guanylate cyclase (sGC), which converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). The intracellular accumulation of cGMP in the smooth muscle from arteries and cavernosal cells promotes vascular and non-vascular smooth muscle relaxation leading to erection [1]. The action of cGMP is terminated by phosphodiesterase type 5 (PDE5), which degrades cGMP into

its inactive form, guanosine monophosphate (GMP) [2]. Multidrug resistance proteins type 4 and 5 (MRP4 and MRP5) which pump cyclic nucleotides out the cell also control the intracellular levels of cGMP or cyclic adenosine monophosphate (cAMP) in platelets [3] and vascular [4] and non-vascular smooth muscle [5]. In the lower urinary tract organs from mice, the non-selective inhibitor of MRP4/MRP5, MK571 increased the relaxing response induced by cGMP-increasing substances in corpus cavernosum [6], urethra and prostate, and cAMP-increasing substances in the bladder [5].

The nucleotides GTP, guanosine diphosphate (GDP), cGMP and GMP, and the nucleoside guanosine are guanine-based purines [7]. Conversely to adenine-based purines such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine, where their extracellular roles are well defined as neurotransmitter (ATP), as substances released from the bladder urothelium (ATP) and platelets granules (ATP and ADP) to sensitize C-fibers in the bladder [8] and

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platelet aggregation [9], respectively, or to induce corpus cavernosum relaxation (adenosine) [10], the extracellular role of guanine-based nucleotides/nucleoside in the peripheral organs/tissues is much less studied.

The majority of the studies of guanine-based purines are focused on guanosine, which is found in *Solanum lycopersicum* (tomatoes) [11] and in mammalian cells and fluids [12–15]. For instance, in primary culture of astrocyte the concentration of guanosine and adenosine in the medium were in the range of, respectively, 120 and 43 nM. In cells exposed to hypoxia/hypoglycemia for 30 min, the concentration of guanosine and adenosine increased, by approximately, 3.5- and 2.5-fold [14]. In another study, the concentration of guanosine was increased significantly (from 7 to 35  $\mu$ M, approximately) in microdialysates obtained from porcine hearts subjected to extracorporeal circulation with 30 min of normothermic ischemia [13].

To date, studies suggest that the hydrolysis of GTP and the extracellular cGMP by E-NTPases (CD39) and ecto-5'-nucleotidases (CD73) [7] are the main pathways that produce guanosine [16], which are the same enzymes responsible to hydrolyze adenine-based nucleotides. In animal models of brain damage such as oxygen/glucose deprivation [17] and glutamate neurotoxicity-induced brain ischemia [18], the neuroprotective role of guanosine involves glutamate reuptake [19, 20], a decrease in glutamate release [21] or an increase in glutamine synthetase activity, which condenses glutamate and ammonia to form glutamine [22].

The peripheral effect of guanosine has also been shown on rat vessel [23], where guanosine concentration-dependent relaxed isolated mesenteric arteries in a mechanism dependent on cGMP accumulation. More recently, the role of guanosine was also investigated into human platelets. Guanosine (250 and 500  $\mu$ M) reduced thrombin, collagen-, and araquidonic acid-induced aggregation accompanied by an increased in the intraplatelet levels of cAMP and phosphorylated protein kinase A [24]. In another study, guanosine (1–4 mmol/L) extracted from tomatoes concentration dependently inhibited ADP-induced human platelets aggregation [11]. Since GTP-cGMP pathway plays a key role in the physiology of erection, this study is aimed to evaluate the direct effect of guanosine in isolated CC from mice.

## Materials and methods

### Animals

C57BL/6J male mice (approximately 30 g) with 12 weeks of age were supplied by the animal facility of the Central Animal House Services of the University of Campinas CEMIB-UNICAMP (São Paulo, Brazil). All experimental protocols were carried out according to the Ethical Principles in

Animal Research adopted by the Brazilian College for Animal Experimentation and approved by the Institutional Committee for Ethics in Animal Research of the University of Campinas (CEUA/UNICAMP protocol number 4719-1).

### Functional assays

After anesthesia with isoflurane, animals were euthanized. The penis was removed from the insertion region of the crura and immediately placed in Krebs–Henseleit nutrient solution. Penile vein, corpus and bulb spongiosum, and connective tissues were removed to isolate the corpus cavernosum. Each penis provided two segments of corpus cavernosum.

Each corpus cavernosum segment obtained was assembled in a 10 mL isolated organ bath containing Krebs–Henseleit nutrient solution (mM) 117 NaCl, 4.7 KCl, 2.5 CaCl<sub>2</sub>, 1.2 MgSO<sub>4</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, and 5.5 Glucose and pH 7.4 at 37 °C, continuously aerated with a carbogenic mixture (95% O<sub>2</sub> and 5% CO<sub>2</sub>), suspended vertically between two metal hooks and connected to a force transducer. Tissues were allowed to equilibrate for 45 min under a resting tension of 2.5 mN. Isometric force was recorded using a PowerLab system, version 7 (ADIInstruments Inc., Sydney, AU).

First, concentration-response curves (CRCs) to guanine-based nucleotides (GTP, GDP, GMP, cGMP) and nucleoside (guanosine) were carried out in tissues pre-contracted with phenylephrine (PE). Since guanosine produced the greatest relaxation in mice CC, CRCs to guanosine in the absence (control) and in the presence of inhibitors of the enzymes nitric oxide synthase (L-NAME, 100  $\mu$ M), sGC (ODQ, 10  $\mu$ M), PDE5 (tadalafil, 300 nM), CD39 (ARL 67156, 100  $\mu$ M), CD73 (alphabetamethylene-ADP, 1  $\mu$ M), and xanthine oxidase (oxypurinol, 100  $\mu$ M) and type 2A (ZM 241385, 1  $\mu$ M) and type 2B (MRS 1754, 1  $\mu$ M) receptors antagonists were also used. In order to compare the effect of guanosine with others, known relaxing substances CRCs to acetylcholine (ACh, 0.001–1  $\mu$ M), sodium nitroprusside (SNP, 0.001–100  $\mu$ M), tadalafil (0.001–100  $\mu$ M), and the stable analog of adenosine (NECA, 0.001–100  $\mu$ M) were also carried out. In another set of experiments CRCs to tadalafil was carried out in the presence of guanosine (100  $\mu$ M).

### Quantification of cyclic nucleotides

Animals were anesthetized and exsanguinated. Corpus cavernosum were isolated and equilibrated for 30 min in Krebs–Henseleit solution at 37 °C, pH 7.4, continuously bubbled with a carbogenic mixture. Then, the tissues were stimulated for 20 min with guanosine (0.1 or 1 mM) or 0.5 M NaOH (vehicle) and immediately frozen in liquid nitrogen. Tissues were pulverized, homogenized in 5% trichloroacetic acid (150  $\mu$ L) and centrifuged for 10 min at 4 °C and 1500 g, and the supernatant collected. The trichloroacetic acid was

extracted from the supernatant through three washes with hydrated ether. Tracer preparation, samples, standard, and incubation with antibody were performed as described in commercial kit (Cayman Chemical Cyclic AMP EIA kit and Cayman Chemical Cyclic GMP EIA kit, Ann Arbor, MI).

### Determination of pharmacological parameters

Based on the formula  $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC}_{50} - X)})$ , non-linear regression was performed for the relaxation induced by GTP, GDP, GMP, cGMP, acetylcholine, sodium nitroprusside, NECA, and tadalafil, and the potency ( $\text{pEC}_{50}$ ) and maximal response values ( $E_{\text{max}}$ ) were determined. As for guanosine, since a non-linear regression was not observed, we only calculated the percentage of relaxation in each concentration studied and, hence, bar graphs were used.

### Stock solutions preparations

L-NAME, ARL 67156, alphabeta-methylene-ADP, acetylcholine, and sodium nitroprusside were prepared in deionized water, whereas guanosine in NaOH (0.5 M). NECA, Tadalafil, and ODQ were prepared in DMSO (99.5%). Oxypurinol was prepared in NaOH (1.0 M). The final concentrations of DMSO and NaOH in the organ bath were, respectively, 0.50% and 7.25 mM. Both vehicles did not alter the tonus induced by PE.

### Statistical analysis

The GraphPad Software Inc. was used for statistical analysis. Data were expressed as mean  $\pm$  standard deviation of  $N =$  number of animals. For the statistical comparisons, unpaired Student  $t$  test or the ANOVA test followed by the Bonferroni test were applied. Statistical significance was considered for values of  $P < 0.05$ .

## Results

### Relaxation induced by nucleotides- and nucleoside-based guanine

In tissues pre-contracted with PE, the nucleotides GTP and GDP (Fig. 1a) and cGMP and GMP (Fig. 1b) and the nucleoside guanosine (Fig. 1c) produced concentration-dependent relaxations when added exogenously. Of these, GMP was the most potent only when comparing to GTP (Table 1). If we compare the maximum concentration used from each agent, guanosine produced the greatest magnitude of relaxation when comparing to the nucleotides (Table 1). Since guanosine

is a byproduct and presented greater relaxing response, all the next protocols were carried out only with guanosine.

First, we have compared the effect of guanosine with substances known to produce CC relaxation. The nitric oxide donor, sodium nitroprusside, (SNP) produced the greatest relaxing response, while ACh was the most potent substance. Considering the maximal response, guanosine was as effective as NECA, tadalafil, and ACh. (Fig. 1d, Table 2). As shown on Fig. 1e and f, respectively, the vehicles DMSO (0.5%, final concentration in the organ bath) and NaOH (7.25 mM, final concentration in the organ bath) did not produce any drop in the contraction induced by PE.

### Effect of adenosine receptor antagonists on guanosine response

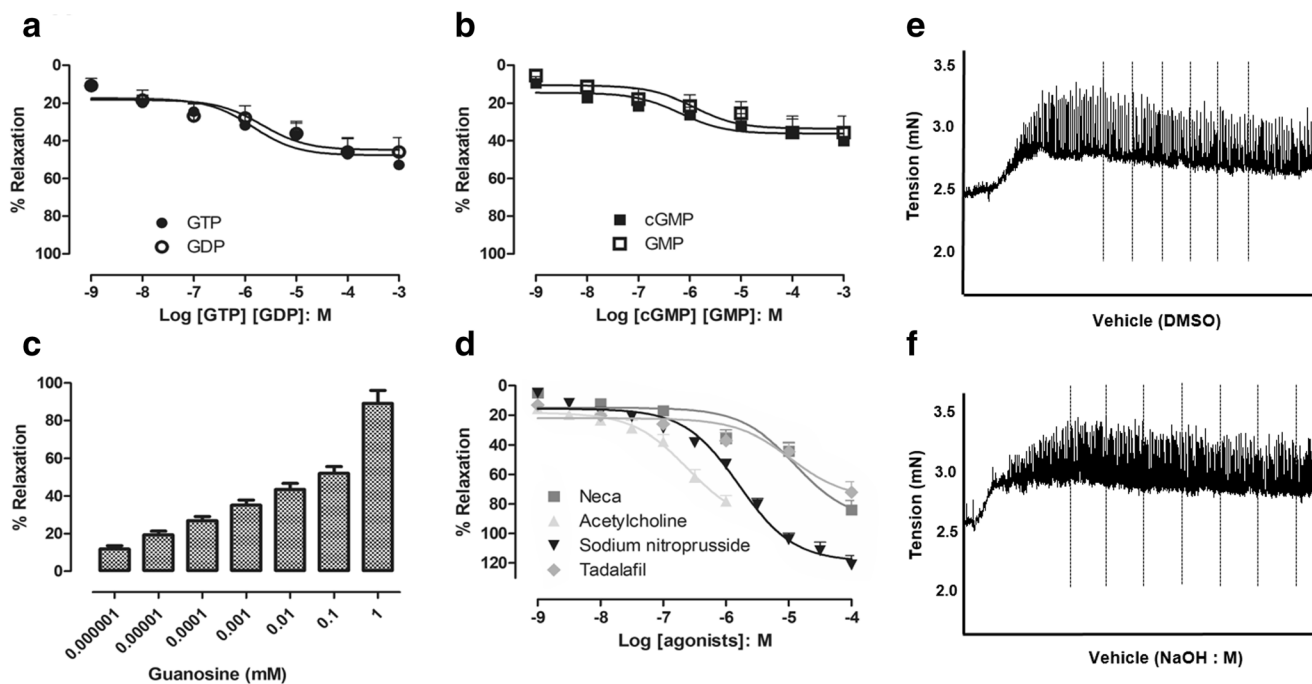
Previous studies have shown that the neuroprotective [25] or the antiplatelet [24] effect of guanosine could be in part due to the activation of adenosine receptors, which stimulate stimulatory G protein (Gs) to accumulate cAMP. However, in mice CC the A2B- and A2A-receptor antagonists, MRS 1754 (1  $\mu\text{M}$ ) and ZM 241385 (1  $\mu\text{M}$ ), respectively did not interfere significantly in the relaxation induced by guanosine (Fig. 2a, b).

To rule out that in mice CC the relaxation induced by guanosine did not involve cAMP accumulation, tissues were stimulated with guanosine (0.1 and 1 mM) and the levels of cAMP were quantified. No increase in the intracellular levels of cAMP was observed (Fig. 2c). The adenylate cyclase activator, forskolin was used as positive control and increased by, approximately, 12-fold the levels of cAMP in mice CC (Fig. 2c).

### Effect of NO-cGMP pathway inhibitors in guanosine-induced relaxation

Previous study carried out in rat mesenteric arteries showed that the potency values of guanosine were reduced in the absence of endogenous NO and its relaxation involved cGMP accumulation [23]. Therefore, we have used inhibitors of NO-sGC-PDE5 pathway in guanosine-induced relaxation. While the inhibitors of NOS (L-NAME, 100  $\mu\text{M}$ , Fig. 3a) and sGC (ODQ, 10  $\mu\text{M}$ , Fig. 3b) reduced significantly the relaxation induced by guanosine, tadalafil (300 nM, Fig. 3c) increased this response.

In another set of experiments, we carried out CRCs to tadalafil in the absence (control) and in presence of guanosine (100  $\mu\text{M}$ ). A 6.6-leftward shift in the relaxing response induced by tadalafil was observed in the presence of guanosine (from  $5.02 \pm 0.18$  to  $5.84 \pm 0.24$ ,  $P < 0.05$ ) without any effect in the  $E_{\text{max}}$  values (Fig. 3d). In CC stimulated with guanosine (1 mM), a 2-fold increase in the intracellular levels of cGMP



**Fig. 1** Concentration-response curves to guanine-based nucleotides, **a** GTP ( $N=7$ ) and GDP ( $N=4$ ), **b** cGMP ( $N=5$ ) and GMP ( $N=5$ ), and the nucleoside, **c** guanosine ( $N=8$ ) in corpus cavernosum pre-contracted with phenylephrine (PE, 10  $\mu$ M). **d** Represents the relaxing response induced by sodium nitroprusside (SNP,  $N=4$ ), acetylcholine (ACh,

$N=8$ ), tadalafil ( $N=8$ ), and NECA ( $N=4$ ). **e** and **f** Represent the original tracings of the lack of effect of vehicles DMSO and NaOH in tissues pre-contracted. The vertical dotted lines refer to the vehicle incubation. Data represent mean  $\pm$  SD of  $N$  = number of animals

was observed (Fig. 3e), thus confirming the involvement of cGMP in the relaxation induced by guanosine.

### Effect of inhibitors of CD39, CD73, and xanthine oxidase

The degradation of GDP to guanosine involves the actions of CD39 and CD73, which hydrolyze GDP into GMP and GMP into guanosine, respectively [7]. To verify whether mice CC has the biochemical apparatus to metabolize the nucleotides, inhibitors of CD39 (ARL 67156, Fig. 4a) and CD73 (alphanbetamethylene-ADP, Fig. 4b) were used. However, no alterations in GDP and GMP-induced relaxation were observed. Yet, oxypurinol (Fig. 4c), a xanthine oxidase (XO)

inhibitor, which degrades guanine into uric acid was also used. However, no alteration in the relaxation induced by guanosine was observed.

### Discussion

The present study is the first to show that guanosine relaxed isolated CC. Guanosine-induced relaxation was greatly reduced by NOS and sGC inhibition and enhanced in the presence of PDE5 inhibitor, thus suggesting that guanosine acts on the NO-sGC pathway to accumulate cGMP. No increase in the cAMP levels were observed in CC stimulated with guanosine.

**Table 1** Emax and pEC50 values of guanine-based nucleotides and nucleoside (guanosine)

	Emax	pEC <sub>50</sub>
GTP	52.8 $\pm$ 13.4	5.84 $\pm$ 0.37
GDP	45.9 $\pm$ 15.4	5.82 $\pm$ 0.52
cGMP	40.1 $\pm$ 16.7	6.18 $\pm$ 0.47
GMP	35.7 $\pm$ 19.6	6.71 $\pm$ 0.57*
Guanosine	90.7 $\pm$ 12.5***	-

\*\*\* $P < 0.0001$  in comparison with the nucleotides

\* $P < 0.05$  in comparison with GTP

**Table 2** Emax and pEC50 values of NECA, SNP, ACh, and tadalafil

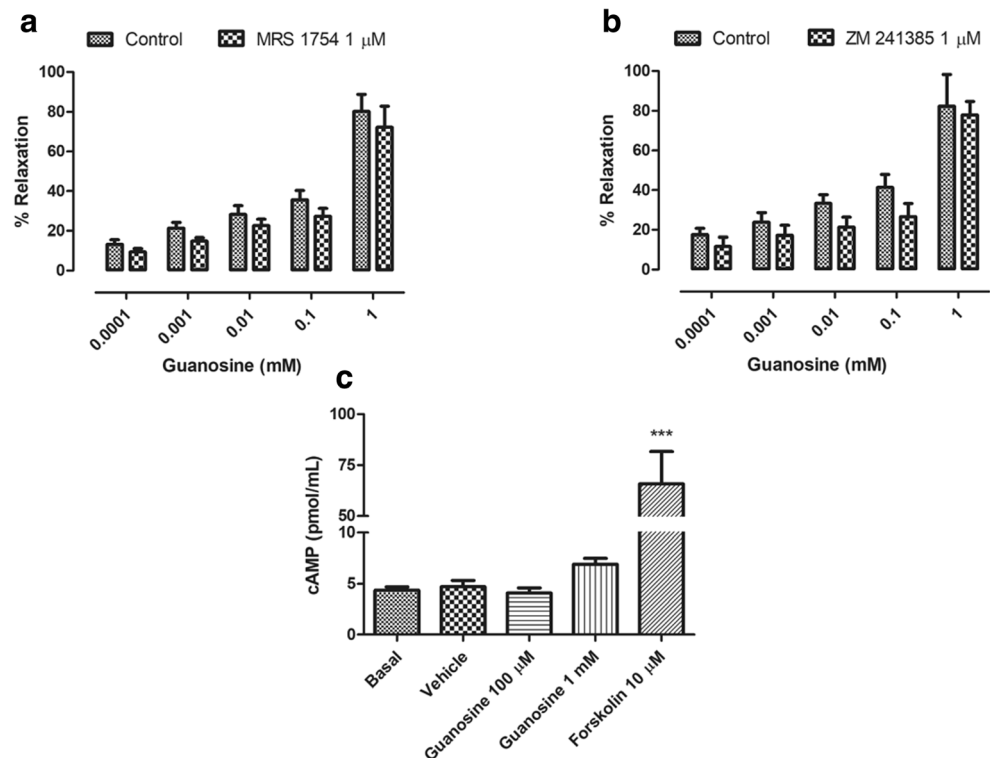
	Emax	pEC <sub>50</sub>
Guanosine	90.7 $\pm$ 12.5	-
NECA	84.0 $\pm$ 12.9	4.90 $\pm$ 0.20
Acetylcholine	78.2 $\pm$ 10.9	6.62 $\pm$ 0.17***
Sodium nitroprusside (SNP)	121.2 $\pm$ 12.8*	5.79 $\pm$ 0.07
Tadalafil	72.1 $\pm$ 20.3	4.97 $\pm$ 0.24

\*\*\* $P < 0.0001$  in comparison with guanosine, NECA, SNP, and tadalafil

\* $P < 0.05$  in comparison with guanosine, NECA, acetylcholine, and tadalafil



**Fig. 2** Concentration-response curve to guanosine (0.0001 to 1 mM;  $n = 5$ ) in the absence and presence of adenosine **a**A2B (MRS 1754- 1  $\mu$ M) and **b** A2A (ZM 241385 1  $\mu$ M) receptor antagonists in tissues pre-contracted with phenylephrine (PE, 10  $\mu$ M). **c** Represents the intracellular levels of cAMP (pmol/mL) in corpus cavernosum stimulates with guanosine (0.1 and 1 mM). Data represent mean  $\pm$  SD of  $N =$  number of animals.  $P < 0.0001$  compared to baseline (\*\*\*)



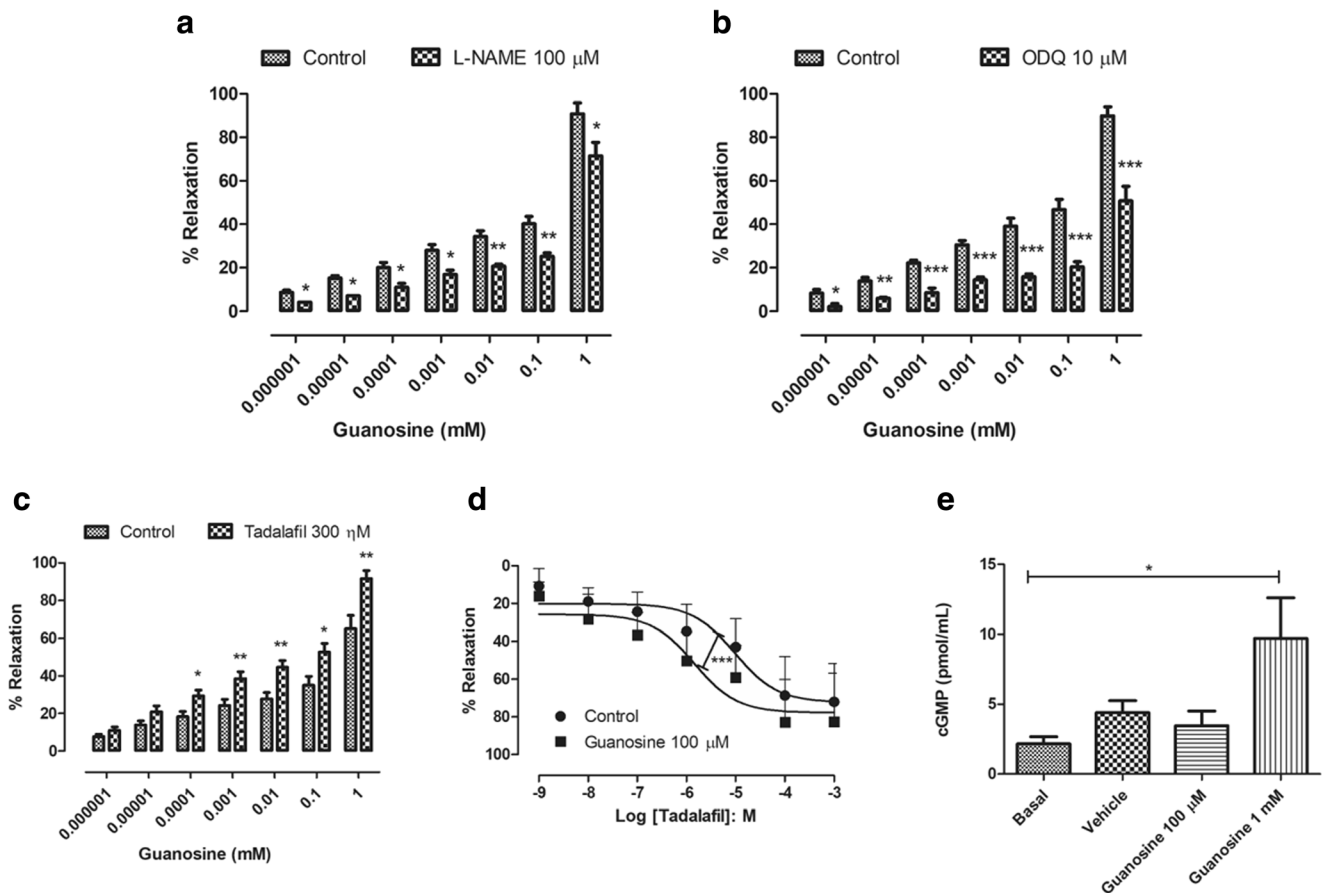
In experiments with binding assays using membrane from rat brain, the saturation curve indicates the presence of a high-affinity binding site for guanosine with  $K_d$  and apparent maximal number of binding sites of, respectively,  $95.4 \pm 11.9$  nM and  $0.57 \pm 0.03$  pmol  $\text{mg}^{-1}$  protein. In competitive assay, guanosine displaced ( $^3\text{H}$ )-guanosine in a monophasic manner with  $\text{IC}_{50}$  equals to 48 nM. Other guanine- and adenine-based nucleotides/nucleosides such as GTP, GMP, GDP, ATP, and adenosine, among others were unable to displace guanosine, thus suggesting that the binding sites of guanosine differs from those of adenosine. Yet, these results suggest that guanosine acts at cell membrane by a specific receptor [26]. Another research group has also shown that guanosine and 6-thioguanosine activate a G-protein-binding receptor in rat brain membrane, but it is not related to the adenosine receptor [27].

Studies show a crosstalk between adenosine and guanosine pathways, where the latter can activate adenosine receptors [28]. The extracellular role of guanine-based nucleotides has been well characterized in the central nervous system. For instance, rats with traumatic brain injury presented a decrease on synaptic (synaptophysin) and plasticity proteins (BDNF and CREB), loss of cresyl violet stain neurons and increased astrogliosis and microgliosis in the hippocampus. Chronic treatment with guanosine (7.5 mg/Kg, ip, daily) prevented these alterations in a mechanism dependent on A1 receptor activation because A1 receptor antagonist DPCPX (1 mg/Kg) reverted the neuroprotection induced by guanosine. No difference was observed when the A2A receptor antagonist,

SCH58261 (0.05 mg/Kg), was used [29]. In hippocampal slices from rats subjected to oxygen/glucose deprivation and reoxygenation (OGD), guanosine (100  $\mu$ M) reduced the production of oxygen species, prevented mitochondrial membrane depolarization, and reduced protein expression of iNOS and NF- $\kappa$ B. The neuroprotective effects were prevented by DPCPX (250 nM). Guanosine also increased glutamate uptake and this response was prevented by ZM 241385 (50 nM), an A2A receptor antagonist [20].

The role of guanosine was also demonstrated peripherally. Guanosine concentration dependently (50–500  $\mu$ M) inhibited platelet aggregation induced by ADP, collagen, arachidonic acid, or TRAP-6, accompanied by increased levels of cAMP and p-PKA activation. The adenosine A2A receptor antagonists, ZM241385 (1 and 10  $\mu$ M) and MSX-2 (1  $\mu$ M), did not interfere in guanosine-induced platelet inhibition [24]. Another study carried out in human platelets compared the antiplatelet activity of adenine- and guanine-based nucleotides/nucleosides. Both GTP and guanosine inhibited thrombin-induced platelet aggregation, although GTP was, approximately, 100 times more potent than guanosine. GTP at 0.1 and 1 mM, but not guanosine, significantly increased the levels of cGMP in thrombin-stimulated platelets. The levels of cAMP were not altered in platelets incubated with GTP or guanosine [30].

Our results showed that guanosine relaxed CC and, conversely to neuronal cells and platelets, this mechanism involved cGMP accumulation. The lower levels of endogenous NO and the oxidation of sGC by ODQ reduced significantly guanosine-induced relaxation. Similar findings were observed

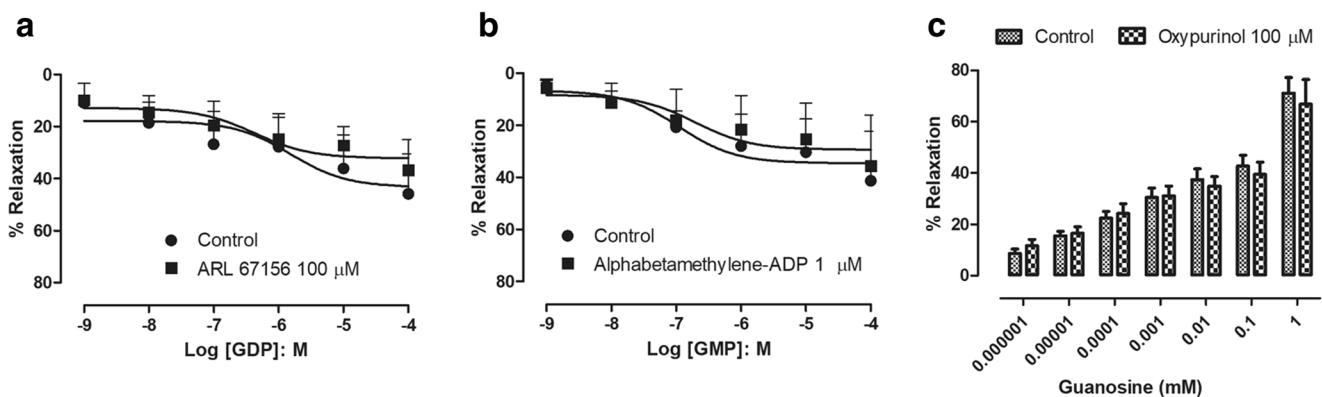


**Fig. 3** Concentration-response curve to guanosine (0.000001 to 1 mM) in the absence and presence of **a** nitric oxide synthase (L-NAME; 100  $\mu$ M;  $N=5$ ), **b** soluble guanylate cyclase (ODQ, 10  $\mu$ M,  $N=6$ ), and **c** phosphodiesterase type 5 (tadalafil, 300 nM,  $N=12$ ) in corpus cavernosum pre-contracted with phenylephrine (PE, 10  $\mu$ M). In another set of experiment, concentration-response curve to tadalafil was

carried out in the presence of guanosine (100  $\mu$ M,  $N=8$ ). **e** Represents the intracellular levels of cGMP (pmol/mL) in tissues stimulated with guanosine (0.1 and 1 mM,  $N=6$ ). Data represent mean  $\pm$  SD of  $N$ = number of animals. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.0001$  in comparison with control or vehicle (NaOH 0.5 M)

in rat mesenteric arteries [23] and in undifferentiated rat pheochromocytoma cells (PC12) [31]. In the first study, guanosine concentration-dependent relaxed mesenteric artery in a mechanism dependent on endogenous NO and sGC activation. In

the second study, guanosine (300  $\mu$ M), through cGMP accumulation, increased the proportion of neurite in PC12 cells in a mechanism independent of NO. Since PC12 does not express nNOS or iNOS, the authors evaluated the role of heme



**Fig. 4** Concentration-response curves to the nucleotides GDP and GMP in the absence and presence of the **a** CD39 inhibitor (ARL 67156; 100  $\mu$ M;  $N=3$ ) and **b** CD73 inhibitor (alphetamethylene-ADP, 1  $\mu$ M;  $N=5$ ), respectively. **c** Represents the effect of guanosine in the

presence of the xanthine oxidase inhibitor (oxypurinol; 100  $\mu$ M;  $N=7$ ) in corpus cavernosum pre-contracted with phenylephrine (PE, 10  $\mu$ M). Data represent mean  $\pm$  SD of the  $N$ = number of animals

oxygenase-1 (HO-1) pathway, which produces carbon monoxide, a gasotransmitter that also activates sGC. Guanosine increases the protein expression of HO-1 and in the presence of ZnPP (100 nM), an inhibitor of HO-1, the cGMP levels induced by guanosine alone or in combination with nerve growth factor were reduced in comparison with PC12 cells untreated with ZnPP [31].

Guanine is a metabolite of guanosine. In SH-SY5y neuroblastoma cells, guanine (50  $\mu$ M) increased the phosphorylation of ERK 1/2 and this response was significantly potentiated by IBMX, a non-selective PDE inhibitor and reduced by L-NAME and ODQ. Yet, guanine increased NO production in this cell line [32]. One may argue whether the relaxation induced by guanosine could be in part due to guanine. To address this question, we carried out experiments to evaluate CC relaxation in the presence of guanine (1 nM to 1 mM, data not shown). However, one particular issue of guanine is its poor solubility in water. We had to use an alkaline solution (NaOH 1 M) to dissolve guanine, but when we added this solution in the organ bath, a rise in pH to nearly 9.5 was observed, resulting in a transient, but intense, fall in the tissue tonus. The effects of pH on smooth muscle tone are not completely understood, it may depend on tissue capacity to regulate the pH change and vary markedly between smooth muscles [33]. In addition, we have used oxypurinol, which inhibits the breakdown of xanthine into uric acid, but no potentiation in guanosine-induced relaxation was observed. This result suggests that at least in CC, the relaxing response induced by guanosine did not involve the accumulation of xanthine byproducts. To date, there are no reports that evaluated the expression or the inhibition of purine nucleoside phosphorylase (PNP), an enzyme that converts guanosine into guanine in CC. Further experiments will be performed to assess the role of PNP inhibition in CC and whether the accumulation of guanine would have any role in CC smooth muscle.

The inhibition of CD39 and CD73 enzymes, which degrade GTP into guanosine, did not affect the relaxation induced by the nucleotides GDP or GMP, thus suggesting that, at least in mice CC, the activity of these nucleotidases is likely low; however, it is not possible to rule out the activity of other phosphatases forming guanosine as well, as it has been reported for adenine-based nucleotides [34]. On the other hand, one study carried out in corpus cavernosum from CD73-deficient mice showed that the intracavernous pressure induced by electrical field stimulation of the cavernosus nerve was decreased in CD73 (–/–) mice as well as the adenosine levels [35]. These results led the authors to conclude that CD73 is a key enzyme in CC to degrade adenine-based nucleotides into the nucleoside adenosine. To date, no study evaluated the role of CD73 or CD39 in the degradation of guanine-based nucleotides in corpus cavernosum.

One limitation of the present study is that we did not evaluate whether guanosine is uptaken by equilibrative nucleoside transporters type 1 and 2 (ENT 1/2) on corpus cavernosum. To the best of our knowledge, there is no study that evaluates the expression of ENT on corpus cavernosum. Second, dipyridamole and NBTI are the most used inhibitors of ENT, but one study that evaluated the role of dipyridamole (10  $\mu$ M) on adenosine-induced mice corpus cavernosum relaxation did not show any effect in the relaxing response [10], thus suggesting that conversely to other cells/organs, where ENT is physiologically relevant, such as human aortic cells [36], human kidney, the liver, endothelial cells [37], and rat epididymal epithelium [38], on CC its role was not well characterized. Besides, dipyridamole is also a phosphodiesterase inhibitor, which could interfere in the interpretation of our results.

In conclusion, our study showed that guanosine relaxes corpus cavernosum in a mechanism dependent of sGC activation to accumulate cGMP. Our study opens the possibility to evaluate the role of guanosine in animal models of erectile dysfunction.

**Authors' contributions** All authors have participated in the research and/or article preparation.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** All experimental protocols were carried out according to the Ethical Principles in Animal Research adopted by the Brazilian College for Animal Experimentation and approved by the Institutional Committee for Ethics in Animal Research of the University of Campinas (CEUA/UNICAMP protocol number 4719-1).

**Abbreviations** *ACh*, Acetylcholine; *ADP*, Adenosine diphosphate; *ARL 67156*, 6-N,N-Diethyl- $\beta$ - $\gamma$ -dibromomethylene-D-adenosine-5'-triphosphate; *ATP*, Adenosine triphosphate; *cAMP*, Cyclic adenosine monophosphate; *CC*, Corpus cavernosum; *CD39*, Ecto-NTPase; *CD73*, Ecto-5'-nucleotidase; *cGMP*, Cyclic guanosine monophosphate; *DPCPX*, 1,3-Dipropyl-8-cyclopentylxanthine; *ENT 1/2*, Nucleoside transporters type 1 and 2; *GDP*, Guanosine diphosphate; *GMP*, Guanosine monophosphate; *GTP*, Guanosine triphosphate; *L-NAME*, N $\omega$ -Nitro-L-arginine methyl ester; *MRP4/MRP5*, Multidrug resistance proteins type 4 and 5; *MRS 1754*, 8-[4-[(4-Cyanophenyl)carbamoymethyl]oxy]phenyl]-1,3-di(n-propyl)xanthine; *NBTI*, S-(4-Nitrobenzyl)-6-thioinosine; *NECA*, 5'-(N-Ethylcarboxamido)adenosine; *NF- $\kappa$ B*, Kappa beta nuclear factor; *NO*, Nitric oxide; *NOS*, Nitric oxide synthase; *ODQ*, 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one; *OGD*, Oxygen/glucose deprivation; *PDE5*, Phosphodiesterase 5; *sGC*, Soluble guanylate cyclase; *SNP*, Sodium nitroprusside; *ZM 241385*, 4-(-2-[7-amino-2-(2-furyl){1,2,4}triazolo{2,3-a}{1,3,5}triazin-5-yl-amino]ethyl)phenol

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