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RESEARCH

Inhibition of uterine contractility by guanine-based purines in non-pregnant rats

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Abstract

Growing evidence pointed out that guanine-based purines are able to modulate smooth muscle contractile activity of blood vessels and gastrointestinal tract. Since, so far, possible guanine-based purine modulation of uterine musculature is unknown, the aim of the present study was to investigate in vitro, using organ bath technique, guanosine and guanine effects on spontaneous uterine contraction, and uterine contraction induced by K^+-depolarization and oxytocin in a non-pregnant rat. Guanosine, but not guanine, reduced the amplitude of spontaneous contraction of the uterine muscle in a dose-dependent manner. The inhibitory response was antagonized by S-(4-nitrobenzyl)-6-thioinosine (NBTI), a membrane nucleoside transporter inhibitor, but persisted in the presence of theophylline, a nonselective adenosine receptor antagonist, or propanolol, β_1/β_2 adrenoreceptor antagonist or blockers of a nitrergic pathway. In addition, potassium channel blockers did not influence guanosine-induced effects. Guanosine was able to inhibit the external calcium (Ca^{2+}) influx-induced contraction, but it did not affect the contraction induced by high-KCl solution, indicating that guanosine does not interact with L-type voltage-gated calcium channel. Guanosine prevented/reduced uterine contractions induced by oxytocin, even in the absence of external calcium. In conclusion, guanosine is able to reduce both spontaneous and oxytocin-induced contractions of rat myometrium, likely subsequently to its intracellular intake. The blockade of extracellular Ca^{2+} influx and reduction of Ca^{2+} release from the intracellular store are the mechanisms involved in the guanosine-induced tocolytic effects.

Keywords Guanosine · Uterus · Contraction · Oxytocin · Calcium

Introduction

The uterine myometrium produces regular spontaneous contractions in response to either endogenous or exogenous chemical stimuli, playing a pivotal role in physiological processes such as sperm and embryo transport and implantation (Aguilar and Mitchell 2010). Abnormality in this physiological process can result in disorders such as dysmenorrhea, endometriosis, preterm birth, post-term pregnancy, and postpartum hemorrhage. These clinical complications can eventually lead to serious problems regarding disability, mortality, and cost to society. Although there is growing awareness of the potential importance of abnormal functions of the uterine muscle layer, there has been relatively little research concerning the role of the myometrium in common disorders of reproduction. Thus, a better understanding of the myometrium physiology is essential to design and test interventions that can prevent or treat the important clinical problems noted above, and there is a clinical need to find better drugs to help control uterine activity.

Most of the endogenous compounds affecting smooth muscle contractile activity in the gastrointestinal tract or in blood vessels seem to also have an effect on the contractile activity of the myometrium. Among these have been described the effects exerted by the purinergic system on motor functions of the uterus. In particular, adenosine and ATP are able to modulate contractile activity in strips of the uterus in a rat, sheep, or guinea pig inducing excitatory and /or inhibitory effects (Gillman and Pennefather 1998; Burnstock 2014).

Guanine-based purines are a group of naturally occurring purines including the guanosine mono-, di-, and

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tri-phosphate nucleotides (GMP, GDP, and GTP, respectively); the nucleoside guanosine; and the nucleobase guanine. Their intracellular roles as the formation of the second messenger cyclic GMP or modulation of activity of G-protein-coupled receptors are well-known; however, growing evidence revealed also their extracellular activity, as neuromodulators in both the central and peripheral nervous system (Zizzo et al. 2011; Di Liberto et al. 2016; Tasca et al. 2018). Among these, it is potentially relevant to the role of guanosine in the modulation of the activity of smooth muscle cells.

Researches provided evidence that guanine-based purines may modulate the contractile activity of smooth muscle vessels (Fujiwara et al. 1989) and of gastrointestinal smooth muscle, the latter either via a direct action on the effectors (Zizzo et al. 2013) or indirectly reducing the cholinergic neurotransmission (Zizzo et al. 2011). So far, data about the possible guanine-based purine effects on uterine musculature are lacking.

In this view, this study was designed to investigate the effects and the action mechanism of guanine-based purines on non-pregnant rat uterus contractility, in consideration that substances able to exert tocolytic action could be promising for treating uterine disorders associated with smooth muscle contraction dysregulation.

Materials and methods

Animals

All animal procedures and care were approved by the Animal Care and Use Ethics Committee of the University of Palermo (69,636.N.JCO) and performed in accordance with national and EU guidelines for the handling and use of experimental animals. In vitro mechanical activity of uterine musculature was measured using the isolated tissue bath technique, as previously described (Zizzo et al. 2017). Briefly, 15 virgin Wistar rats (200–250 g, Envigo, S Pietro al Natisone- Italy) in oestrus phase, as determined by examination of a vaginal smear, were euthanized using 2% isoflurane anesthesia followed by cervical dislocation. After laparotomy, the uterine horns were rapidly excised and immediately placed into physiological Krebs saline solution, and longitudinal uterine strips (2 mm × 10 mm) were dissected from each horn, followed by the mechanical removal of the endometrial layer. Longitudinally oriented uterine strips were suspended in 10 ml four-channel organ bath containing oxygenated (95% O_2 and 5% CO_2) and warmed (37 °C) Krebs solution. Strips were anchored at the distal end to an organ holder and at the proximal end secured with a silk thread to a force transducer (FORT 10, Ugo Basile, Biological Research Apparatus, Comerio VA, Italy) for the isometric recording of muscular activity (PowerLab/400 system, Ugo Basile, Italy). Strips were allowed to equilibrate at 1 g tension for at least 40 min before the addition of the experimental drugs. All strips developed spontaneous mechanical activity.

After the equilibration period, preparations were challenged with high-KCl solution (60 mM) until stable responses were obtained. Strips not responding to KC1 were discarded.

Cumulative concentration–response curves for guanine and guanosine, 10 µM–3 mM each, were constructed. Drugs were applied for approximately 6 min. Preliminary experiments, comparing cumulative and noncumulative concentration–response curves to guanosine, have shown no receptor desensitization (data not shown).

The guanosine concentration–response curve was repeated in the presence of the following: (i) NBTI (10 µM), a nucleoside transporter inhibitor; (ii) theophylline (30 µM), a nonselective adenosine receptor antagonist; (iii) propanolol (1 µM) β_1/β_2, adrenoreceptor antagonist; (iv) L-NAME, (100 µM) a blocker of the NO synthase; and (vi) ODQ (10 µM), soluble guanylyl cyclase inhibitor. Drugs acting as blockers or antagonists were left in contact with the tissue for 30 min, before adding guanosine. Each preparation was tested with a single antagonist or blocker.

To investigate the effect of guanosine on the extracellular calcium influx, after the equilibrium, the preparation was washed with calcium-free solution, and cumulative concentration–response curve to $CaCl_2$ (0.1–10 mM) was performed. Then, the submaximal concentration of guanosine (1 mM) was added to the organ bath for 10 min before performing a second cumulative concentration–response curve to $CaCl_2$.

In subsequent experiments, to evaluate the preventive effects of guanosine treatment, strips were pre-treated for 5 min with guanosine (0.1 mM, corresponding to EC50), and then the high-KCl solution (60 mM) or oxytocin (5 nM) was added in the continued presence of the guanosine. To investigate the effect of guanosine on calcium release from the intracellular calcium stores, calcium-free solution (1 mM Ethylene Glycol Tetraacetic Acid (EGTA)) was used. After equilibrium, Krebs solution was replaced with the calcium-free solution containing 1 mM of EGTA until the complete abolition of spontaneous activity. Then, oxytocin was added to the organ bath in the absence or in the presence of guanosine. To evaluate the possible action of guanosine on the opening of L-type Ca^{2+} channels, the uterine strips were exposed to high K^+ solution to depolarize membrane potential. Moreover, the effects of guanosine on the contraction induced by oxytocin (5 nM) were also tested. Guanosine was added to the plateau phase of the induced contractions.

Lastly, to evaluate the possible involvement of K^+ channels on guanosine effects, the response to the submaximal concentration of guanosine (1 mM) was repeated in the presence of the following: (i) Tetraethylammonium (TEA, 20 mM), nonselective K^+ channel blocker; (ii) apamin (100 nM), a blocker

of small conductance Ca^{2+}-activated K^+ channels; and (iii) iberiotoxin (IbTX, 100 nM), a blocker of large and intermediate conductance Ca^{2+}-activated K^+ channels.

The time of exposition and concentrations of the drugs used were determined from preliminary experiments and from the literature (Zizzo et al. 2010, 2011, 2013).

Drugs

The following drugs were used: apamin, $CaCl_2$,ethylene glycol tetraacetic acid (EGTA), guanine, guanosine, iberiotoxin (IbTX), Nω-Nitro-L-Arginine methyl ester (L-NAME), 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one (ODQ), S-(4-Nitrobenzyl)-6-thioinosine (NBTI), oxytocin, propranolol, theophylline, and tetraethylammonium (TEA) (Sigma-Aldrich, Inc., St. Louis, USA). Guanosine were dissolved in 10% solution of 1 N NaOH, and guanine was dissolved in 30% solution of 1 N NaOH. NBTI, ODQ, and IbTX were dissolved in dimethyl sulphoxide and further diluted in Krebs. The maximal final concentration of NaOH or dimethyl sulphoxide in the organ bath did not affect the contractility of the uterine strips. All the other drugs were dissolved in distilled water. The working solutions were prepared fresh on the day of the experiment by diluting the stock solutions in Krebs and were added to the organ bath.

Statistical analysis

Data are given as means ± SD; *n* in the result section refers to the number of animals on which observations were made. The responses induced by guanosine were estimated as a decrease in the amplitude of the slow phasic contractions during 6 min after drug administration, normalized to the amplitude of the slow phasic contractions measured 6 min prior to the treatment, set as 100%. Indeed, the amplitude of the plateau phase of the contraction induced by high-KCl solution or by oxytocin was taken as 100% contraction to determine the effects of guanosine. Responses were fitted to sigmoid curves (Prism 5.0, Graph-PAD, San Diego, CA, USA), and EC_{50} values with 95% confidence limits (CLs) were determined. Statistically significant differences were calculated by Student's *t*-test or by means of analysis of variance, followed by Bonferroni's test, when appropriate. A probability value less than 0.05 was regarded as significant. Since the study has an exploratory character rather than a hypothesis-design study, the *p* values should be considered descriptive (Motulsky 2014; Michel et al. 2020).

Results

Longitudinal strips from rat uterus, once mounted in the organ bath, developed a spontaneous mechanical activity characterized by rhythmic contractions with an amplitude of 2.51 ± 0.22 g and frequency of 0.91 ± 0.03 c.p.m (contractions per minute) ($n = 15$). The cumulative addition of guanosine from 10 μM to 3 mM inhibited spontaneous activity in a concentration-dependent manner, decreasing both the amplitude and the frequency of the contractions (Fig. 1A, B, C). Parallel experiments using a vehicle alone showed that it has no effect on the basal uterine contractions. The maximal response consisted in the abolition of the spontaneous contractions without affecting the muscular tone ($n = 15$). The EC_{50} for guanosine was 0.12 mM (95% CL 0.08–0.3 mM; $n = 15$). The effects of guanosine were rapidly reversible. On the contrary, guanine (10 μM–3 mM) or vehicle did not affect spontaneous mechanical activity (Fig. 1A, B, C). Since guanosine could be transported into cells to induce the response, as observed in the intestine and in retinal ganglion cells (Benowitz et al. 1998; Zizzo et al. 2011, 2013), guanosine effects were tested in the presence of NBTI, a membrane nucleoside transporter inhibitor.

Contractile responses to guanosine were deeply antagonized by pretreatment of the preparations with NBTI (10 μM), (Fig. 2A, $p < 0.05$), which per se did not significantly modify the spontaneous mechanical activity, suggesting that guanosine effects are dependent on intracellular mechanisms after its uptake.

To evaluate if guanosine may act viaadenosine or adrenergic receptor activation, guanosine effects were observed in the presence of theophylline, a nonselective adenosine receptor antagonist, or propranolol, a β_1/β_2 adrenoreceptor antagonist. As shown in Fig. 2, the inhibitory effects induced by guanosine were not affected by the blockade of adenosine receptors by theophylline (1 μM) (Fig. 2B, $p = 0.76$) or by propanolol (1 μM) (Fig. 2C, $p = 0.42$), which antagonized the inhibitory effects induced by the respective agonist in the same preparation (data not shown).

Moreover, we tested the possible involvement of NO in guanosine responses since NO acts as a relaxant agent in uterine muscle cells (Chaud et al. 1997). L-NAME (100 μM), a blocker of the NO synthase, or ODQ (10 μM), NO-sensitive soluble guanylyl cyclase inhibitor, did not modify the inhibitory responses induced by guanosine, implying that NO synthesis is not involved in guanosine-induced inhibitory effects (Fig. 2D, $p = 0.94$).

Effect of guanosine on Ca^{2+}-dependent contractile responses

To investigate if guanosine could interfere with extracellular calcium influx, we studied calcium-dependent contractile responses. In calcium-free solution addition of $CaCl_2$ (0.1–10 mM) resulted in a concentration-dependent contractile response, which was significantly inhibited by the submaximal concentration of guanosine (1 mM) as shown in Fig. 3. To evaluate the hypothesis of a modulation of the opening of L-type Ca^{2+} channels, the uterine strips were exposed to high K^+ solution that, depolarizing membrane potential, induces opening of calcium channels. Application of high-KCl solution

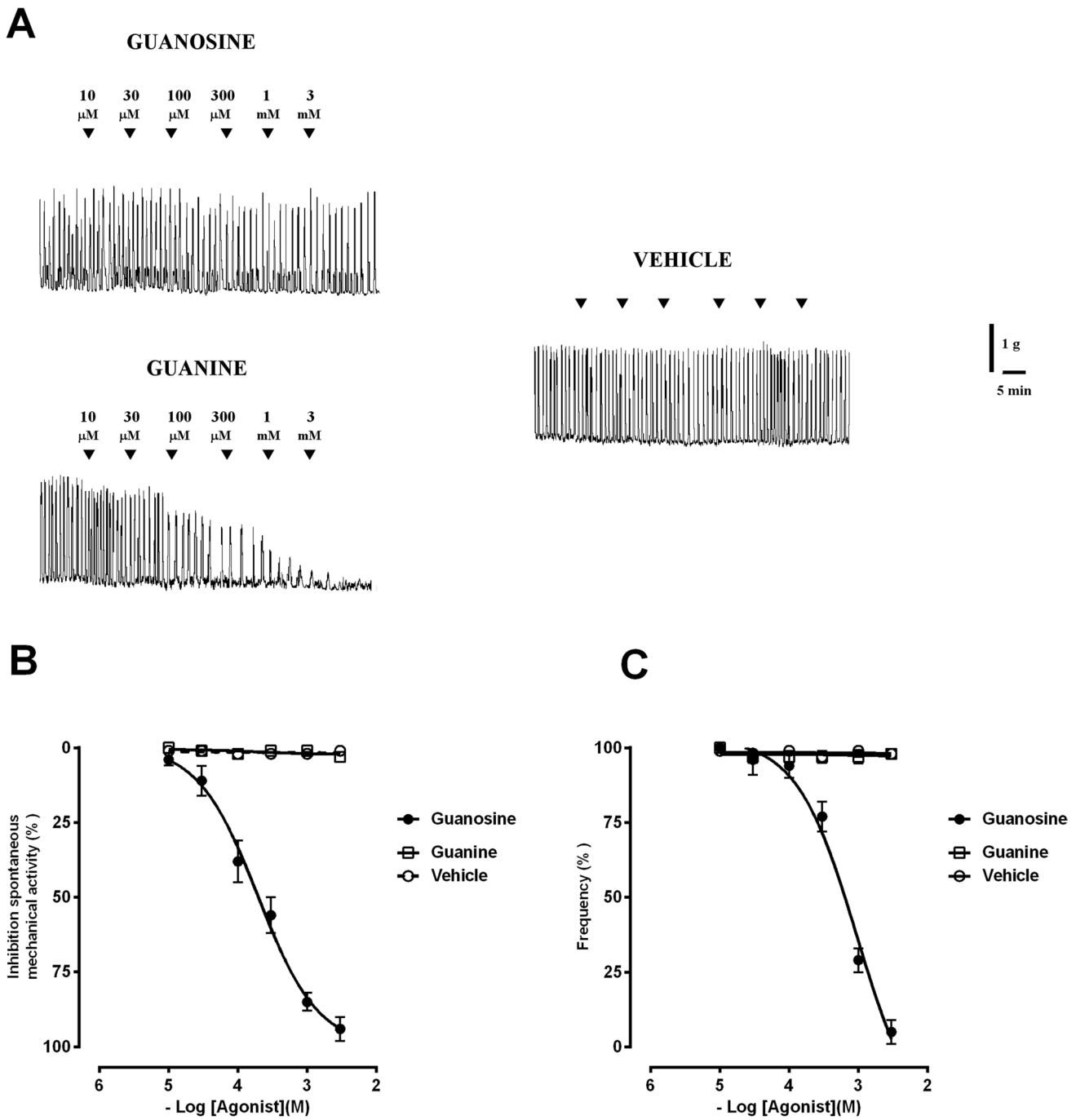

Fig. 1 **A** Original tracings showing the inhibitory effects induced by increasing concentrations of guanine guanosine or vehicle (10% solution of 1 N NaOH) on isolated rat uterine preparations. Concentration–response curves for the effects induced by guanosine (10 µM–3 mM) or guanine (10 µM–3 mM) or vehicle on the amplitude (**B**) and on the frequency (**C**) of spontaneous contractions in isolated rat uterine preparations ($n = 5$ for each). Data are mean ± SD and expressed as the percentage of the inhibition of spontaneous activity, with 100% inhibition corresponding to total suppression of spontaneous contractions

(60 mM) caused a contraction characterized by an initial rapid increase in force followed by a decline and plateau phase, maintained throughout the application time.

The ability of the high-KCl solution to contract uterine muscle was not modified by pretreatment with guanosine 0.1 mM ($p = 0.13$) as well as in the presence of the maximal concentration (3 mM, $p = 0.08$). In addition, the application of the guanosine (10 µM–3 mM) during the plateau phase did not affect the force produced by the high-KCl solution (Fig. 4A, B).

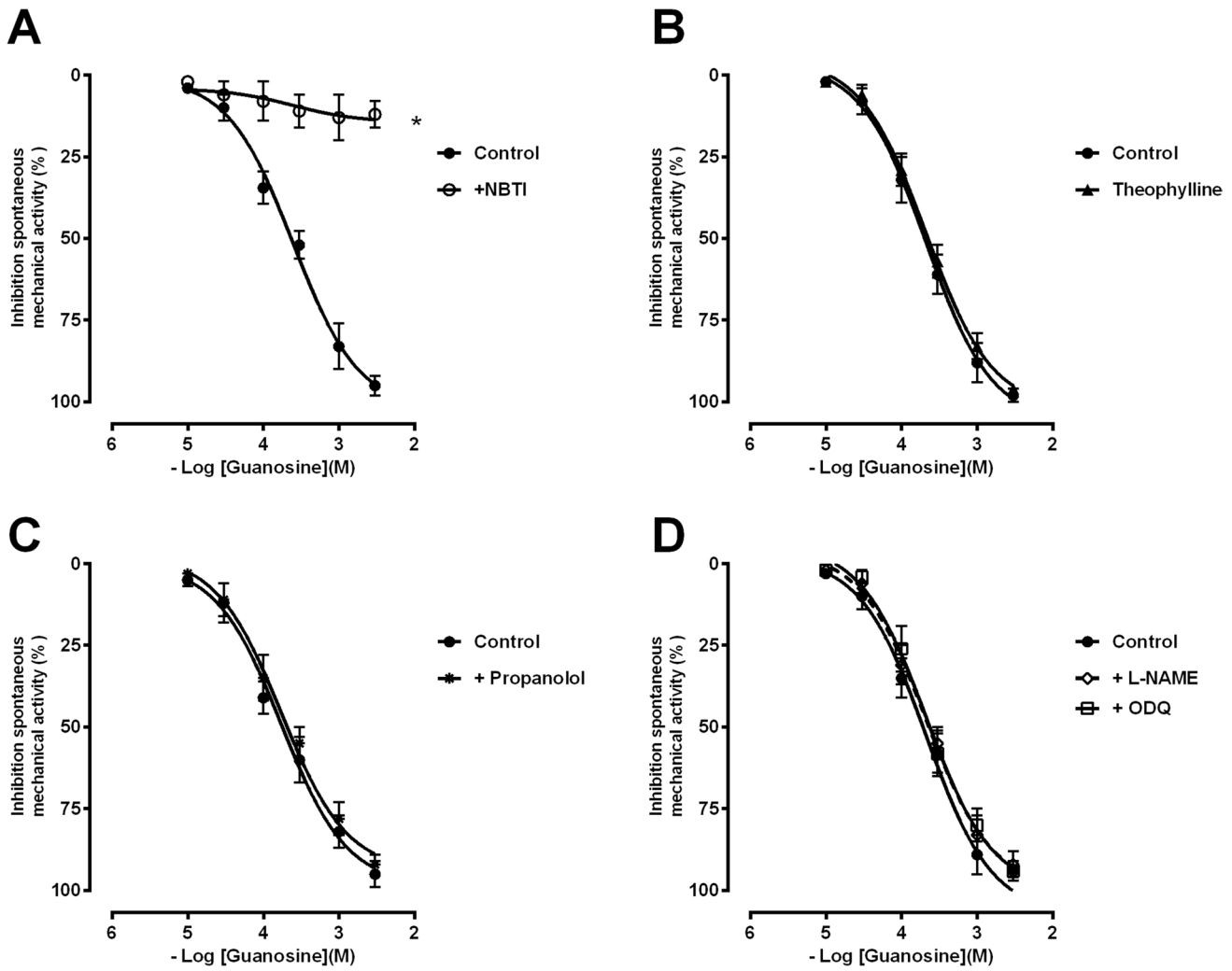

Fig. 2 Concentration–response curves for the inhibitory effects induced by guanosine (10 µM–3 mM) before and after: **A** 10 µM NBTI, a membrane nucleoside transporter inhibitor; **B** 30 µM theophylline, a nonselective adenosine receptor antagonist; **C** 1 µM propranolol, a β_1/β_2 adrenoreceptor antagonist; and **D** 100 µM L-NAME, a blocker of the NO synthase or 10 µM ODQ, soluble guanylyl cyclase inhibitor ($n=4$ for each). Data are mean ± SD and expressed as the percentage of the inhibition of spontaneous activity, with 100% inhibition corresponding to total suppression of spontaneous contractions

Effects of guanosine on uterine contractions induced by oxytocin

The application of 5 nM oxytocin produced a contractile response, characterized by an increase in force followed by a decline and a plateau with rapidly oscillating force production.

Guanosine (0.1 mM) pretreatment induce a reduction of 50% of the oxytocin-induced contraction (Fig. 5A, $p<0.05$). In the uterus, oxytocin can still produce a contraction in the absence of external calcium due to Ca^{2+} release from the sarcoplasmic reticulum (Trujillo et al. 2000). Then, uterine smooth muscle strips were incubated in Ca^{2+}-free solution containing 1 mM EGTA. After 20 min in zero Ca^{2+} solution, spontaneous activity was abolished, and the application of 5 nM oxytocin induced a small increase in force (Fig. 5A, $p<0.05$). The application of 0.1 mM guanosine in the continued absence of external calcium prevented oxytocin-induced transient contraction. In addition, the application of the guanosine (10 µM–3 mM) at the plateau phase of oxytocin-induced contraction caused, in a concentration-dependent manner, a decrease in the amplitude of the contractile response (Fig. 5B, $p<0.05$).

Effects of K^+ channel blockers on guanosine-induced response

Guanosine effects on rat uterine contractions were tested in the presence of apamin (100 nM,) or iberiotoxin (IbTx, 100 nM), in the blocker of small or large and intermediate conductance Ca^{2+}-activated K^+ channels, respectively, or in the presence of

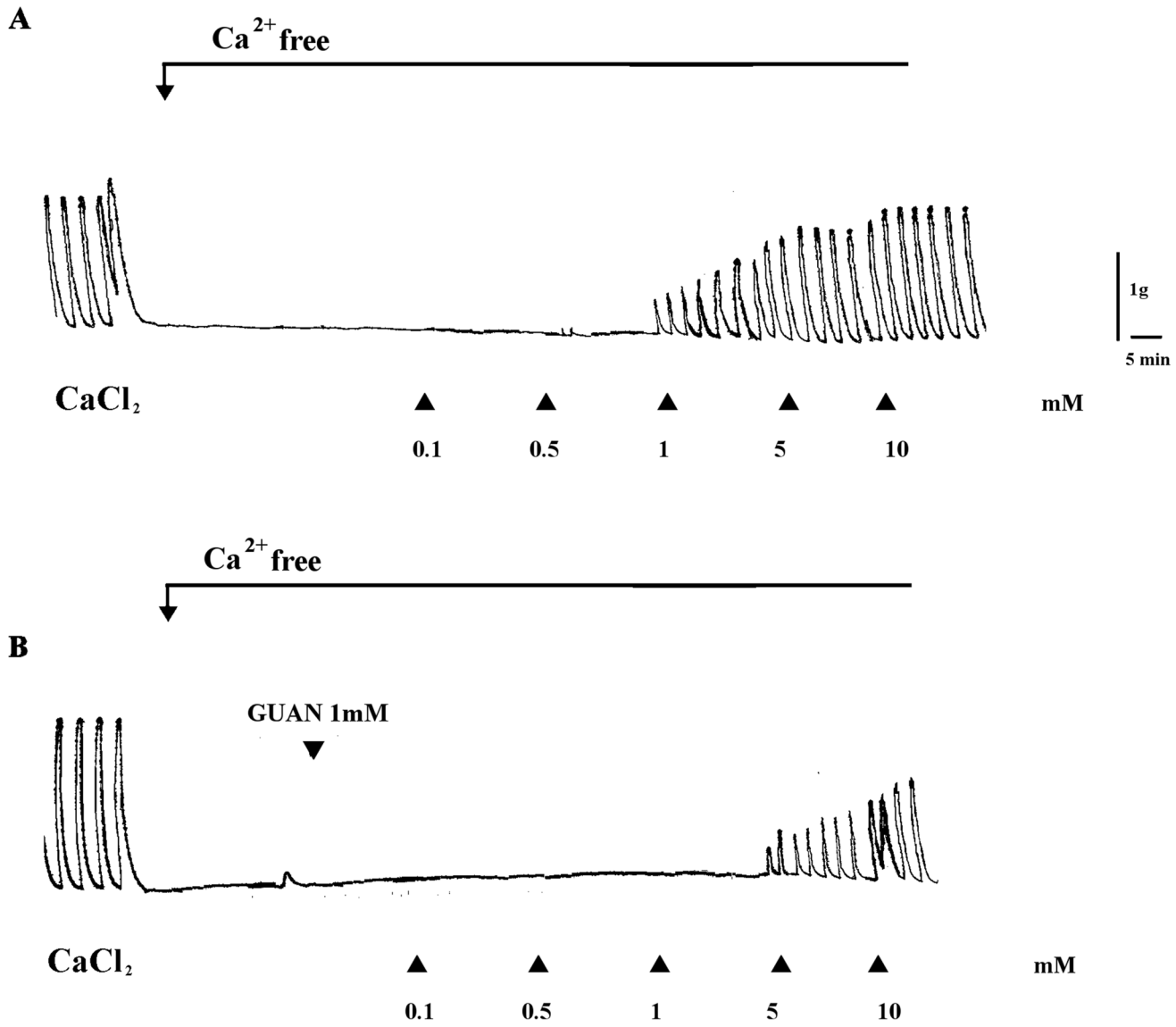

Fig. 3 Original tracing showing the inhibitory effect of guanosine on calcium-dependent contractile responses. **A** Addition of calcium chloride ($CaCl_2$, 0.1–10 mM) in the bathing solution resulted in a concentration-dependent increase in the contraction of the isolated rat uterus. **B** Pretreatment with 1 mM guanosine inhibited Ca^{2+}-dependent contractile responses

TEA (20 mM), non-selective K^+ channel inhibitor. As shown in Fig. 6, the inhibitory effect induced by guanosine was not affected by apamin ($p=0.17$) or IbTx ($p=0.07$) as well as by TEA ($p=0.13$). TEA, per se, induced a marked increase of the amplitude of mechanical activity, while none of the other blockers had effects on the strip tone or on the amplitude and frequency of spontaneous contractions (Fig. 6).

Discussion

This study indicates that guanine-based purines could be considered as locally acting regulators of uterine physiology showing a consistent reversible tocolytic activity in non-pregnant rat uterus. The main finding is that guanosine reversibly inhibits, in a concentration-dependent manner, rat uterine spontaneous contractile activity as well as oxytocin-induced contractions. These effects seem to be related to an inhibition of extracellular calcium influx and calcium mobilization from intracellular stores (Fig. 7).

The guanine-based purinergic system, in addition to the well-characterized adenine-based purinergic system, has been, more recently, highlighted as an extracellular signaling system regulating important functions in the central and peripheral nervous system. Current evidence suggests that guanine-based purines can act as modulators of neurotransmitter release in the central and enteric nervous system

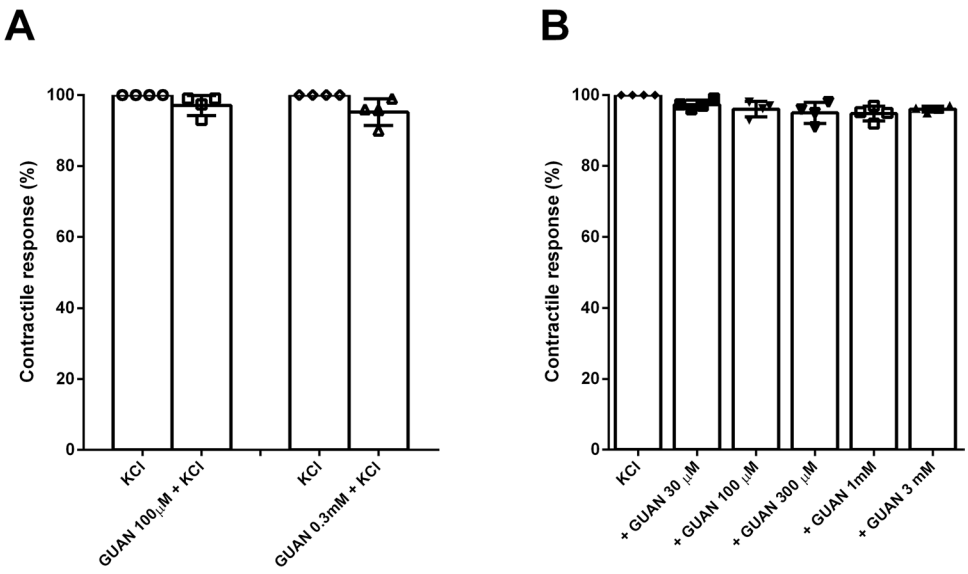

Fig. 4 Guanosine effects on uterine contraction induced by high-KCl solution. **A** Histogram showing the effects of guanosine (0.1 mM or 3 mM) pretreatment on the contractile response to high-KCl solution (60 mM). **B** Effects of guanosine (10 µM–3 mM) on contractile response to 60 mM KCl. Data are mean ± SD ($n = 4$ each) and expressed as the percentage of the amplitude of the plateau phase of the contraction induced by high-KCl solution, taken as 100%

(Zizzo et al. 2011; Di Liberto et al. 2016). Moreover, guanosine has been shown to affect gastrointestinal and vessel smooth muscle contractility [5,7,8].

In rat myometrium, guanosine is able to inhibit spontaneous uterine contractions, while guanine was without any effect, implying that, although guanosine can be converted by ecto-purine nucleotide phosphorylase into guanine in the extracellular media (Rathbone et al. 2008), in our preparation, guanosine itself is responsible for the observed effects.

Some studies pointed to the existence of a putative selective guanosine receptor in rat brain membranes belonging to the G-protein-coupled receptor family (Traversa et al. 2003; Volpini et al. 2011). In our experiments, the responses did not undergo desensitization; therefore, it seems unlikely that guanosine would act through the activation of membrane receptors linked to the G protein. Moreover, the inhibition of purine transport with NBTI prevented guanosine effects, indicating that guanosine must be transported into cells to induce a response as already observed in retinal ganglion cells as well as in the gut (Benowitz et al. 1998; Zizzo et al. 2011, 2013).

It is reported that the adenine-based purinergic system can modulate motor function in the uterus through specific purinergic (P1 and P2) receptor subtypes, exerting excitatory or inhibitory effects. In particular, in rat uterus, inhibitory effects induced by adenosine viaP1 purinoceptor activation have been documented (Gillman and Pennefather 1998). In the central nervous system, it has been suggested an interplay between guanosine and adenosine, being ultimately the purinergic P1 receptor signaling the responsible for guanosine-induced effects. However, we can discard such a

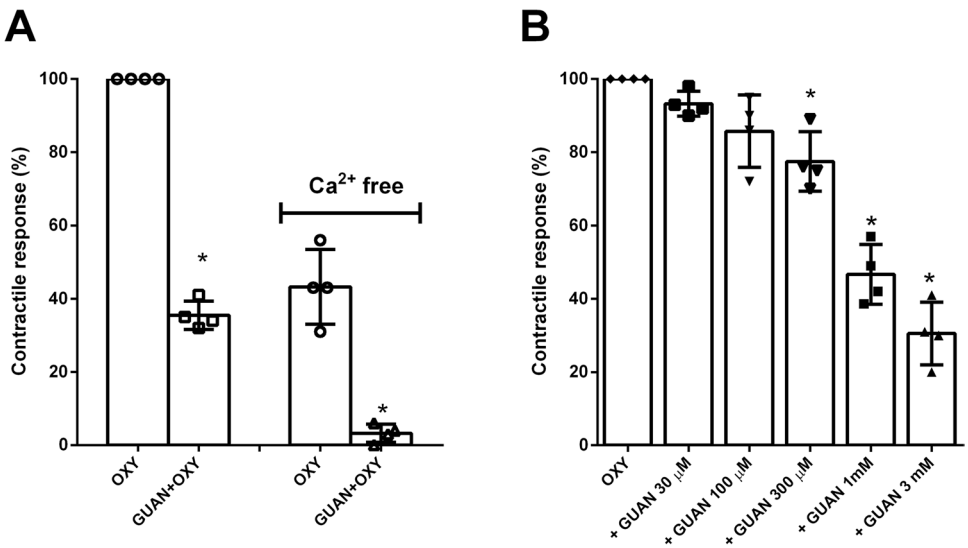

Fig. 5 Guanosine effects on uterine contraction induced by oxytocin. **A** Histogram showing the effects of guanosine (0.1 mM) pretreatment on the contractile response 5 nM oxytocin (OXY) in normal condition or in Ca^{2+} free solution. **B** Histogram showing the effects of guanosine (10 µM–3 mM) on contractile response to 5 nM oxytocin. Data are mean ± SD ($n = 4$ each) and expressed as the percentage of the amplitude of the plateau phase of the contraction induced by oxytocin, taken as 100%

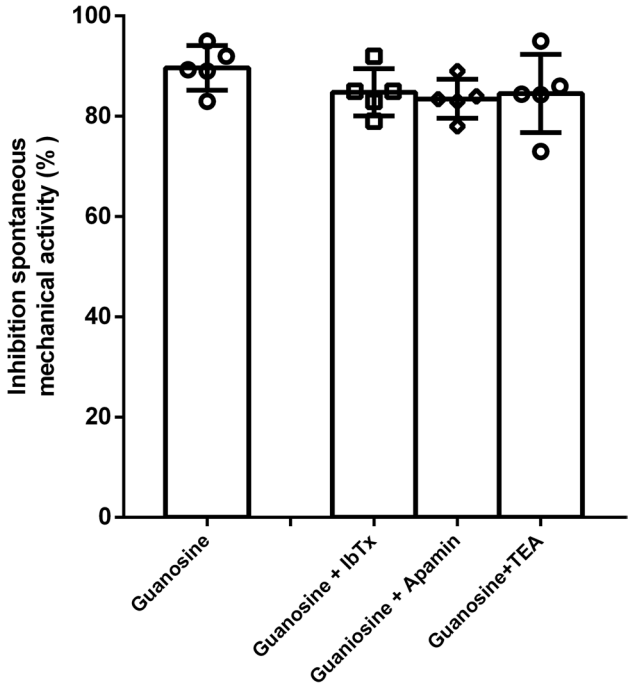

Fig. 6 Guanosine effects and K^+ channel blockers. Histogram showing the inhibitory effects induced by guanosine (1 mM) on the spontaneous contraction before and after tetraethylammonium (TEA, 20 mM), non-selective K^+ channel blocker; apamin (100 nM), blocker of small conductance Ca^{2+}-activated K^+ channels; and iberiotoxin (IbTx,100 nM), blocker of large and intermediate conductance Ca^{2+}-activated K^+ channels ($n=5$ for each). Data are mean ± SD and expressed as the percentage of the inhibition of spontaneous activity, with 100% inhibition corresponding to total suppression of spontaneous contractions

possibility in rat myometrium since the guanosine inhibitory responses were unaffected by pretreatment with a nonselective adenosine receptor antagonist. Moreover, a contribution of β-receptor activation in the guanosine effects in our preparations can be excluded.

Among the factors that modulate myometrium, contractile activity can comprise NO, which is a potent relaxant agent, likely playing a role in the maintenance of uterine quiescence (Chaud et al. 1997). Both cGMP-dependent or -independent pathways account for smooth muscle relaxation. The observation that the effects induced by guanosine remained unaltered in the presence of a NO synthase blocker or of the soluble guanylyl cyclase inhibitor indicates that synthesis of NO and cGMP pathway are not downstream events to guanosine to inhibit uterine contractions.

Spontaneously active uterine contractions are dependent upon the increase in intracellular calcium concentration due to extracellular calcium influx, mainly via voltage-operated channels or viarelease from its intracellular stores. Guanosine is able to inhibit calcium-dependent contractile responses, interfering with extracellular calcium ion influx. The exposure of the uterine strips to high K^+ solution increases intracellular Ca^{2+} concentration by depolarizing membrane potential, resulting in the opening of L-type Ca^{2+} channels, and hence contraction (Wray and Shmygol 2007). The application of guanosine had no effect on KCl-induce tonic uterine contractions suggesting that this purine does not interact with L-type voltage-gated calcium channel.

Oxytocin is the main actor in the onset of preterm labor among the physiological pathways. This hormone acts on uterine smooth muscle promoting contraction via G-protein-coupled receptors, activating phospholipase C resulting in increased production of inositol trisphosphate (IP_3) that, binding sarcoplasmic reticulum membrane receptors, stimulates Ca^{2+} release from the intracellular stores into the cytosol. Elevated cytosolic Ca^{2+} further encouraged extracellular Ca^{2+} influx hence a further rise in the intracellular Ca^{2+} (Trujillo et al. 2000). Our study demonstrated that guanosine significantly reduced oxytocin-induced uterine contraction both in the presence of external calcium and in the absence of external Ca^{2+}, being only the intracellular Ca^{2+} source available. Since myometrium does not have functional ryanodine receptors (Dabertrand et al. 2006), the effect must be on the IP_3 pathway. Thus, it is possible to speculate that guanosine might suppress the uterine contractile response by modulating the calcium release from intracellular stores.

In hippocampal slices, it was shown that guanosine effects may depend on the direct or indirect activation of K^+ channels, mainly vialarge conductance Ca^{2+}-activated

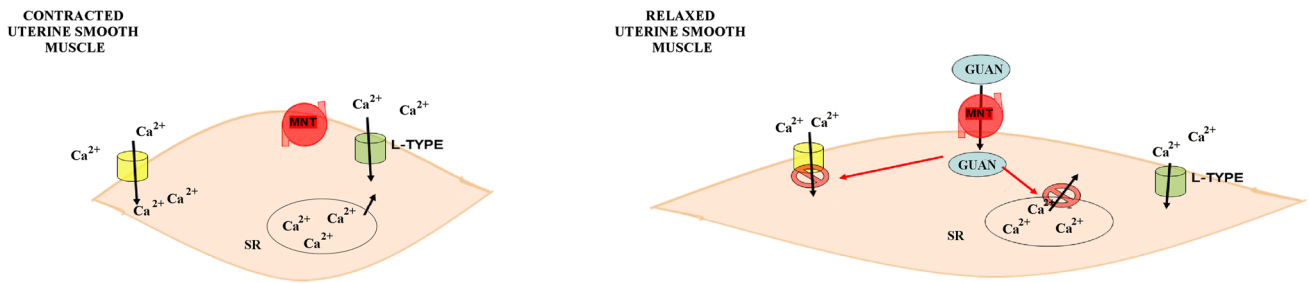

Fig. 7 Proposed mechanism of action of guanosine on myometrial contractions. Abbreviations: guanosine (GUAN), membrane nucleoside transporter (MNT), L-TYPE voltage-dependent Ca^{2+} channels (L-TYPE), sarcoplasmic reticulum (SR)

potassium channels (Oleskovicz et al. 2008; Dal-Cim et al. 2013). These K^+ channels seem to be not involved in guanosine-induced inhibitory effects on rat myometrium as suggested by the observation that iberiotoxin, a blocker of large and intermediate conductance Ca^{2+}-activated potassium channels, unaffected guanosine response. Moreover, neither apamin, a blocker of small conductance Ca^{2+}-activated potassium channels, nor TEA, which at the used concentration acts as aspecific blocker of potassium channels, were able to modify guanosine response, indicating that likely, an increase of potassium conductance is not a mechanism to guanosine to inhibit rat myometrium mechanical activity. Further studies are needed to identify the intracellular mechanism/s activated by guanosine.

In conclusion, our result indicated that guanosine is able to reduce both spontaneous and oxytocin-induced contractions of rat myometrium subsequently to its intracellular intake. The blockade of extracellular Ca^{2+} influx and reduction of Ca^{2+} release from the intracellular store are the mechanisms involved in the guanosine-induced tocolytic effects.

Although the precise mechanism of guanine-based purine should be clarified in the future research, results of the present study suggest that guanosine has the potential to be investigated as a promising drug effective in the treatment of uterine spasmodic disorders. Moreover, even if our study has been conducted in non-pregnant rat myometrium, the potent inhibitory effects on oxytocin-induced contraction might also suggest a potential effect of guanosine on contractions in the early and/or at-term pregnant uterus.

Author contribution MGZ designed and performed the study, analyzed and interpreted the data, prepared figures, and drafted and edited the manuscript. AC performed the experiments and analyzed the data. RS participated to supervision, experimental design, interpreted the data, and edited and revised the manuscript. All the authors contributed to the critical revision and approved the final version of the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval All animal care was approved by the Animal Care and Use Ethics Committee of the University of Palermo and performed in accordance with national.

Consent to participate Not applicable.

Consent for publication The authors authorize the submission and publication of this article *in Naunyn–Schmiedeberg's Archives of Pharmacology.*

Conflict of interest The authors declare no competing interests.

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