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RESEARCH PAPER

Mechanisms involved in the antinociception induced by systemic administration of guanosine in mice

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Background and purpose: It is well known that adenine-based purines exert multiple effects on pain transmission. However, less attention has been given to the potential effects of guanine-based purines on pain transmission. The aim of this study was to investigate the effects of intraperitoneal (i.p.) and oral (p.o.) administration of guanosine on mice pain models. Additionally, investigation into the mechanisms of action of guanosine, its potential toxicity and cerebrospinal fluid (CSF) purine levels were also assessed.

Experimental approach: Mice received an i.p. or p.o. administration of vehicle (0.1 mM NaOH) or guanosine (up to 240 mg·kg^{-1}) and were evaluated in several pain models.

Key results: Guanosine produced dose-dependent antinociceptive effects in the hot-plate, glutamate, capsaicin, formalin and acetic acid models, but it was ineffective in the tail-flick test. Additionally, guanosine produced a significant inhibition of biting behaviour induced by i.t. injection of glutamate, AMPA, kainate and trans-ACPD, but not against NMDA, substance P or capsaicin. The antinociceptive effects of guanosine were prevented by selective and non-selective adenosine receptor antagonists. Systemic administration of guanosine (120 mg·kg^{-1}) induced an approximately sevenfold increase on CSF guanosine levels. Guanosine prevented the increase on spinal cord glutamate uptake induced by intraplantar capsaicin.

Conclusions and implications: This study provides new evidence on the mechanism of action of the antinociceptive effects after systemic administration of guanosine. These effects seem to be related to the modulation of adenosine A_1 and A_{2A} receptors and non-NMDA glutamate receptors.

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Abbreviations: A_1, adenosine receptor type 1; A_{2A}, adenosine receptor type 2A; ABPs, adenine-based purines; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AOPCP, α,β-methyleneadenosine 5′-diphosphate; DMSO, dimethyl sulfoxide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; GBPs, guanine-based purines; HBSS, Hanks balanced salt solution; HPLC, high-performance liquid chromatography; L-NOARG, *N*-nitro-L-arginine; MPE, maximum possible effect; NMDA, N-methyl-D-aspartate; NO, nitric oxide; SCH58261, 5-amino-2-(2-furyl)-7-phenylethyl-pyra-zolo-[4,3-e]-1,2,4-triazolo[1,5c]pyrimidine; TFL, tail-flick latency; trans-ACPD, (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid

Introduction

The purinergic system involves adenosine and ATP as major endogenous effectors, acting on P1 and P2 receptors respectively (Burnstock, 2007). It is well known that extracellular ATP and adenosine have an important role in pain signalling both in the periphery and the central nervous system (CNS) (Sawynok, 1998; Sawynok and Liu, 2003; Inoue *et al.*, 2005). ATP can stimulate sensory nerve endings causing pain and, by acting via $P2X_3$ and $P2X_{2/3}$ receptors, is associated with the initiation of acute, inflammatory, neuropathic and visceral pain (Burnstock, 2007). Adenosine and its analogues induce antinociceptive effects in several pain paradigms, and alleviate manifestations of neuropathic pain in nerve injury models in rodents (Sawynok, 1998; McGaraughty and Jarvis, 2005). Adenosine regulates pain transmission in the spinal cord and

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periphery, and a number of agents can alter the extracellular availability of adenosine subsequently modulating pain transmission, particularly by activating the widely distributed central adenosine receptors (Sawynok and Liu, 2003).

Although ATP and adenosine are usually considered the main effectors of the purinergic system (Burnstock, 2007), extracellular guanine-based purines (GBPs) exert biological effects unrelated to direct G-proteins modulation, including the modulation of glutamate activity (Souza and Ramirez, 1991; Schmidt *et al.*, 2007), trophic effects on neural cells (Ciccarelli *et al.*, 2001), and behavioural effects (Schmidt *et al.*, 2000; Lara *et al.*, 2001; Schmidt *et al.*, 2005). Concerning *in vitro* effects on the glutamatergic system, GBPs inhibit the binding of glutamate and analogues (Baron *et al.*, 1989; Burgos *et al.*, 1998), prevent cell responses to excitatory amino acids (Souza and Ramirez, 1991), present neuroprotective effects in cultured neurons submitted to hypoxia and increase glutamate uptake in cultured astrocytes (Frizzo *et al.*, 2001; 2002; 2003). *In vivo*, GBPs prevent glutamate-induced seizures and neurotoxicity (Malcon *et al.*, 1997; Regner *et al.*, 1998; Schmidt *et al.*, 2000; 2005; 2008; 2009b; Lara *et al.*, 2001; Saute *et al.*, 2006), and are anxiolytic/amnesic in rodents (Roesler *et al.*, 2000; Vinadé *et al.*, 2003; 2004; 2005). Several of these effects seem to be related to conversion to guanosine (Soares *et al.*, 2004; Schmidt *et al.*, 2005; 2008). Recently, we showed that intracerebroventricular (i.c.v.) administration of guanosine or GMP is anti-nociceptive against several chemical and thermal pain models in mice (Schmidt *et al.*, 2008). Additionally, we have shown that i.t. administration of guanosine produces significant inhibition of glutamate-, non-NMDA glutamate receptor agonist- and substance P-induced biting behaviour (Schmidt *et al.*, 2009b). Importantly, most of these effects seem to be related, at least partially, to a guanosine-induced modulation of the glutamatergic pathways.

The present study was designed to investigate the antinociceptive effects of intraperitoneal (i.p.) or oral (p.o.) administration of guanosine in mice. Attempts have been made to further investigate some of the possible mechanisms that underlie the antinociceptive property of guanosine, especially the purinergic and glutamatergic mechanisms. We also assessed the acute toxicity induced by systemic administration of guanosine.

Methods

Animals

All animal care and experimental procedures were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. We followed the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983), as well as our institutional guidelines for experiments with animals, designed to avoid suffering and limit the number of animals. The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects of drug treatments.

Male adult Swiss albino mice (3–4 months of age, 30–50 g) were kept on a 12-h light/dark cycle (light on at 7:00) at 22 ± 1°C, housed in plastic cages (five per cage), with tap water and commercial food *ad libitum*. In all nociceptive behavioural experiments, the animals were acclimatized to the laboratory for at least 1 h before testing.

Drug administration

Experiments were performed according to Schmidt *et al.* (2000): 20 min before the experiment, animals were placed individually in acrylic boxes, which served as observation chambers. After this adaptation period, treatments were given as follows: i.p. or p.o. administration (10 mL·kg^{-1}) of vehicle (0.1 mN NaOH) or guanosine (up to 240 mg·kg^{-1}) and submitted to tests of nociception, 30 or 45 min thereafter respectively. Morphine (6 mg·kg^{-1}) and/or dexamethasone (30 mg·kg^{-1}) were used as positive controls. The doses and time of drug administration were selected on the basis of published data, as well as our previous results (Schmidt *et al.*, 2000; 2009a; Lara *et al.*, 2001; Vinadé *et al.*, 2003; 2004; 2005). In order to investigate the role of adenosine receptors in the mechanism of action of guanosine, part of the animals were also pretreated 15 min prior to the treatments with an i.p. injection of the non-selective (A_1 and A_{2A}) adenosine receptor antagonist caffeine (10 mg·kg^{-1}), the selective A_1 adenosine receptor antagonist DPCPX (1 mg·kg^{-1}) or the selective A_{2A} adenosine receptor antagonist SCH58261 (0.5 mg·kg^{-1}). Adenosine (100 mg·kg^{-1}) was used as a positive control. Caffeine, adenosine, DPCPX and SCH58261 doses were adapted from elsewhere (Lara *et al.*, 2001; Dall'Igna *et al.*, 2007; Schmidt *et al.*, 2009a). In order to minimize the number of animals, mechanistic studies of guanosine effects and toxicity tests were only performed with i.p. treatment.

Capsaicin-induced nociception

The method used for capsaicin-induced licking was similar to that described by Sakurada *et al.* (1993). Thirty or 45 min after i.p. or p.o. vehicle, morphine or guanosine 7.5 to 240 mg·kg^{-1} treatments, respectively, 20 µL of capsaicin (1.6 µg per paw) were injected under the plantar surface of the right hindpaw (i.p.) using a Hamilton microsyringe with a 26 gauge needle. Animals were observed individually for 5 min after capsaicin administration for the time spent licking the injected paw, considered as indicative of nociception. Considering that the capsaicin test involves peripheral and central mechanisms of nociception, pain induced by capsaicin may be prevented by glutamate receptor antagonists (Sakurada *et al.*, 1998) and capsaicin induces glutamate and aspartate release from dorsal spinal cord (Jeftinija *et al.*, 1991; Sorkin and McAdoo, 1993; Ueda *et al.*, 1993), the capsaicin pain model was chosen for a wide dose-response curve of guanosine as well as for mechanistic studies.

Tail-flick test

Nociception was assessed with a tail-flick apparatus (Albrasch Electronic Equipments, Brazil), as described in detail elsewhere (D'Amour and Smith, 1941). A source of light was positioned above the tail and the time that the mouse took to withdraw its tail from the noxious stimulus was recorded. The

trial was automatically terminated when the mouse tail deflected activating a photocell that turns off the light. A cut-off time of 10 s was employed in order to prevent tissue damage. At day one, the animals were habituated to the tail-flick apparatus with three separate measurements (data not shown). At day two, baseline tail-flick latency (TFL) was measured for each mouse prior to treatments; animals displaying at least two TFL of 10 s under the basal conditions were excluded from the study. Immediately after the third TFL measurement, animals received i.p. or p.o. treatments (vehicle, morphine or guanosine 30, 60 or 120 mg·kg^{-1}) and, after 30 or 45 min respectively, were submitted to the tail-flick.

Hot-plate test

Response latencies were measured according to the method described by Eddy and Leimback (1953), with minor modification. The hot-plate apparatus (Ugo Basile, model-DS 37, Italy) was maintained at 55 ± 0.5°C. Animals were placed on the heated surface surrounded by a glass cylinder of 24 cm diameter, and the time between placement and the occurrence of licking hindpaws or jumping was recorded as response latency. At day one, the animals were habituated with the apparatus. At day two, mice were tested and animals displaying baseline latencies of more than 15 s were excluded from the study. An automatic 20 s cut-off was used to prevent tissue damage. Each animal was tested before administration of drugs in order to obtain the baseline. Thirty or 45 min after i.p. or p.o. vehicle, morphine or guanosine 30, 60 or 120 mg·kg^{-1} treatments, respectively, animals were placed on the heated surface and response latency recorded as described above.

Formalin-induced nociception

The formalin test was carried out as described by Hunskaar and Hole (1987). Animals received 20 µL of a 2.5% formalin solution (0.92% of formaldehyde), injected i.pl. under the plantar surface of the right hindpaw. Animals were pretreated with i.p. or p.o. administration of vehicle, morphine, dexamethasone or guanosine 30, 60 or 120 mg·kg^{-1}, 30 or 45 min before formalin injection respectively. After i.pl. injection of formalin, the animals were observed from 0–5 min (neurogenic phase) and 15–30 min (inflammatory phase) and the time spent licking the injected paw was timed with a chronometer and considered as indicative of nociception.

Acetic acid-induced abdominal constriction

The abdominal constriction was induced according to Corrêa *et al.* (1996) and resulted in contraction of the abdominal muscle together with a stretching of the hind limbs in response to an i.p. injection of acetic acid (1.6%). Mice were pretreated with i.p. or p.o. vehicle, morphine or guanosine 30, 60 or 120 mg·kg^{-1}, 30 or 45 min before the irritant injection. After the challenge, the abdominal constrictions were counted cumulatively over a period of 20 min. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions, that is, the difference between control animals (mice pretreated with vehicle) and animals pretreated with guanosine.

Glutamate-induced nociception and paw oedema

The procedure used was similar to Beirith *et al.* (2002). Thirty or 45 min after i.p. or p.o. treatments respectively (vehicle, morphine or guanosine 30, 60 or 120 mg·kg^{-1}), 20 µL of glutamate solution (10 µmol per paw prepared in saline) was injected i.pl. under the plantar surface of the right hindpaw. The mice were observed individually for 15 min following glutamate injection, and the amount of time spent in licking the injected paw was considered as indicative of nociception. In order to verify whether the antinociceptive activity produced by i.p. guanosine in glutamate-induced nociception was associated with the development of oedema formation, we measured the paw oedema by comparing the difference between the weight of the glutamate-treated paw and the weight of the nontreated contralateral paw. For this purpose, animals were killed 15 min after glutamate injection by cervical dislocation, and both paws were cut at the ankle joint and weighed on an analytical balance. To address some mechanisms involved in local effect caused by guanosine on glutamate-induced nociception and paw oedema, separate groups of animals were treated with i.pl. guanosine (200 nmol) or vehicle, both locally co-administered with glutamate (10 µmol per paw).

Analysis of the mechanisms involved in guanosine action on the glutamate test

To explore the possible involvement of the nitric oxide-L-arginine-cGMP pathway in the antinociceptive action caused by guanosine, mice were pretreated with L-arginine (600 mg·kg^{-1}, i.p., a nitric oxide precursor) or D-arginine (600 mg·kg^{-1}, i.p., an inactive isomer of L-arginine), and 20 min later received guanosine (60 mg·kg^{-1}, i.p.), *N*-nitro-L-arginine (L-NOARG; 75 mg·kg^{-1}, i.p., an inhibitor of nitric oxide synthesis) or vehicle (0.1 mN NaOH, i.p.). The nociceptive responses to i.pl. glutamate were recorded 30 min after guanosine, L-NOARG or vehicle. A separate group of animals were pretreated with L-NOARG (30 mg·kg^{-1}, i.p.), methylene blue (a non-specific inhibitor of NO/guanylyl cyclase; 1 mg·kg^{-1}, i.p.) or vehicle (saline, i.p.) and after 15 min received guanosine (60 mg·kg^{-1}, i.p.) or vehicle (0.1 mN NaOH, i.p.), 30 min before i.pl. glutamate injection (Duarte and Ferreira, 2000; Abacioglu *et al.*, 2001).

Spinal algogen-induced nociception

To test the hypothesis that spinal excitatory amino acids, substance P or capsaicin might be involved in the antinociceptive effects induced by guanosine, we assessed the effect of guanosine (30, 60 or 120 mg·kg^{-1} or vehicle, i.p.) given 30 min prior to the biting response induced by an i.t. injection of 5 µL of these algogens. The nociceptive response was elicited by glutamate (175 nmol per site, i.t.), NMDA (a selective agonist of NMDA-subtype of glutamatergic ionotropic receptors, 450 pmol per site, i.t.) (Urca and Raigorodsky,

1988), AMPA (a selective agonist of AMPA-subtype of glutamatergic ionotropic receptors, 135 pmol per site, i.t.) (Brambilla *et al*., 1996), kainate (a selective agonist of kainate-subtype of glutamatergic ionotropic receptors, 110 pmol per site, i.t.), trans-ACPD (a metabotropic glutamate agonist, 50 nmol per site, i.t.) (Boxall *et al*., 1998), substance P (NK_1 receptor-selective agonist, 135 ng per site) (Sakurada *et al*., 1990) or capsaicin ($TRPV_1$ receptor agonist, 30 ng per site) (Sakurada *et al*., 1996). A group of mice received only vehicle (saline) by i.t. route, and no significant biting behaviour was observed (data not shown). Immediately after the i.t injection of each agonist, mice were placed individually in observation chambers, and the amount of time (s) the animal spent biting itself was noted: glutamate (3 min); AMPA (1 min); kainate (4 min); NMDA (5 min); substance P and capsaicin (6 min); and trans-ACPD (15 min). A bite was defined as a single head movement directed at the flanks or hind limbs, resulting in contact of the animal's snout with the target organ.

Hole-board test

The hole-board apparatus (Ugo Basile, Italy) consisted of a gray Perspex panels (40 × 40 cm, 2.2 cm thick) with 16 equidistant holes 3 cm in diameter on its floor. Photocells below the surface of the holes automatically recorded the number of head-dips. The board was positioned 15 cm above the table, and divided into nine squares of 10 × 10 cm with a water-resistant marker. Thirty min after i.p. treatments (vehicle or guanosine 30, 60 or 120 $mg{\cdot}kg^{-1}$), each animal was placed singly in the centre of the board facing away from the observer and the behaviour recorded for 5 min. The numbers of head-dips, crossings (number of squares crossed with all four paws), rearings, groomings, and defecations were recorded, as well as the latency to start locomotion (Vinadé *et al*., 2003).

Motor status

In order to evaluate non-specific muscle relaxant or neurotoxic effects, we evaluated the effects of guanosine (30, 60 or 120 $mg{\cdot}kg^{-1}$ or vehicle, i.p.) in the rotarod test and on spontaneous locomotion. The rotarod apparatus (Ugo Basile, Italy) consisted of a rotating (18 rpm) bar (2.5 cm diameter), subdivided by disks into six compartments. As previously described (Vinadé *et al*., 2003), mice were initially trained to remain on the rotarod apparatus for 120 s. Those not remaining on the bar for at least two out of three consecutive trials were discarded. On the day after training, the latency to fall from the rotarod (one trial with a maximum of 60 s) was determined 30 min after i.p. treatments. The method for assaying spontaneous locomotion was adapted from Creese *et al*. (1976). Activity cages (45 × 25 × 20 cm, Albarsch Electronic Equipment, Brazil), equipped with three parallel photocells, automatically recorded the number of crossings. Animals were individually habituated to an activity cage for 10 min before treatments; animals returned to the activity cages 30 min after i.p. treatments, and crossings were recorded for 15 min.

Sleeping time

In order to investigate potential sedative properties of guanosine, mice were pretreated with guanosine (30, 60 or 120 $mg{\cdot}kg^{-1}$) or vehicle 30 min before an i.p. injection of sodium pentobarbital (30 $mg{\cdot}kg^{-1}$). The sleeping time (time elapsed between loss and recuperation of righting reflex) was recorded. Criterion for recuperation of righting reflex was that animals had to regain normal upright posture when challenged for three consecutive times to remain on their backs (Costa-Campos *et al*., 1998).

Body temperature

Temperature was measured by using a flexible probe of a digital thermometer inserted 2 cm into the rectum, before and 30 min after i.p. injection of guanosine (30, 60 or 120 $mg{\cdot}kg^{-1}$) or vehicle.

General toxicity and lethal dose

To investigate the potential toxicity of guanosine, mice received a single i.p. administration of guanosine (7.5 to 960 $mg{\cdot}kg^{-1}$) or vehicle and were observed thereafter up to 72 h. The body weight gain of animals was recorded every 24 h, as an indication of general toxicity. After 72 h, mice were slightly anesthetized for blood collection by heart puncture, the serum obtained by centrifugation at 5000 g for 10 min (haemolysed serum was discarded) and used for biochemical assays [commercial kits, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as markers for early acute hepatic damage; serum urea and creatinine levels, as index of renal function].

Cerebrospinal fluid (CSF) sampling

Another group of mice was similarly treated with i.p. or p.o. administration of vehicle or guanosine (60 $mg{\cdot}kg^{-1}$). After 30 or 45 min for i.p. or p.o. treatments, respectively, mice were anesthetized with sodium thiopental (60 $mg{\cdot}kg^{-1}$, 10 $mL{\cdot}kg^{-1}$, i.p.) and placed in a stereotaxic apparatus; CSF samples were drawn (10–20 μL per mouse) by direct puncture of the *cisterna magna* with an insulin syringe (27 gauge × 1/2 in length), under a magnifying glass. The samples were centrifuged at 10 000 g in an Eppendorf centrifuge for 5 min to obtain cell-free supernatants and stored at –70°C.

HPLC procedure

High-performance liquid chromatography (HPLC) was performed with aliquots of CSF cell-free supernatants in order to measure the purine concentration according to Domanski *et al*. (2006). The following purines were assessed: adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenosine, guanosine triphosphate (GTP), guanosine diphosphate (GDP), guanosine monophosphate (GMP), guanosine, inosine monophosphate (IMP), inosine, hypoxanthine, xanthine and uric acid. Analyses were performed with a Shimadzu Class-VP chromatography system, consisting of a quaternary gradient pump with vacuum degassing and piston desalting modules, a Shimadzu SIL-10AF auto injector valve with 50 μL loop, and an UV detector. Separations were achieved on a Supelco C18 250 mm × 4.6 mm, 5 μm particle size column. The mobile

phase flowed at a rate of 1.2 mL/min and the column temperature was 24°C. Buffer composition remained unchanged (A: 150 mmol/L phosphate buffer, pH 6.0, containing 150 mmol/L potassium chloride; B: 15% acetonitrile in buffer A). The gradient profile was modified to the following content of buffer B in the mobile phase: 0% at 0.00 min, 2% at 0.05 min, 7% at 2.45 min, 50% at 10.00 min, 100% at 11.00 min, and 0% at 12.40 min. Samples of 10 µL were injected into the injection valve loop. Absorbance was read at 254 nm. CSF concentrations of purines are expressed as mean ± SEM in µM.

Glutamate uptake

Mice were treated with guanosine (60 mg·kg^{-1}, i.p.) or vehicle (0.1 mM NaOH); after 30 min, animals received an i.pl. injection of capsaicin or vehicle (DMSO 5%). Five min thereafter, animals were decapitated, their brains and spinal cords were immediately removed and submerged in a ice-cold Hank's balanced salt solution (HBSS), containing (in mM): 137 NaCl, 0.63 Na_2HPO_4, 4.17 $NaHCO_3$, 5.36 KCl, 0.44 KH_2PO_4, 1.26 $CaCl_2$, 0.41 $MgSO_4$, 0.49 $MgCl_2$ and 5.55 glucose, adjusted to pH 7.2. Cortices and spinal cords were dissected into a Petri dish filled with ice-cold HBSS. Coronal cortical slices and transversal spinal cord slices (0.4 mm) were obtained using a McIlwain tissue chopper, and sections were separated with the help of a magnifying glass. Slices were transferred to 24-multiwell dishes, containing 500 µL of HBSS solution and pre-incubated for 15 min (cortex) or 120 min (spinal cord) at 35°C. Subsequently, slices were washed with 1 mL HBSS, and the total glutamate uptake was assessed by addition of 0.33 µCi·mL^{-1} L-(^{3}H)glutamate with 100 µM unlabeled glutamate in HBSS solution at 35°C. Incubation was stopped after 7 min by two ice-cold washes with 1 mL HBSS immediately followed by the addition of 0.5 N NaOH, which was kept overnight. Lysates were taken for determination of intracellular content of L-(^{3}H)glutamate through scintillation counting. To determine the sodium-independent glutamate uptake, parallel assays were performed under ice using *N*-methyl-D-glucamine instead of sodium chloride in the incubation medium. Sodium-dependent glutamate uptake was obtained by subtracting the sodium-independent uptake from the total in order to obtain the specific uptake. Protein was measured using the method of Peterson *et al*. (1977) using bovine albumin as standard. The experiments were performed in triplicate.

Statistical analysis

Data are expressed as mean ± standard error (SEM), except the ID_{50} values (i.e. the dose of guanosine necessary to reduce the nociceptive response by 50% relative to the control value) and LD_{50} (i.e. the dose of guanosine necessary to induce mortality in 50% of mice), which are reported as geometric means accompanied by their respective 95% confidence limits. The ID_{50} and LD_{50} values were determined by linear regression from individual experiments, using linear regression GraphPad software (GraphPad software, San Diego, CA, USA). For tail-flick and hot-plate experiments, data are expressed as mean percent of maximum possible effect (% MPE) ± SEM, according to the following formula (Calcagnetti *et al*., 1990): % MPE: 100 × (postdrug latency – baseline latency)/(cutoff time – baseline latency). Data were submitted to Kolmogorov-Smirnov test for normality evaluation. Differences were assessed by one-way analysis of variance (ANOVA) plus the *post-hoc* Student–Newman–Keuls test when necessary. All results with $P < 0.05$ were considered statistically significant.

Materials

Guanosine, adenosine, caffeine, L-glutamic acid hydrochloride (glutamate), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainic acid (kainate), N-methyl-D-aspartate (NMDA), Substance P, and (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD), capsaicin and dexamethasone were purchased from Sigma Chemicals (St. Louis, MO, USA). 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) was purchased from Tocris (Northpoint, UK). SCH58261 (5-amino-2-(2-furyl)-7-phenylethyl-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5c] pyrimidine) was provided by S. Weiss (Vernalis, UK). Sodium thiopental and morphine sulphate were acquired from Cristália (SP, Brazil). Guanosine was dissolved in 0.1 mN NaOH and buffered to pH 7.4. The amount of NaOH caused no detectable effect. Capsaicin was diluted in 5% dimethyl sulfoxide (DMSO). DPCPX and SCH58261 were diluted in 10% DMSO. All other solutions were dissolved in saline (NaCl 0.9%) and buffered with 0.1 N NaOH or 0.1 N HCl to pH 7.4 when necessary. Receptor nomenclature used in this manuscript follows Alexander *et al*. (2008).

Results

Effects of guanosine in pain models

The results at Figure 1 show that a single systemic (i.p. or p.o.) administration of guanosine (7.5 to 240 mg·kg^{-1}) produces antinociception against i.pl. capsaicin-induced pain. Figures 2 and 3 show that i.p. or p.o. administration of guanosine (30 to 120 mg·kg^{-1}) is antinociceptive in the hot-plate- (Figures 2B and 3B), i.pl. glutamate- (Figures 2C and 3C), i.p. acetic acid- (Figures 2D and 3D) and i.pl. formalin (Figures 2E/F and 3E/F)-induced pain models, but not in the tail-flick test (Figures 2A and 3A). Neither i.p. nor p.o. administration of 0.1 mN NaOH (vehicle) affected nociception as compared with control (sham) animals (data not shown). Mean ID_{50} values and maximal inhibitions for i.p. guanosine against capsaicin, glutamate, acetic acid and formalin (first and second phases) tests are presented in Table 1.

Effects of guanosine against spinal algogen-induced nociception

Figure 4 shows that i.p. administration of guanosine significantly inhibited the nociceptive response induced by i.t. injection of glutamate (4A), AMPA (4B), kainate (4C), and trans-ACPD (4D), when compared with the control group (0.1 mN NaOH). Mean ID_{50} values and maximal inhibitions for the antinociceptive effect of guanosine against spinal glutamate, AMPA, kainate, and trans-ACPD are described in Table 2. In contrast, systemic administration of guanosine

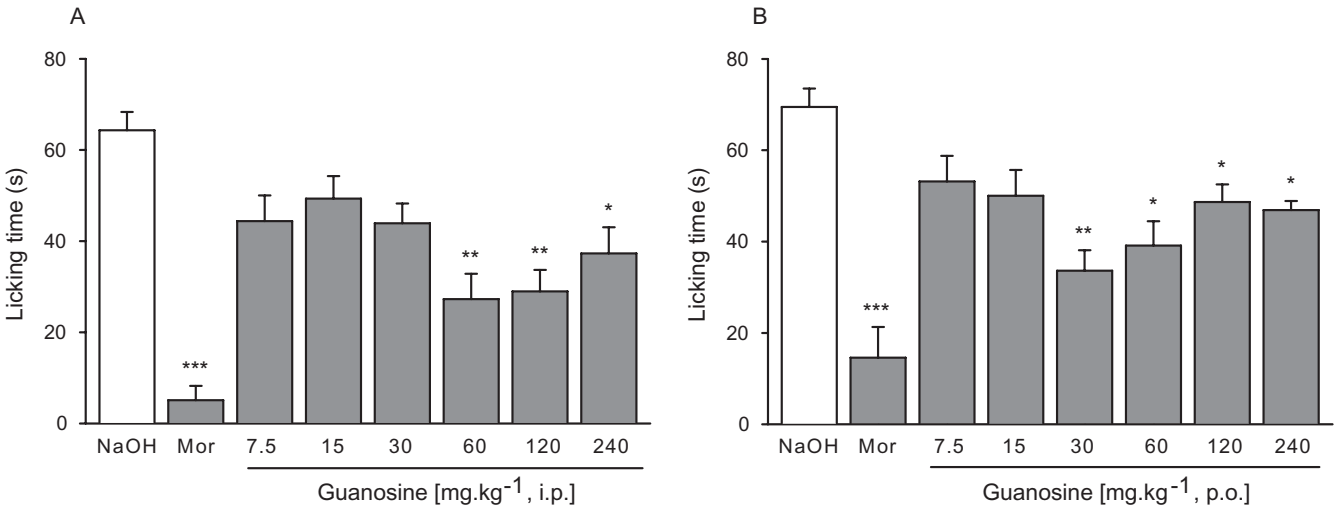

Figure 1 Effects of i.p. (A) or p.o. (B) administration of vehicle (0.1 mN NaOH), morphine (Mor – 6 mg·kg^{-1}) or guanosine (7.5 to 240 mg·kg^{-1}) in the i.pl. capsaicin test in mice. Columns represent mean time spent in licking the injected hindpaw, and vertical bars represent SEM. n = 8–10. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with vehicle, one-way ANOVA/Student–Newman–Keuls.

produced no significant effect against NMDA (4E), substance P (4F) and capsaicin (4G) mediated biting response in mice.

Role of adenosine receptors in guanosine-induced antinociception

As shown in Figure 5A, i.p. adenosine (100 mg·kg^{-1}), as well as guanosine (60 mg·kg^{-1}), produced antinociceptive effects against capsaicin-induced pain, an effect prevented by pretreatment with the non-selective adenosine receptor antagonist caffeine (10 mg·kg^{-1}). Figure 5B shows that the selective A_1 adenosine receptor antagonist DPCPX (0.1 mg·kg^{-1}), but not the selective A_{2A} adenosine receptor antagonist SCH58261 (0.5 mg·kg^{-1}), prevented antinociception induced by adenosine in the i.pl. capsaicin pain test. Both selective adenosine receptor antagonists prevented guanosine-induced antinociception (Figure 5B). Notably, adenosine receptor antagonists had no antinociceptive effect *per se*.

Mechanisms involved in guanosine effects on the glutamate test

The results presented in Figure 6A show that pretreatment of mice with the nitric oxide precursor L-arginine (600 mg·kg^{-1}, i.p.) completely reversed the antinociception caused by L-NOARG (75 mg·kg^{-1}, i.p.), but not by guanosine (60 mg·kg^{-1}, i.p.). D-arginine did not affect antinociception produced by either L-NOARG or guanosine (data not shown). Figure 6B shows that methylene blue (1 mg·kg^{-1}, i.p.), a non-specific inhibitor of NO/guanylyl cyclase, did not inhibit guanosine-induced antinociception. Methylene blue by itself did not modify glutamate-induced nociceptive behaviour.

Effects of guanosine on glutamate-induced paw oedema

The results presented at Figure 7 show that i.p. administration of guanosine (60 and 120 mg·kg^{-1}) caused a significant inhibition of the paw oedema induced by i.pl. injection of glutamate (Figure 7A). Mean ID_{50} value (95% confidence limits) for i.p. guanosine against glutamate-induced paw oedema was 73 (49–107) mg·kg^{-1}, and maximal inhibition of 50 ± 10%. However, when co-injected intraplantarly in association with glutamate, guanosine (100, 200 or 400 nmol) did not affect licking behaviour (data not shown) or paw oedema (Figure 7B) induced by glutamate.

Effects of guanosine on CSF purine levels

As shown in Figure 8, systemic administration of guanosine (30, 60 or 120 mg·kg^{-1}) produced a significant increase in CSF guanosine levels. Intraperitoneal (Figure 8A) or p.o. (Figure 8B) administration of guanosine produced up to a 6.8- and 7.8-fold increase in CSF guanosine levels respectively. However, guanosine did not affect inosine, xanthine, hypoxanthine, uric acid, adenosine, ATP, ADP, AMP, GTP, GDP, GMP and IMP CSF levels (data not shown).

Effects of guanosine on cortical and spinal cord glutamate uptake

Figure 9 shows the effects of i.p. guanosine or vehicle followed by i.pl. capsaicin or vehicle on glutamate uptake by mice cortical (Figure 9A) and spinal cord (Figure 9B) slices. Capsaicin produced a significant increase in spinal cord glutamate uptake, an effect partially prevented by guanosine pretreatment. No significant effects on cortical glutamate uptake were observed. Importantly, systemic administration of guanosine did not affect basal cortical or spinal cord glutamate uptake (data not shown).

Guanosine lethal dose and general toxicity

Intraperitoneal guanosine LD_{50} was well over 960 mg·kg^{-1}, the highest dose here used. Additionally, no significant differences were seen in body weight of mice treated with guanosine (data not shown).

Guanosine (up to 960 mg·kg^{-1}) did not cause signs of renal impairment (Figure 10A,B) after a single i.p. administration. However, doses of guanosine higher than 240 mg·kg^{-1}

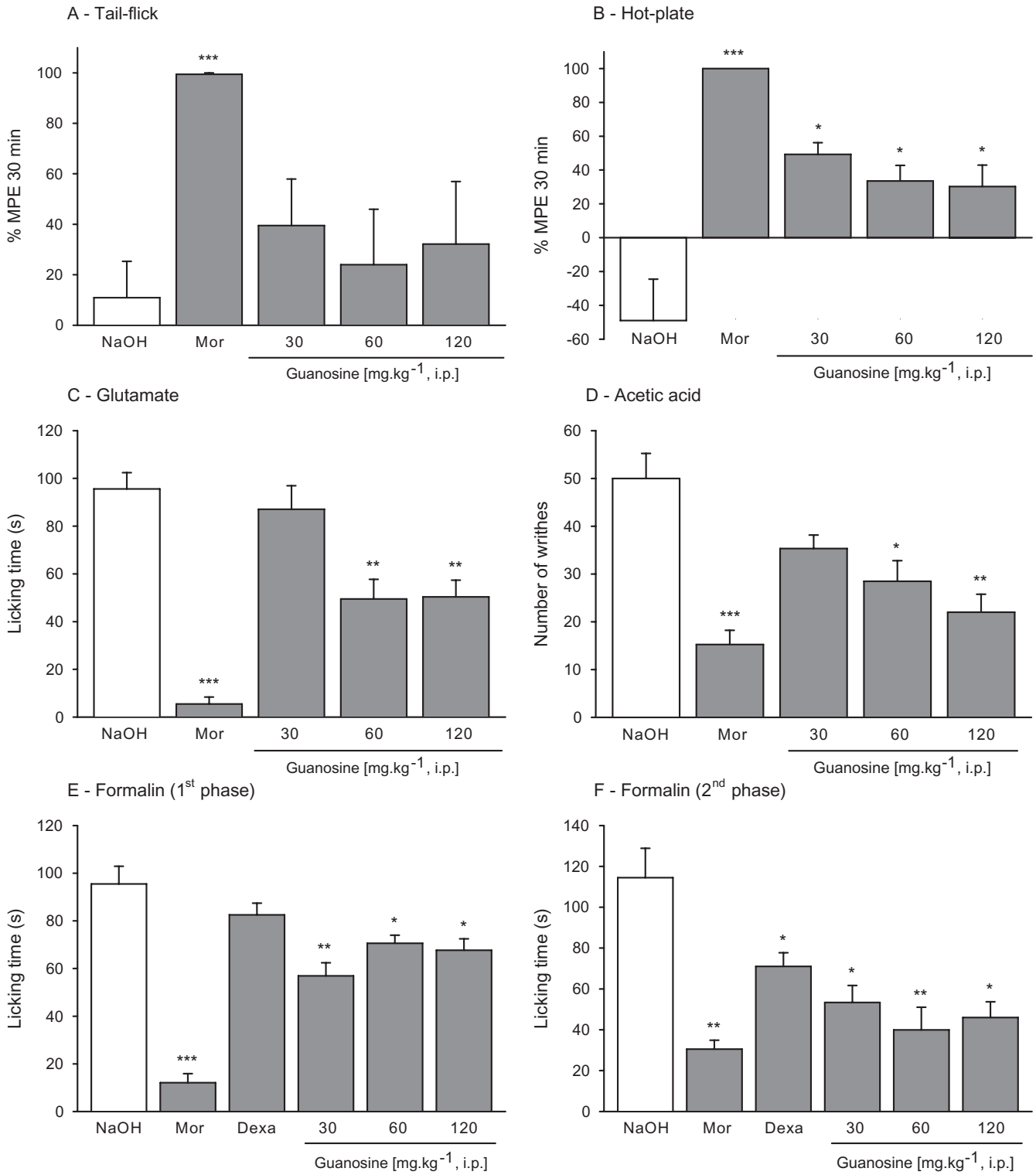

Figure 2 Effects of i.p. administration of vehicle (0.1 mN NaOH), morphine (Mor – 6 mg·kg^{-1}) or guanosine (30, 60 or 120 mg·kg^{-1}) in the tail-flick (A), hot-plate (B), i.pl. glutamate (C), i.p. acetic acid (D), and formalin (E-neurogenic phase and F-inflammatory phase) tests in mice. Dexamethasone (Dexa – 30 mg·kg^{-1}) was also administered in the formalin test. (A and B) Columns represent mean percent of maximum possible effect (% MPE), and vertical bars represent SEM. (C–F) Columns represent mean time spent in licking the injected hindpaw, and vertical bars represent SEM. $n = 8$–10. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with vehicle, one-way ANOVA/Student–Newman–Keuls.

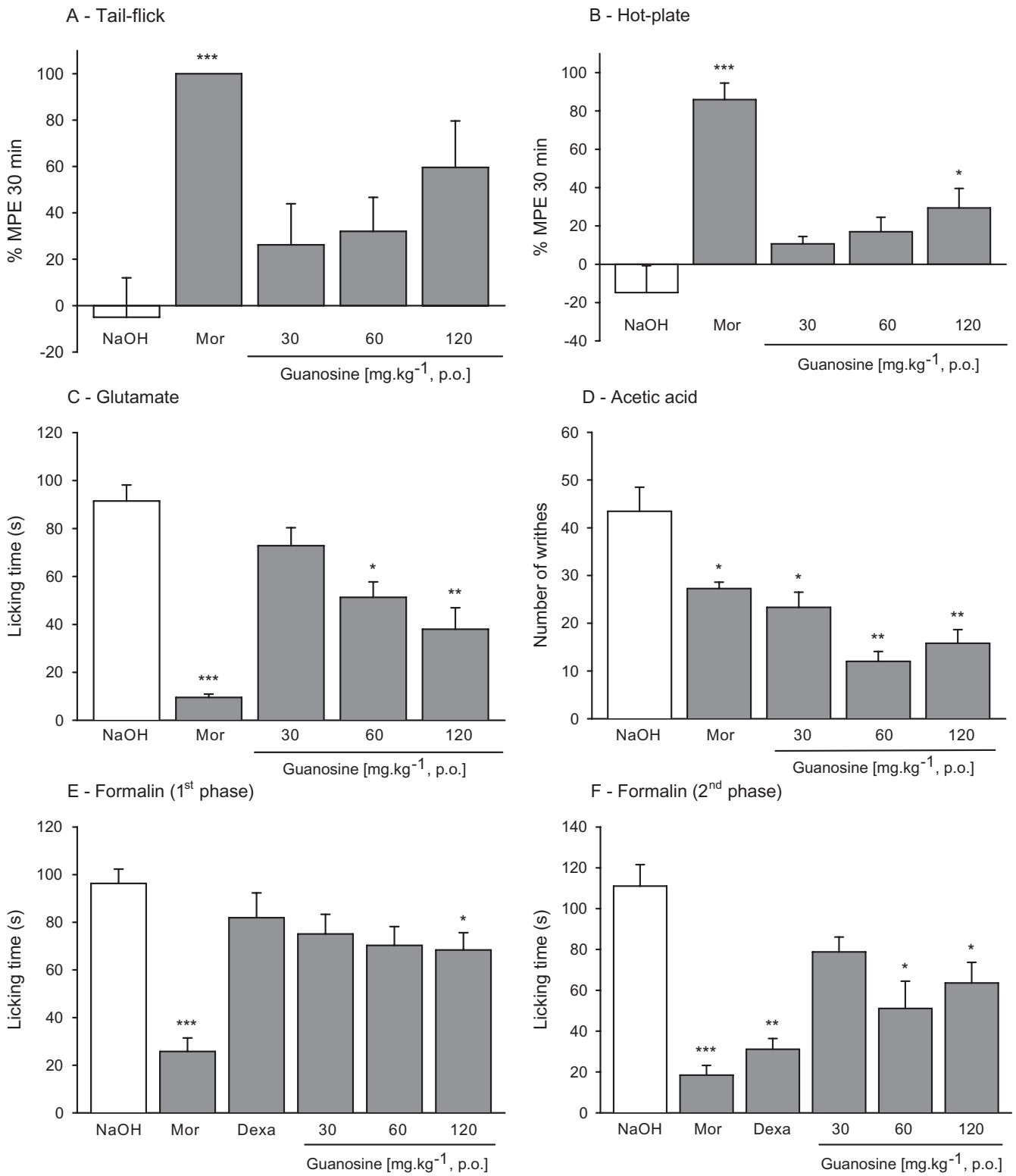

Figure 3 Effects of p.o. administration of vehicle (0.1 mN NaOH), morphine (Mor – 6 mg·kg^{-1}) or guanosine (30, 60 or 120 mg·kg^{-1}) in the tail-flick (A), hot-plate (B), i.pl. glutamate (C), i.p. acetic acid (D), and formalin (E-neurogenic phase and F-inflammatory phase) tests in mice. Dexamethasone (Dexa – 30 mg·kg^{-1}) was also administered in the formalin test. (A and B) Columns represent mean percent of maximum possible effect (% MPE), and vertical bars represent SEM. (C–F) Columns represent mean time spent licking the injected hindpaw, and vertical bars represent SEM. $n = 8$–10. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with vehicle, one-way ANOVA/Student–Newman–Keuls. MPE, maximum possible effect.

Table 1 Effects of systemic guanosine in pain models

Pain model	*Guanosine (i.p.)*	*Guanosine (p.o.)*
Mean ID_{50} ($mg \cdot kg^{-1}$ – 95% confidence limits)		
Capsaicin	84 (31–230)	88 (29–267)
Glutamate	107 (83–137)	85 (64–112)
Acetic acid	48 (13–173)	42 (21–83)
Formalin (first phase)	76 (22–262)	119 (103–138)
Formalin (second phase)	66 (33–133)	83 (49–139)
Maximal Inhibitions (%)		
Capsaicin	58 ± 14	52 ± 12
Glutamate	48 ± 15	58 ± 13
Acetic acid	56 ± 10	72 ± 6
Formalin (first phase)	40 ± 8	29 ± 11
Formalin (second phase)	57 ± 17	43 ± 20

Data shown are mean ID_{50} values (95% confidence limits) and maximal inhibition (±SEM) for i.p. or p.o. guanosine against capsaicin, glutamate, acetic acid and formalin (first and second phases) pain tests. Differences were assessed by linear regression from individual experiments.

produced a significant increase of serum AST levels (Figure 10C). This effect was likewise observed on serum ALT levels at the highest dose (Figure 10D). Importantly, guanosine significantly reduced the pentobarbital-induced sleeping time (NaOH: 33.3 ± 6.0 min; guanosine 30 $mg \cdot kg^{-1}$: 8.9 ± 3.1 min; guanosine 60 $mg \cdot kg^{-1}$: 10.5 ± 4.4 min; and guanosine 120 $mg \cdot kg^{-1}$: 20.7 ± 5.9 min; $P = 0.029$), but no significant effects were observed on core temperature (NaOH: 35.2 ± 0.2°C; guanosine 30 $mg \cdot kg^{-1}$: 35.8 ± 0.3°C; guanosine 60 $mg \cdot kg^{-1}$: 35.9 ± 0.4°C; and guanosine 120 $mg \cdot kg^{-1}$: 35.7 ± 0.4°C; $P = 0.35$).

In the hole-board model, i.p. guanosine (30, 60 or 120 $mg \cdot kg^{-1}$) did not affect latency to first head-dip, number of head-dips, crossings, rearings, groomings and defecations (Table 3). Guanosine did not induce motor deficits or ataxia, as evaluated by the performance in the rotarod test, and did not affect locomotion (Table 3).

Discussion and conclusions

Guanosine and its effects in several pain models

The present study clearly demonstrates that systemically administered guanosine produces significant inhibition of pain-related behaviour induced by several algogens in mice. Additionally, guanosine prevents biting behaviour induced by i.t. administration of glutamate and non-NMDA agonists, but not against NMDA, substance P or capsaicin. We also demonstrate that these antinociceptive effects may involve adenosine receptors and spinal cord glutamate uptake, but are not related to the nitric oxide-L-arginine-cGMP pathway.

Adenosine-derived purines have been considered important targets for the development of new drugs for pain management, as the nucleoside adenosine and its analogues induce antinociceptive effects following both systemic and central administration (Sawynok and Liu, 2003). Considering that guanosine and adenosine closely interact in modulating several CNS functions (Dobolyi *et al.*, 2000), we proposed that guanosine might well play a role in pain transmission. Recently, we demonstrated that i.c.v. guanosine-based purines produced consistent antinociceptive effects in several pain models (Schmidt *et al.*, 2008). We also demonstrated that GMP-induced antinociception was prevented by the 5′-nucleotidase inhibitor AOPCP, suggesting that its effects result from conversion to guanosine. In the present study, the role of systemic administration of guanosine on nociception was investigated.

Guanosine was effective against several pain models, including those based on thermal or chemical stimuli. Guanosine produced antinociception in all chemical models and the hot-plate test, but not in the tail-flick test. In a previous study (Schmidt *et al.*, 2008), however, we have found that i.c.v. guanosine was antinociceptive in the tail-flick test. This somewhat conflicting finding may be related to CNS levels of guanosine, which are more prominently increased following i.c.v. guanosine (Schmidt *et al.*, 2008). Although these thermal models are essentially based on short-lasting, phasic noxious stimuli, some differences exist between these tests. While the tail-flick and hot-plate tests are both thermal threshold tests, the former refers predominantly to a spinal reflex with modest control by supraspinal structures, while hot-plate is a more complex pain model, producing two behavioural components (i.e. paw licking and jumping) considered to be supraspinally integrated responses (Le Bars *et al.*, 2001). These differences may also contribute to the lack of efficacy of systemic guanosine in the tail-flick test. Intraplantar or i.p. injection of algogenic chemical agents (capsaicin, glutamate, formalin or acetic acid) usually produces a longer duration or tonic stimulus as compared with phasic pain tests, in which thermal thresholds are determined. These tonic pain tests, particularly the acetic acid-induced pain model, are very sensitive methods to test new molecules whose pharmacodynamic properties are unknown (Le Bars *et al.*, 2001). Therefore, our results indicate that guanosine is a potential new analgesic and, considering its effects on the second phase of formalin test, guanosine may produce anti-inflammatory effects as well.

Guanosine-induced antinociception and the purinergic system

Adenine- and guanine-based purines share some metabolism steps (i.e. nucleoside transporters and ecto-nucleotidases), and, consequently, may respond similarly in certain conditions (i.e. ischemia) (Ciccarelli *et al.*, 1999; 2001). Previous studies have suggested involvement of the adenosine system in the effects of guanosine, as guanosine stimulated the release of adenosine in cultured astrocytes and both are released under excitotoxic conditions (Ciccarelli *et al.*, 1999). In contrast, studies indicate that the guanosine-induced enhancement of neurite outgrowth in PC12 cells was not affected by adenosine receptor antagonists (Gysbers and Rathbone, 1996), nor were the effects of guanosine on glutamate uptake (Frizzo *et al.*, 2001), seizures (Lara *et al.*, 2001), learning and memory (Roesler *et al.*, 2000; Vinadé *et al.*, 2004) and guanosine-induced antinociception (Schmidt *et al.*, 2008). Moreover, i.c.v. (Schmidt *et al.*, 2008), i.t. (Schmidt *et al.*, 2009b) and systemic administration of guanosine failed to increase CSF ABPs levels. Conversely, in the present study, a pretreatment with non-selective and selective A_1/A_{2A} adenosine receptor antagonists significantly affected guanosine-induced nociception. Therefore, at least for antinociception, adenosine receptors seem to be relevant.

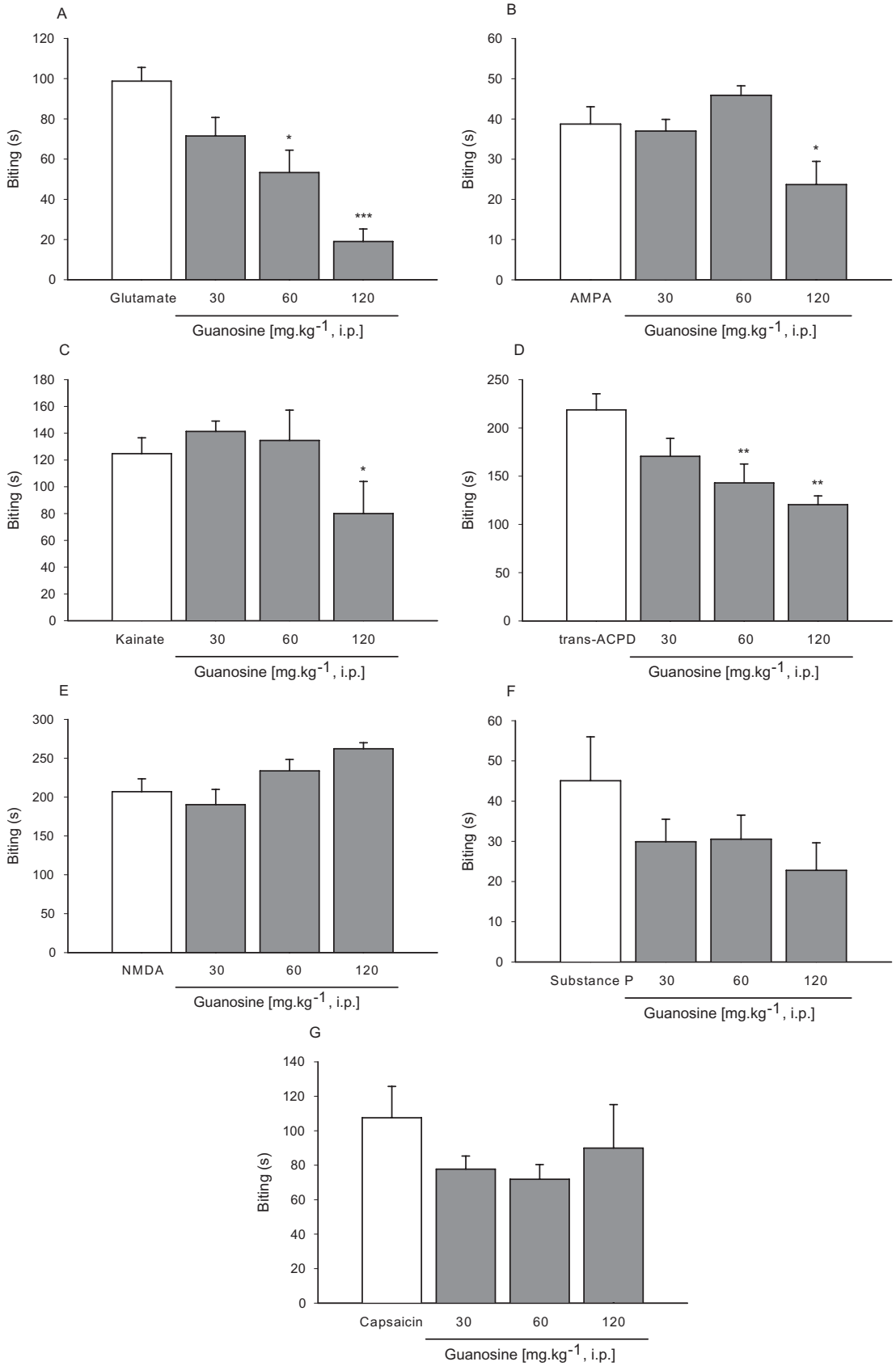

Figure 4 Effects of i.p. vehicle (0.1 mN NaOH) or guanosine (30, 60 or 120 mg·kg^{-1}) in the glutamate (A, 175 nmol per site, i.t.)-, AMPA (B, 135 pmol per site, i.t.)-, kainate (C, 110 pmol per site, i.t.)-, trans-ACPD (D, 50 nmol per site, i.t.)-, NMDA (E, 450 pmol per site, i.t.)-, substance P (F, 135 ng per site, i.t.)- or capsaicin (G, 30 ng per site, i.t.)-induced biting in mice. Columns represent mean, and vertical bars represent SEM. $n = 8–10$. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with vehicle, one-way ANOVA/Student–Newman–Keuls. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartate.

Table 2 Effects of guanosine against spinal algogens

Spinal algogen	*Guanosine (i.p.)*
Mean ID_{50} ($mg \cdot kg^{-1}$ – 95% confidence limits)	
Glutamate	65 (53–81)
AMPA	152 (122–188)
Kainate	122 (106–140)
Trans-ACPD	126 (90–177)
Maximal inhibition (%)	
Glutamate	81 ± 8
AMPA	47 ± 15
Kainate	36 ± 20
Trans-ACPD	45 ± 4

Dtata shown are mean ID_{50} values (95% confidence limits) and maximal inhibition (±SEM) for i.p. guanosine against spinal algogen-induced nociception. Vehicle (0.1 mN NaOH) or guanosine was i.p. administered 30 min prior to the biting response induced by an i.t. injection of 5 μL of several algogens. Differences were assessed by linear regression from individual experiments.

It is well known that activation of A_1 adenosine receptors, widely distributed in superficial layers of the dorsal spinal cord and afferent terminals of nociceptors, causes antinociception following nerve injury and inflammation, and decreases C fibre-driven responses in dorsal horn neurons (Reeve and Dickenson, 1995; Schulte *et al.*, 2003). However, the role of A_{2A} adenosine receptors, present on spinal presynaptic terminals of sensory afferents, for pain processing is less clear. Recent studies have demonstrated that A_{2A} adenosine receptors may also be involved in the modulation of pain transmission (Poon and Sawynok, 1998; Yoon *et al.*, 2005). However, controversy remains about the role of A_{2A} adenosine receptors on pain transmission, as other studies found opposing effects (Ledent *et al.*, 1997; Bastia *et al.*, 2002; Zahn *et al.*, 2007). Further studies about the actual role of A_{2A} adenosine receptors on pain transmission, and the molecular mechanisms involved on guanosine-induced antinociception (i.e. direct or indirect activation of adenosine receptors and/or guanosine specific receptors) are needed to elucidate these issues. Nevertheless, this study demonstrates that guanosine-induced analgesia involves adenosine receptors; importantly, both A_1 and A_{2A} receptors seem to be relevant, differing from adenosine, which produces analgesia largely mediated by A_1 receptors (Sawynok and Liu, 2003).

We demonstrated that administration of guanosine produced a significant increase in CSF levels of oxypurines (Schmidt *et al.*, 2008), which can not be excluded to play some role in the antinociceptive effects of guanosine. However, this study failed to demonstrate an increase in CSF levels of oxypurines following a single i.p. or p.o. guanosine administration. A previous study has demonstrated that an i.p. administration of guanosine increased the amounts of both guanosine and guanine at the spinal cord, with a peak around 30 min (Jiang *et al.*, 2008). As extracellular guanine also exerts several biological effects (Rathbone *et al.*, 2008), the antinociceptive effects of guanosine may be regulated by its conversion to guanine by a membrane-located purine nucleoside phosphorylase. Therefore, a role for guanine in the antinociceptive effects of guanosine can not be excluded at this point.

Guanosine-induced antinociception and glutamatergic receptors

Glutamate and its receptors play crucial roles in pain transmission and the modulation of glutamate receptors may have therapeutic potential for several categories of pain (Millan, 1999). *In vitro*, guanosine has been shown to prevent ischemic injury (Frizzo *et al.*, 2002), and NMDA-induced excitotoxicity (Ciccarelli *et al.*, 2001). *In vivo*, guanosine prevents seizures and toxicity induced by drugs that overstimulate the glutamatergic system (Baron *et al.*, 1989; Malcon *et al.*, 1997; Schmidt *et al.*, 2000), is amnesic and anxiolytic in rodents (Vinadé *et al.*, 2003), and neuroprotective against stroke and spinal cord injury (Jiang *et al.*, 2003; 2007; 2008; Chang *et al.*, 2008). Although the overall effects of guanosine may be related to attenuating glutamatergic overstimulation, its precise mechanism of action remains unclear. In this study, guanosine produced a significant inhibition of the biting behaviour induced by i.t. injection of glutamate or non-NMDA agonists (AMPA, kainate and trans-ACPD), but not against NMDA. Thus, we suggest that the antinociceptive effect caused by guanosine may involve an interaction with the glutamatergic system and its receptors, and/or with their signal transduction mechanisms.

Guanosine-induced antinociception and the nitric oxide-L-arginine-cGMP pathway

In the present study we aimed to further characterize the mechanisms through which guanosine exerts its antinociceptive action in the glutamate model of nociception. Results show that systemic administration of guanosine prevented pain and produced antioedematogenic effects against i.pl. glutamate; however, local administration of guanosine failed to affect the nociception and paw oedema induced by glutamate. Additionally, pretreatment with the nitric oxide precursor L-arginine, the non-specific inhibitor of NO/guanylyl cyclase methylene blue or the inhibitor of nitric oxide synthesis L-NOARG, did not prevent the antinociception caused by guanosine. Altogether, these results indicate that the nitric oxide-L-arginine-cGMP pathway is not involved in the antinociceptive effects of systemic guanosine.

Guanosine-induced antinociception and glutamate uptake

Recently, we demonstrated that an i.pl. administration of capsaicin caused a significant decrease in cortical glutamate uptake, an effect prevented by i.c.v. guanosine (Schmidt *et al.*, 2008). As guanosine has been shown to stimulate glutamate uptake *in vitro* (Frizzo *et al.*, 2002), it was possible that the *in vivo* antinociceptive effect of i.c.v. guanosine against capsaicin could result from its effect on glutamate removal from the synaptic cleft, decreasing the activation of glutamatergic receptors. However, in the present study, we showed that an i.pl. capsaicin produced an increase in spinal cord glutamate uptake, an effect prevented by guanosine. Surprisingly, no significant effects were observed on cortical glutamate uptake. Notably, neither i.c.v. nor i.t. guanosine altered basal glutamate uptake at both brain and spinal cord. It is not possible to establish whether the changes in the spinal cord glutamate uptake were responsible for nociceptive behaviour. However, considering our results and previous data (Schmidt

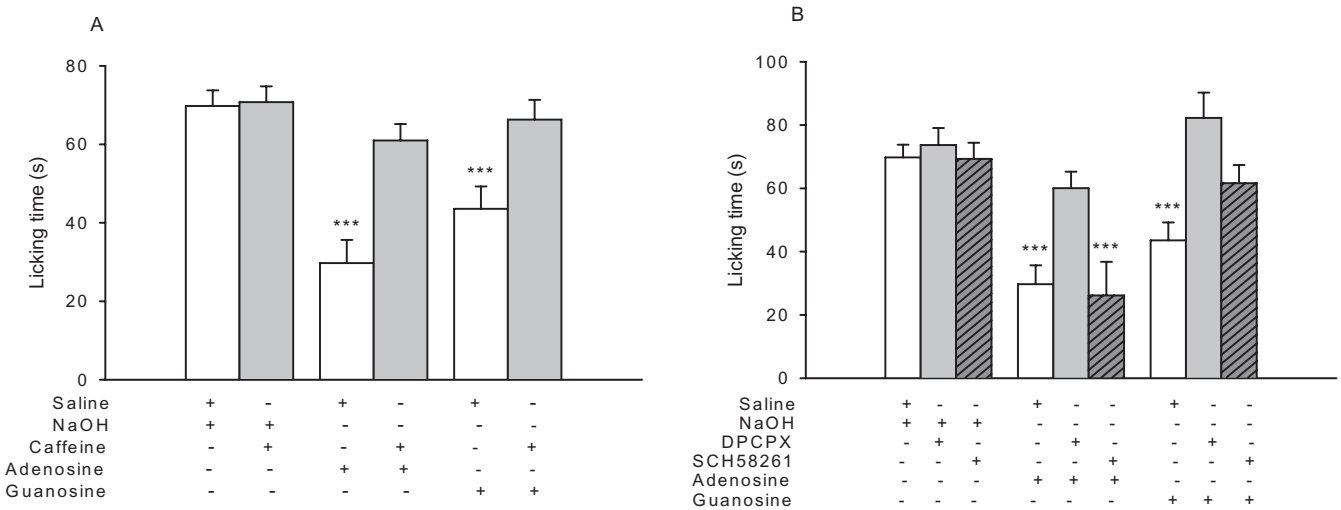

Figure 5 Effects of caffeine (10 mg·kg^{-1}, i.p., A), DPCPX (1 mg·kg^{-1}, i.p., B) or SCH58261 (0.5 mg·kg^{-1}, i.p., B) 15 min before vehicle (0.1 mN NaOH), adenosine (100 mg·kg^{-1}, i.p.) or guanosine (60 mg·kg^{-1}, i.p.) in the i.pl. capsaicin test in mice. Columns represent mean time spent in licking the injected hindpaw, and vertical bars represent SEM. n = 14–18. ***P < 0.001 compared with vehicle, one-way ANOVA/Student–Newman–Keuls. DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; SCH58261, 5-amino-2-(2-furyl)-7-phenylethyl-pyra-zolo-[4,3-e]-1,2,4-triazolo[1,5c]pyrimidine.

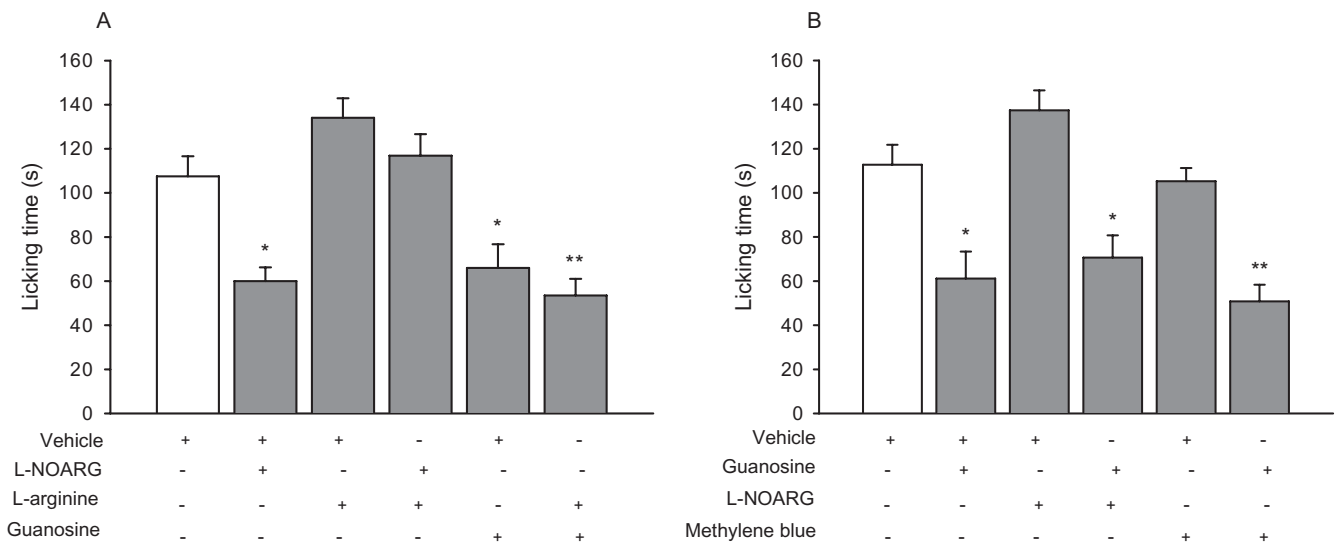

Figure 6 Effects of L-arginine (600 mg·kg^{-1}, i.p.) 20 min before L-NOARG (75 mg·kg^{-1}, i.p.), vehicle (0.1 mN NaOH) or guanosine (60 mg·kg^{-1}, i.p.) in the i.pl. glutamate-induced nociception (A). Effects of L-NOARG (30 mg·kg^{-1}, i.p.), methylene blue (1 mg·kg^{-1}, i.p.) or vehicle (saline, i.p.) 15 min before guanosine (60 mg·kg^{-1}, i.p.) or vehicle (0.1 mN NaOH, i.p.) (B). The total time spent in licking the hindpaw was measured for 15 min after i.pl. injection of glutamate. Columns represent mean, and vertical bars represent SEM. n = 8–10. *P < 0.05 and **P < 0.01 compared with vehicle, one-way ANOVA/Student–Newman–Keuls. L-NOARG, *N*-nitro-L-arginine.

et al., 2008), we may argue that these changes were probably produced by the nociceptive stimulus and its modulation by guanosine, rather than an underlying mechanism of action responsible for guanosine effects.

Guanosine-induced antinociception and cellular mechanisms

There is data supporting the existence of specific receptor-like binding sites for guanosine on membrane preparations from rat brain (Traversa *et al.*, 2002; 2003). If so, it is arguable that guanosine through its specific binding site, could promote its extracellular effects by activating intracellular cAMP-dependent and independent pathways (Tomaselli *et al.*, 2005). Additionally, guanosine could act as an alternative source of energy for neural cells after metabolism, as previously demonstrated in spinal cord cultures (Jurkowitz *et al.*, 1998; Litsky *et al.*, 1999). However, intracellular mechanisms underlying guanosine antinociceptive effects remain to be investigated.

Systemic guanosine administration and general toxicity

Regarding side effects, our results show that guanosine did not induce obvious behavioural disturbances, altered

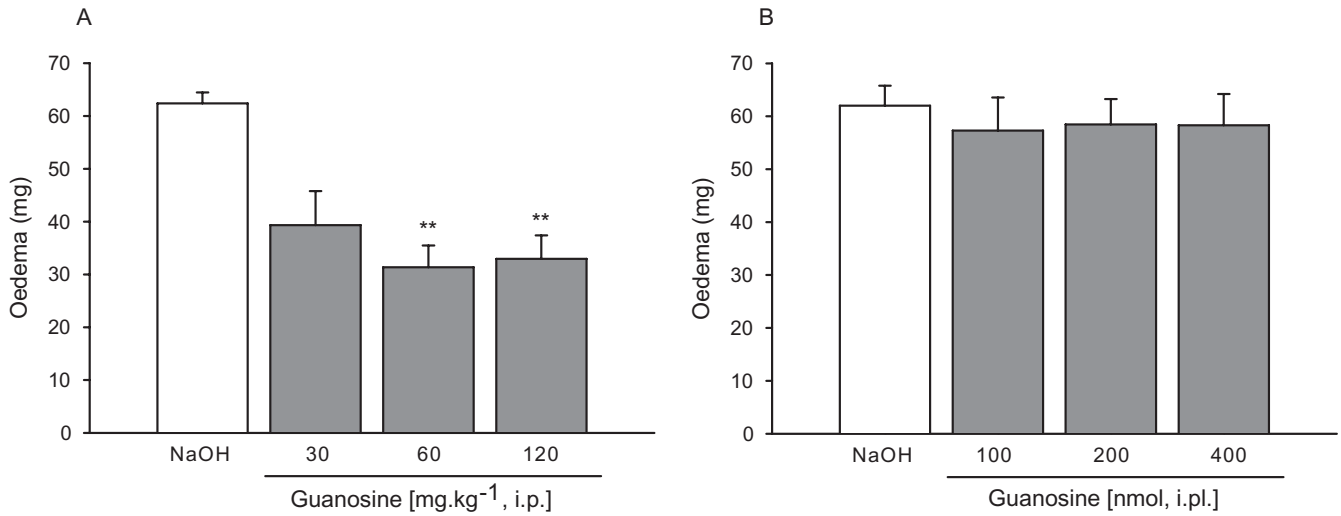

Figure 7 Effects of i.p. vehicle (0.1 mN NaOH) or guanosine (30, 60 or 120 mg·kg^{-1}) in the i.pl. glutamate-induced paw oedema in mice (A). Effects of i.pl. administration of vehicle (0.1 mN NaOH) or guanosine (100, 200, 400 nmol) against i.pl. glutamate-induced paw oedema in mice (B). Columns represent mean weight difference (injected–non-injected paw), and vertical bars represent SEM. $n = 8$. $^{**}P < 0.01$ compared with vehicle, one-way ANOVA/Student–Newman–Keuls.

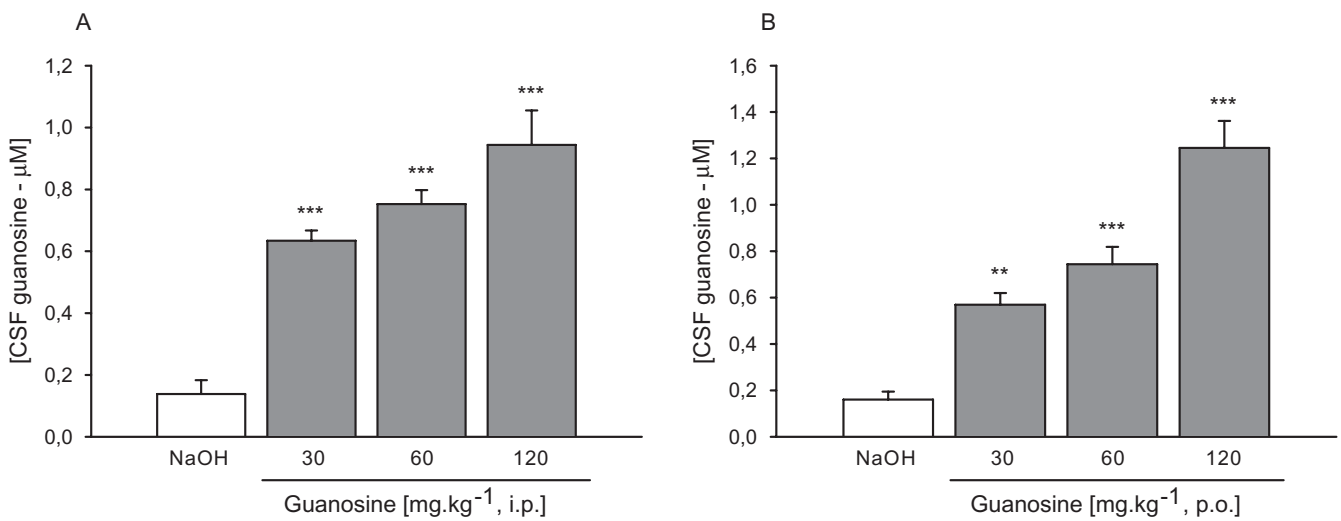

Figure 8 Effects of i.p. (A) or p.o. (B) vehicle (0.1 mN NaOH) or guanosine (30, 60 or 120 mg·kg^{-1}) on CSF purine concentration. The columns represent mean (µM), and vertical bars represent SEM. $n = 8$. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with vehicle, one-way ANOVA/Student–Newman–Keuls. CSF, cerebrospinal fluid.

coordination or locomotion, consistent with previous data (Vinadé *et al.*, 2003). The minor toxic potential of guanosine was also demonstrated by the low index of mortality (none at 960 mg·kg^{-1}), and lack of alterations in weight body gain or core temperature up to 72 h after guanosine administration. Additionally, no CNS depressant activity of guanosine was observed in the barbiturate-induced sleeping time. Actually, guanosine produced a CNS excitant effect that resembles some adenosine receptor antagonists (El Yacoubi *et al.*, 2003). Although no evidences of renal impairment were noted, some hepatic toxicity was observed in doses higher than 240 mg·kg^{-1}; at 960 mg·kg^{-1} both serum AST and ALT were significantly increased. Although these effects were not observed at antinociceptive doses, future studies may focus on potential adverse effects of guanosine including those involved on liver metabolism.

Conclusions and perspectives

In summary, this is the first study demonstrating antinociceptive effects after systemic guanosine administration. Because guanosine is an endogenous compound apparently well tolerated and orally active, it could eventually be developed as a drug useful for managing pain. This study also provides new evidence on the role of extracellular guanosine in the CNS, and indicates that the antinociceptive effects of guanosine are likely to involve the adenosine and glutamatergic systems. Ongoing experiments on the antinociceptive effects of

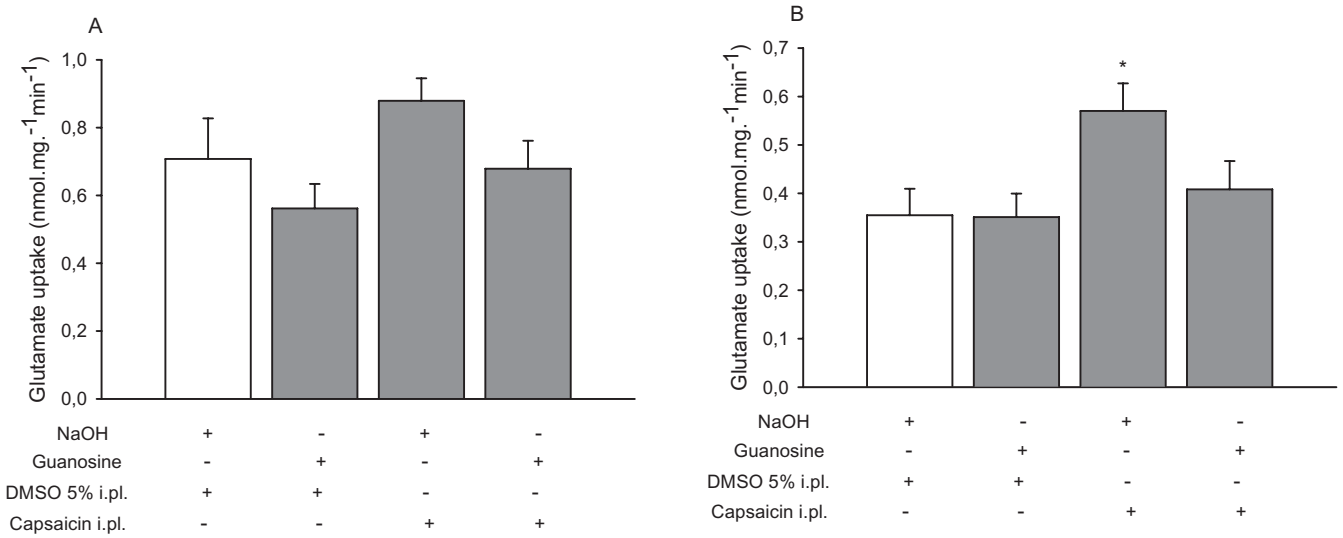

Figure 9 Effects of i.p. guanosine and i.pl. capsaicin on glutamate uptake by mice cortical (A), and spinal cord (B) slices. Mice were treated with an i.p. injection of vehicle (0.1 mN NaOH) or guanosine (60 mg·kg^{-1}); after 30 min, animals received an i.pl. injection of vehicle (DMSO 5%) or capsaicin. After behavioural evaluation, the mice were killed and the cortical and spinal cord slices processed for glutamate uptake assay. Data are mean ± SEM. $n = 12$. *$P < 0.05$ compared with vehicle, one-way ANOVA/Student–Newman–Keuls. DMSO, dimethyl sulfoxide.

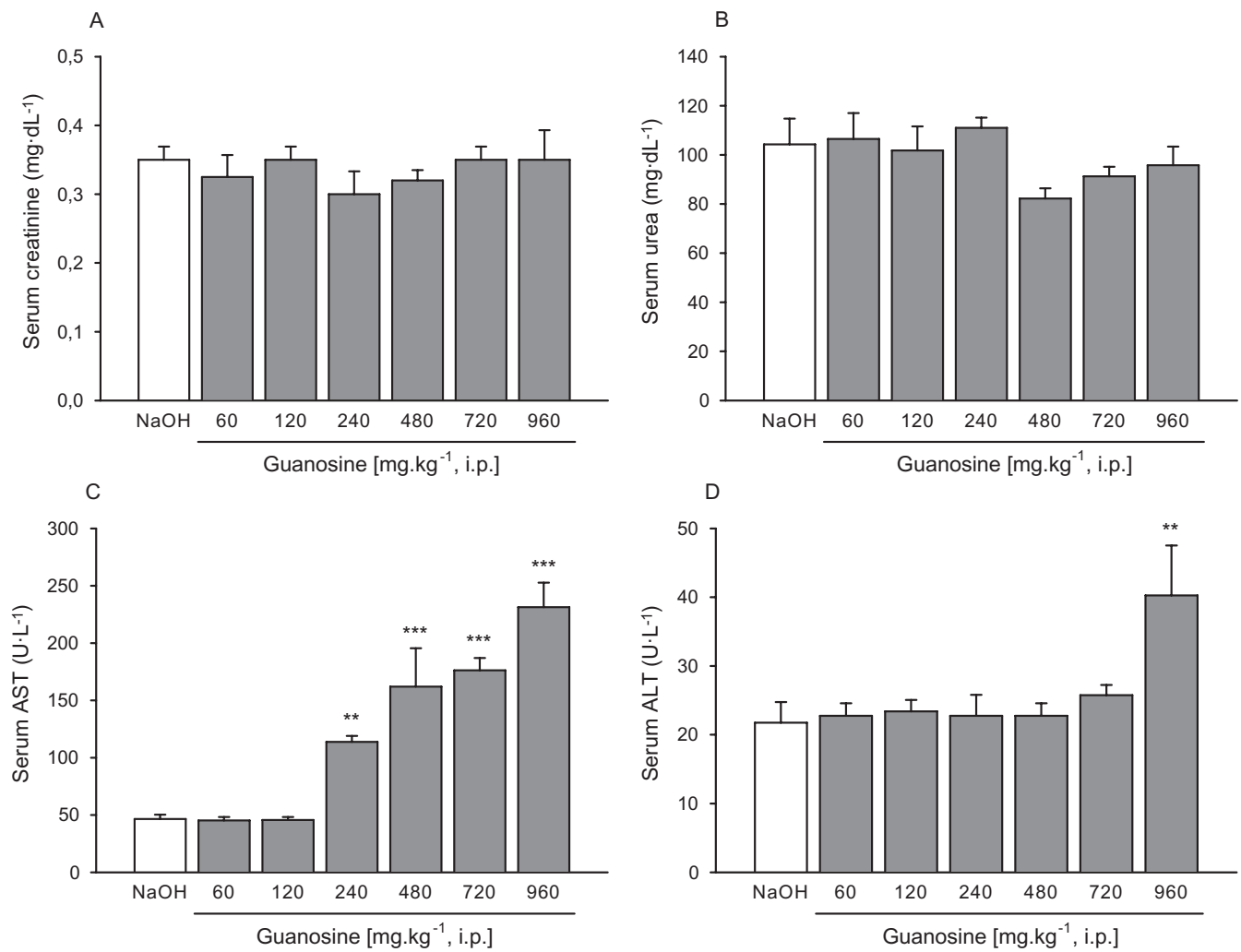

Figure 10 Effects of i.p. vehicle (0.1 mN NaOH) or guanosine (60 to 960 mg·kg^{-1}) on serum levels of creatinine (A), urea (B), aspartate aminotransferase (AST – C), and alanine aminotransferase (ALT – D) in mice. Mice received an i.p. injection of vehicle or guanosine 72 h before blood sampling. Data are mean ± SEM. $n = 8$. **$P < 0.01$ and ***$P < 0.001$ compared with vehicle, one-way ANOVA/Student–Newman–Keuls. AST, aspartate aminotransferase.

Table 3 Effects of i.p. guanosine on the hole-board, rotarod and spontaneous locomotion in mice.

Treatment	*0.1 mN NaOH*	*Guanosine (mg·kg^{-1}, i.p.)*		
		30	*60*	*120*
Latency to head-dip (s)	6.7 (1.9)	5.6 (1.3)	7.1 (1.3)	6.3 (1.5)
Head-dips (*n*)	81.8 (6.1)	87.5 (5.9)	88.7 (4.6)	76.8 (5.5)
Squares crossed (*n*)	44.8 (5.4)	51.5 (7.9)	39.8 (6.7)	48.5 (6.6)
Rearings (*n*)	1.4 (0.8)	2.0 (0.9)	1.3 (0.6)	2.2 (0.8)
Groomings (*n*)	1.6 (0.5)	1.4 (0.5)	1.0 (0.5)	1.3 (0.3)
Fecal boli (*n*)	0.8 (0.4)	1.3 (0.5)	1.6 (0.6)	1.1 (0.5)
Latency to fall (s)	59.0 (0.8)	60 (0)	57.7 (2.4)	55.6 (3.7)
Crossings (*n*)	152 (17)	165 (25)	153 (11)	159 (24)

Vehicle (0.1 mN NaOH) or guanosine (30, 60 or 120 mg·kg^{-1}) were i.p. administered 30 min prior to the behaviour measurements: latency to the first head-dip; head-dips; squares crossed; rearings; groomings; fecal boli (hole board); latency to fall (rotarod); number of crossings (spontaneous locomotion). Data are mean ± SEM. n = 7–8; differences were assessed by one-way ANOVA followed by Student–Newman–Keuls test.

guanosine against chronic pain models, and the mechanisms underlying these effects, should provide additional data on the potential of guanosine as a new analgesic strategy.

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Conflicts of interest

None.

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