



The NMDA antagonist MK-801 induces hyperalgesia and increases CSF excitatory amino acids in rats: Reversal by guanosine

André P. Schmidt^{a,b,*}, Adriano B.L. Tort^{a,c}, Patrícia P. Silveira^a, Ana Elisa Böhmer^a, Gisele Hansel^a, Luisa Knorr^a, Cristhine Schallenberger^a, Carla Dalmaz^a, Elaine Elisabetsky^d, Rosa H. Crestana^e, Diogo R. Lara^f, Diogo O. Souza^a

^a Department of Biochemistry, ICBS, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

^b Anesthesia and Perioperative Medicine Service at Hospital de Clínicas de Porto Alegre (HCPA), Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

^c Department of Mathematics and Center for Biodynamics, Boston University, Boston, MA 02215, USA

^d Department of Pharmacology, ICBS, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

^e Department of Physiology, ICBS, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

^f Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:

Received 4 June 2008

Received in revised form 2 September 2008

Accepted 18 September 2008

Available online 25 September 2008

Keywords:

Guanosine

MK-801

Riluzole

Glutamate

Aspartate

Pain

Tail flick test

ABSTRACT

Excitatory amino acids (EAAs) and their receptors play a central role in the mechanisms underlying pain transmission. NMDA-receptor antagonists such as MK-801 produce antinociceptive effects against experimental models of chronic pain, but results in acute pain models are conflicting, perhaps due to increased glutamate availability induced by the NMDA-receptor antagonists. Since guanosine and riluzole have recently been shown to stimulate glutamate uptake, the aim of this study was to examine the effects of guanosine or riluzole on changes in nociceptive signaling induced by MK-801 in an acute pain model. Rats received an i.p. injection of vehicle, morphine, guanosine, riluzole or MK-801 or a combined treatment (vehicle, morphine, guanosine or riluzole+MK-801) and were evaluated in the tail flick test, or had a CSF sample drawn after 30 min. Riluzole, guanosine, and MK-801 (0.01 or 0.1 mg/kg) did not affect basal nociceptive responses or CSF EAAs levels. However, MK-801 (0.5 mg/kg) induced hyperalgesia and increased the CSF EAAs levels; both effects were prevented by guanosine, riluzole or morphine. Hyperalgesia was correlated with CSF aspartate and glutamate levels. This study provides additional evidence for the mechanism of action of MK-801, showing that MK-801 induces hyperalgesia with parallel increase in CSF EAAs levels.

© 2008 Elsevier Inc. All rights reserved.

1. Introduction

N-methyl-D-aspartate (NMDA) receptors and the excitatory amino acids (EAAs) aspartate and glutamate are implicated in the generation and maintenance of central states of hypersensitivity, whereas glutamate antagonists may prevent hyperalgesia or an enhanced pain state (Bennett, 2000). A number of studies have demonstrated that NMDA antagonists can decrease nociceptive behaviors in animal models of neuropathic pain and potentiate the analgesic effects of opiates (Fisher et al., 2000). However, the effects of NMDA antagonists on acute nociception and the neurobiological mechanisms that mediate their action are still unclear, and of conflicting results have been reported (Al-Amin et al., 2003). Moreover, noncompetitive

NMDA-receptor antagonists, such as MK-801, induce several unusual behaviors in rodents, such as hyperlocomotion, stereotyped movements, ataxia, and amnesia (Dall'igna et al., 2003; Tort et al., 2004). Interestingly, evidence suggests that noncompetitive antagonism of NMDA receptors is also associated with a paradoxical glutamatergic activation of non-NMDA receptors induced by increased glutamate availability, which could underlie the behavioral effects observed (Moghaddam et al., 1997; Tort et al., 2004). However, the role of glutamate and aspartate in the effects of NMDA antagonists has not been studied in pain models to our knowledge.

Extracellular guanine-based purines (GBPs), mainly the nucleoside guanosine, have been shown to exert biological effects not directly related to G-protein activity, such as trophic effects on neural cells (Ciccarelli et al., 2001) and antagonism of the glutamatergic system (Baron et al., 1989; Burgos et al., 1998; Malcon et al., 1997; Regner et al., 1998). In vitro, GBPs inhibit the binding of glutamate and its analogs, prevent cell responses to excitatory amino acids, and present neuroprotective effects in several brain preparations submitted to excitotoxic conditions (Baron et al., 1989; Burgos et al., 1998; Caciagli

* Corresponding author. Avenida Ramiro Barcelos, 2600-Anexo, 90035-003 Porto Alegre, RS, Brazil. Tel.: +55 51 33085557/33085558; fax: +55 51 33085540/33085535.

E-mail address: aschmidt@ufrgs.br (A.P. Schmidt).

et al., 2000; Frizzo et al., 2001, 2002, 2003; Souza and Ramirez, 1991). In vivo, GBPs prevent seizures induced by glutamatergic agents and present amnesic effects in rodents (Lara et al., 2001; Schmidt et al., 2000, 2005; Soares et al., 2004; Vinadé et al., 2003, 2005). Several studies indicate that these anti-glutamatergic effects seem to be directly related to a guanosine-induced glutamate removal from the synaptic cleft (glutamate uptake) (Frizzo et al., 2001, 2002, 2003; Schmidt et al., 2007; Soares et al., 2004).

Given the pivotal role of EAAs and their receptors in the mechanisms underlying pain transmission, the occurrence of paradoxical behavioral effects of NMDA-receptor noncompetitive antagonists, and the reported antagonism of glutamatergic activity by guanosine, we studied the effects of systemic administration of guanosine and the noncompetitive NMDA-receptor antagonist MK-801 on pain in the tail flick model in rats. Riluzole, a well-known glutamate release inhibitor and glutamate uptake stimulator (Frizzo et al., 2004), and morphine, a well-known opioid receptor agonist, were used as positive controls. Additionally, the effects of guanosine, riluzole, morphine and MK-801 on cerebrospinal fluid (CSF) EAAs levels were determined.

2. Materials and methods

2.1. Animals

Male adult Wistar rats (3–4 months of age, 250–350 g) were used. Animals were kept on a 12 h light/dark cycle (light on at 7:00 am) at a constant temperature of 22 ± 1 °C, in plastic cages (five per cage) with tap water and commercial food pellets ad libitum. All behavioral procedures were conducted between 8:00 and 10:00 am. The ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983) and our institutional protocols for experiments with animals, designed to avoid suffering and limit the number of animals sacrificed, were followed throughout. This study was conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments.

2.2. Drugs

Guanosine was purchased from Sigma Chemicals (St Louis, MO, USA). 5-Methyl-10-11-dihydro-5H-dibenzo[a,b]cyclohepta-5-10-imine maleate (MK-801 or dizocilpine) was obtained from RBI-Research Biochemicals (Natick, MA, USA). Riluzole was obtained

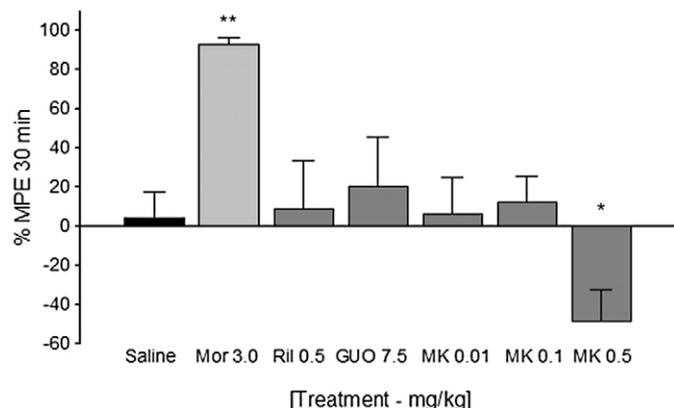


Fig. 1. Effects of i.p. vehicle (saline 0.9%), morphine (Mor – 3 mg/kg), riluzole (Ril – 0.5 mg/kg), guanosine (GUO – 7.5 mg/kg), and MK-801 (MK – 0.01, 0.1 and 0.5 mg/kg) on tail flick latency 30 min after treatments. The columns represent % of Maximum Possible Effect (% MPE) and vertical bars represent SEM. $N=20$ animals per group. * = $P<0.01$ and ** = $P<0.001$ compared to vehicle (control), Kruskal–Wallis followed by Dunn's test.

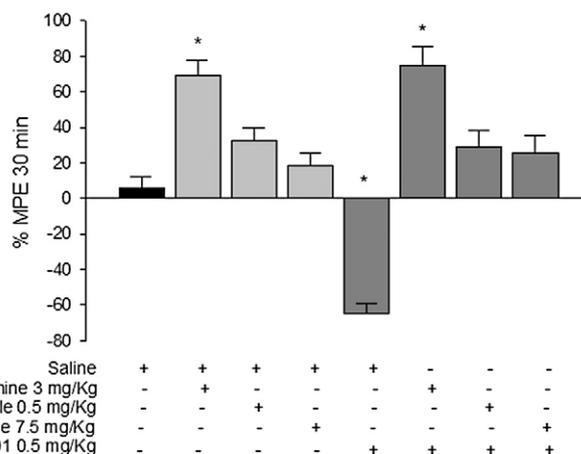


Fig. 2. Effects of i.p. vehicle (saline 0.9%)+vehicle, morphine (3 mg/kg)+vehicle, riluzole (0.5 mg/kg)+vehicle, guanosine (7.5 mg/kg)+vehicle, vehicle+MK-801 (0.5 mg/kg), riluzole+MK-801 or guanosine+MK-801 on tail flick latency 30 min after treatments. The columns represent % of Maximum Possible Effect (% MPE) and vertical bars represent SEM. $N=20$ animals per group. * = $P<0.01$ compared to control (saline), Kruskal–Wallis followed by Dunn's test.

from Tocris (Ballwin, MO, USA) and morphine from Cristália (São Paulo, Brazil).

2.3. Tail flick test

Nociception was assessed with a tail flick apparatus (Albrasch Electronic Equipments), as described in detail elsewhere (D'Amour and Smith, 1941). Briefly, a source of heat was positioned above the tail, focused on a point 2.3 cm rostral to the tip of the tail and the latency to withdraw the tail from the noxious luminous stimulus was automatically recorded. The light intensity was adjusted in order to obtain a baseline tail flick latency (TFL) of 3–4 s; a cut-off time of 10 s was employed in order to prevent tissue damage. A rat that did not flick by 10 s was considered as fully responsive to the analgesic. On day one, the animals were habituated with the tail flick apparatus through three separate measures (data not shown). On day two, baseline tail flick latency was measured for each rat prior to the treatments. Immediately after the third TFL measurement, the animals received an i.p. injection of vehicle (saline – NaCl 0.9%), morphine (3 mg/kg), guanosine (0.75, 2.5, or 7.5 mg/kg), MK-801 (0.01, 0.1 or 0.5 mg/kg) or riluzole (0.5 mg/kg). After 30 min, three new tail flick measures were taken at the tail flick apparatus. A separate group of animals received a pretreatment with vehicle, morphine (3 mg/kg), riluzole (0.5 mg/kg), or guanosine (0.75, 2.5 or 7.5 mg/kg) 15 min before MK-801 (0.5 mg/kg) administration. These groups were submitted to the tail flick 30 min after MK-801 administration. MK-801 doses and the 30 min endpoint for tail flick were adapted from elsewhere (Schmidt et al., 2000; Lara et al., 2001; Tort et al., 2004). Data are expressed as mean percent of Maximum Possible Effect (% MPE) \pm SEM, according to the following formula (Calcagnetti et al., 1990): % MPE: $100 \times (\text{post drug latency} - \text{baseline latency}) / (\text{cut-off time} - \text{baseline latency})$.

2.4. CSF sampling

Groups of rats were treated similarly with i.p. administration of vehicle, morphine (3 mg/kg), riluzole (0.5 mg/kg), or guanosine (0.75, 2.5 or 7.5 mg/kg) 15 min before MK-801 i.p. administration; 30 min after MK-801 treatment, rats were anesthetized with sodium thiopental (40 mg/kg, i.p.), placed in a stereotaxic apparatus, and CSF samples (40–60 μ L per rat) were drawn by direct puncture of the cisterna magna with an insulin syringe (27 gauge \times 1/2 in length) (Portela et al., 2002). In order to obtain cell-free supernatants, all samples were centrifuged at 10,000 g

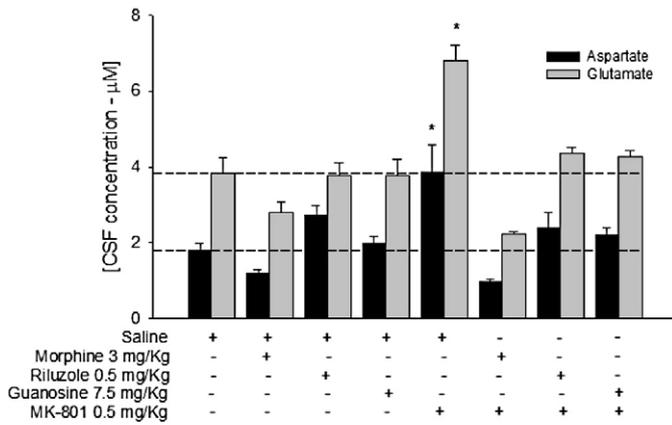


Fig. 3. Effects of i.p. vehicle (saline 0.9%)+vehicle morphine (3 mg/kg)+vehicle, riluzole (0.5 mg/kg)+vehicle, guanosine (7.5 mg/kg)+vehicle, vehicle+MK-801 (0.5 mg/kg), morphine+MK-801, riluzole+MK-801, or guanosine+MK-801 on CSF levels of aspartate and glutamate 30 min after treatments. The columns represent means (μM) and vertical bars represent SEM. Dashed lines represent control values. $N=10$ animals per group. * = $P<0.01$ compared to control (Veh+Veh), ANOVA followed by Tukey–Kramer’s test.

in an Eppendorf centrifuge during 5 min and samples stored ($-70\text{ }^{\circ}\text{C}$) until EAAs quantification by HPLC.

2.5. HPLC procedure

High-performance liquid chromatography (HPLC) was performed with CSF cell-free supernatant aliquots to quantify aspartate and glutamate levels (according to Joseph and Marsden, 1986). Briefly, samples were derivatized with *o*-phthalaldehyde and separation was carried out with a reverse phase column (Supelcosil LC-18, 250 mm \times 4.6 mm, Supelco) in a Shimadzu Instruments liquid chromatograph (50 μL loop valve injection). The mobile phase flowed at a rate of 1.4 mL/min and column temperature was 24 $^{\circ}\text{C}$. Buffer composition is A: 0.04 mol/L sodium dihydrogen phosphate monohydrate buffer, pH 5.5, containing 20% of methanol; B: 0.01 mol/L sodium dihydrogen phosphate monohydrate buffer, pH 5.5, containing 80% of methanol. The gradient profile was modified according to the content of buffer B in the mobile phase: 0% at 0.00 min, 25% at 13.75 min, 100% at 15.00–20.00 min, 0% at 20.01–25.00 min. Absorbance was read at 360 nm and 455 nm, excitation and emission respectively, in a Shimadzu fluores-

cence detector. Samples of 50 μL were used and concentration was expressed in μM (as mean \pm SEM).

2.6. Statistical analysis

Kruskal–Wallis followed by the post-hoc Dunn multiple comparisons test with chi-square distribution was used for behavioral data. One-way ANOVA followed by the post-hoc Tukey–Kramer multiple comparisons test was used for neurochemical data. Correlations are presented as Pearson’s coefficient. $P<0.05$ was considered for statistically significant differences.

3. Results

Fig. 1 shows the effects of each drug alone on tail flick latency in rats. As expected, morphine (3.0 mg/kg), which was taken as a positive control, suppressed nociceptive responses in the tail flick test ($P<0.001$). Neither guanosine (0.75, 2.5 or 7.5 mg/kg), MK-801 (0.1 or 0.01 mg/kg) nor riluzole (0.5 mg/kg) treatments altered tail flick latency in rats. However, MK-801 (0.5 mg/kg) induced an increase in nociceptive response ($P<0.01$), compatible with a hyperalgesic profile. As can be observed in Fig. 2, pretreatment with morphine (3 mg/kg), riluzole (0.5 mg/kg) or guanosine (7.5 mg/kg) prevented the MK-801-induced hyperalgesia ($P<0.01$). Note that guanosine and riluzole specifically counteracted MK-801-induced hyperalgesia, as their pain scores were not different from saline controls, in contrast to morphine, which caused analgesia (Fig. 2). Guanosine (0.75 or 2.5 mg/kg) also partially prevented MK-801-induced hyperalgesia (% MPE = 11.5 ± 7.1 and 21.6 ± 7.0 , respectively – $P<0.05$ – data not shown).

Fig. 3 shows that MK-801 induces a significant increase of CSF aspartate and glutamate levels, an effect completely prevented by pretreatment with morphine (3 mg/kg), guanosine (7.5 mg/kg) or riluzole (0.5 mg/kg) in doses that per se do not affect CSF aspartate and glutamate levels. Guanosine (0.75 or 2.5 mg/kg) also prevented MK-801-induced increase of CSF aspartate and glutamate levels (guanosine 0.75 mg/kg: $2.5 \pm 0.9\text{ }\mu\text{M}$ and $4.1 \pm 0.4\text{ }\mu\text{M}$, respectively – $P<0.05$ and guanosine 2.5 mg/kg: $1.9 \pm 0.5\text{ }\mu\text{M}$ and $5.1 \pm 0.5\text{ }\mu\text{M}$, respectively – $P<0.05$). MK-801 (0.01 or 0.1 mg/kg) did not alter CSF aspartate and glutamate levels.

Fig. 4 shows that there was a statistically significant correlation between CSF aspartate and glutamate levels and pain scores in animals that received MK-801 treatment with vehicle, morphine, guanosine or riluzole pretreatment (Pearson’s coefficient $r=0.556$;

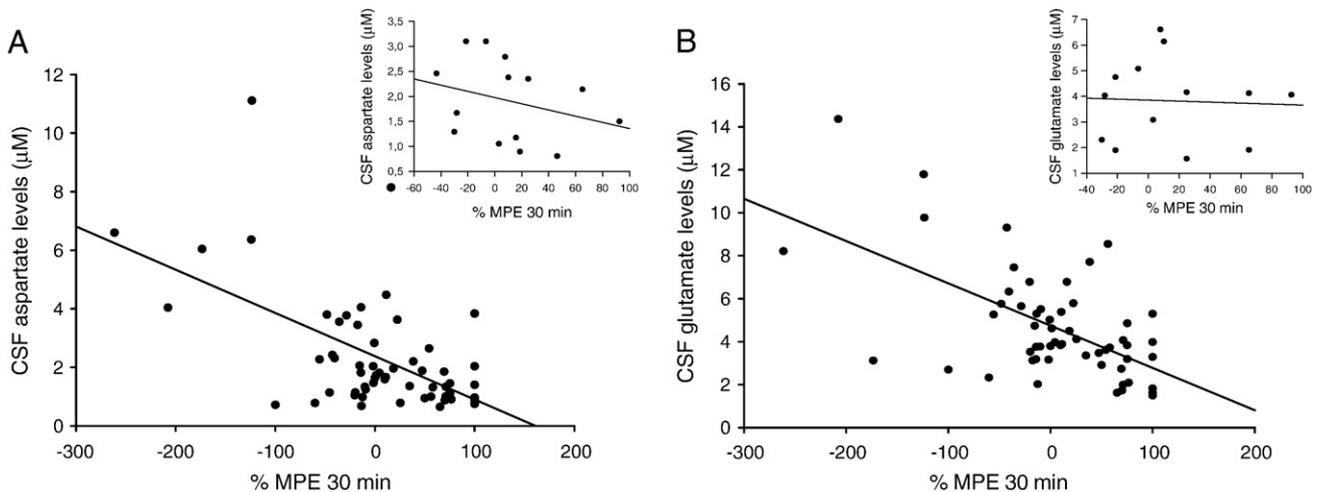


Fig. 4. Correlation between CSF levels of aspartate or glutamate and % of Maximum Possible Effect (% MPE) on the tail flick test. (A) Aspartate CSF levels (Pearson’s coefficient $r=0.556$; $P<0.0001$) (insert: control values – Pearson’s coefficient $r=0.29$; $P=0.314$); (B) glutamate CSF levels (Pearson’s coefficient $r=0.588$; $P<0.0001$) (insert: control values – Pearson’s coefficient $r=0.046$; $P=0.88$).

$P < 0.0001$ and $r = 0.588$; $P < 0.0001$, respectively). This correlation was not observed in the control group (Pearson's coefficient $r = 0.29$; $P = 0.314$ and $r = 0.046$; $P = 0.88$, respectively).

4. Discussion

The administration of MK-801 (0.5 mg/kg) to rats induced a hyperalgesic state in the tail flick test; this was prevented by pretreatment with morphine, guanosine or riluzole. Guanosine, riluzole or low doses of MK-801 did not affect the tail flick latency. Furthermore, this high dose of MK-801 induced an increase in CSF glutamate and aspartate levels; this was also prevented by pretreatment with morphine, guanosine or riluzole. A significant correlation was found between increased levels of CSF aspartate and glutamate and hyperalgesia induced by MK-801. These results support the notion that a CNS EAAs release may contribute to the atypical behavioral effects commonly induced by moderately high doses of MK-801.

The noncompetitive NMDA antagonist MK-801 has been shown to reduce nociceptive response in neuropathic pain models (Fisher et al., 2000). However, consistent with our data, some previous reports suggest that the NMDA antagonists are ineffective in the tail flick test (Lutfy et al., 1997; Suh et al., 2000; Zhao and Kamei, 1996). Activation of the non-NMDA receptors is necessary for transmission of phasic pain, whereas, activation of NMDA and/or non-NMDA receptors may be involved in mediation of tonic/chronic pain states (Lutfy et al., 1997).

Despite reducing glutamate neurotransmission at NMDA receptors, MK-801 may promote increased activation of non-NMDA receptors consequent to an increased efflux and reduced uptake of EAAs in the CNS (Longuemare et al., 1996). It has been suggested that the EAAs efflux could result from disinhibition of GABAergic or other inhibitory inputs to glutamatergic neurons (Moghaddam et al., 1997; Tort et al., 2004), or from effects on astrocytes not related to the NMDA receptor (Longuemare et al., 1996). Accordingly, Moghaddam et al. have demonstrated that NMDA antagonists provoke an increase in EAAs efflux in prefrontal cortex (PFC) and nucleus accumbens (NAc) (Moghaddam et al., 1997). In this context, non-NMDA-receptor antagonists, or inhibitors of glutamate/aspartate release and/or stimulators of glutamate uptake such as lamotrigine and riluzole have been shown to counteract the behavioral and neurochemical effects of NMDA-receptor antagonists (Anand et al., 2000).

In vitro, high doses of MK-801 inhibit glutamate uptake by both astrocyte and neuronal cultures and induce glutamate efflux from astrocytes. These effects seem to be related to a MK-801-induced membrane depolarization (Longuemare et al., 1996; Moghaddam et al., 1997). Previous reports have demonstrated that pathological conditions not only reduce the electrochemical driving force for glutamate uptake but also stimulate reversal of the transporter such that glutamate is released from the intracellular space to the extracellular space along with its concentration gradient (Longuemare et al., 1996). It is therefore possible that disruption of glutamate transport plays an important role in the increased extracellular EAAs availability and in the neurotoxic effects of MK-801. Likewise, the effects of high doses of MK-801 on EAAs uptake and release by glia and neurons (which could explain the increase in CSF EAAs levels) may contribute to the development of hyperalgesia in the tail flick test in the present study.

The nucleosides guanosine and adenosine interact closely in modulating the glutamatergic system. Given the central role of adenosine in pain transmission and modulation (Sawynok and Liu, 2003), a role for guanosine in pain transmission and nociception could also be considered. In the present study, guanosine did not alter nociception in the tail flick test. The tail flick test is a model designed to investigate phasic pain, mainly peripheral and spinally mediated responses (Lutfy et al., 1997). It remains to be determined whether guanosine has antinociceptive effects in chronic and neuropathic pain models, which actively depend on glutamatergic neurotransmission.

Guanosine and other guanine-based purine effects on the brain have been studied in several in vivo and in vitro models; they exhibit inhibition of glutamate (and analogs) binding (Baron et al., 1989; Souza and Ramirez, 1991), neuroprotection against excitotoxicity (Frizzo et al., 2002; Malcon et al., 1997; Regner et al., 1998), and anticonvulsant properties against glutamatergic agent-induced seizures in rodents (Lara et al., 2001; Schmidt et al., 2000, 2005; Soares et al., 2004; Vinadé et al., 2003, 2005). These anti-glutamatergic effects of guanosine are likely to result from glutamate removal from the synaptic cleft, an outcome of the guanosine-induced stimulation of astrocytic glutamate uptake (Frizzo et al., 2001, 2002, 2003). Since NMDA antagonists such as MK-801 may stimulate the efflux of glutamate and inhibit its uptake, with the overall effect contributing to the hyperalgesia observed in the tail flick test, we suggest that guanosine prevents MK-801-induced hyperalgesia by enhancing glutamate removal from the synaptic cleft, leading to less activation of non-NMDA glutamatergic receptors. Accordingly, we have previously shown that guanosine attenuates MK-801-induced potentiation of kainate toxicity (Lara et al., 2001) and selectively inhibits MK-801-induced hyperlocomotion without any effect on hyperlocomotion produced by amphetamine or caffeine (Tort et al., 2004). Deutsch et al. (2008) have recently shown that guanosine reduces MK-801-induced increase in voltage threshold for electrically-precipitated tonic hindlimb extension in unstressed mice. This modulatory effect was interpreted as resulting from an increased astrocytic glutamate uptake induced by guanosine and subsequent reduced proportion of open NMDA receptor-associated ion channels. It is noteworthy that MK-801 is an "open-channel blocker" and that its pharmacological effects are closely dependent on its access to the open-state channel where it binds to a specific hydrophobic domain (Deutsch et al., 2001). As we recently suggested (Schmidt et al., 2008), guanosine-induced enhancement of glutamate removal from the synaptic cleft, and consequent decrease of paradoxical non-NMDA glutamate receptors activation by MK-801, are alternative interpretations of such results.

Similarly to guanosine, riluzole stimulates glutamate uptake in rat spinal cord synaptosomes (Azbill et al., 2000). This raises the possibility that stimulated glutamate uptake, in addition to an inhibitory action on glutamate release, could contribute to the reversal of MK-801-induced hyperalgesia by riluzole showed here. This hypothesis was supported by the clear increase in CSF EAAs levels observed after high dose of MK-801, which was completely prevented by pretreatment with guanosine or riluzole. An additional interpretation of our results is that the nociceptive input, enhanced by MK-801, specifically caused the increased CSF EAAs, since pretreatment with morphine, an opioid receptor agonist, prevented MK-801-induced behavioral and neurochemical effects. However, there has been growing evidence that opioids also modulate the glutamatergic system, indirectly affecting glutamate receptors, transporters and its extracellular availability (Abarca et al., 2000; Niederberger et al., 2003; Schmidt and Schmidt, 2002; Yamamoto et al., 2003). Therefore, new studies are needed to further clarify the exact correlation between MK-801-induced neurochemical (increased extracellular EAAs availability) and atypical behavioral effects.

In conclusion, this study provides additional evidence for the mechanism of action of MK-801 in the CNS, showing that MK-801 induces hyperalgesia, with parallel increase in CSF EAAs levels in rats. These results suggest that some of the atypical behaviors induced by MK-801, and perhaps other NMDA antagonists, is related to a paradoxical increase of EAAs in the CNS. The prevention of MK-801 effects by guanosine, riluzole or morphine could result, at least partially, from a decrease in extracellular glutamate and aspartate availability. Since guanosine is an endogenous compound and apparently well tolerated and devoid of obvious CNS toxicity, we suggest that the usefulness of guanosine in the management of pain states associated with overstimulation of the glutamatergic system deserves to be further investigated.

Acknowledgments

The authors thank Vitor H. Cereser for technical assistance. This research was supported by the FINEP research grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” # 01.06.0842-00, CNPq, CAPES, FAPERGS, UFRGS.

References

- Abarca C, Silva E, Sepúlveda MJ, Oliva P, Contreras E. Neurochemical changes after morphine, dizocilpine or riluzole in the ventral posterolateral thalamic nuclei of rats with hyperalgesia. *Eur J Pharmacol* 2000;403:67–74.
- Al-Amin HA, Saadé NE, Khani M, Atweh S, Jaber M. Effects of chronic dizocilpine on acute pain and on mRNA expression of neuropeptides and the dopamine and glutamate receptors. *Brain Res* 2003;981:99–107.
- Anand A, Charney DS, Oren DA, Berman RM, Hu XS, Cappiello A, et al. Attenuation of the neuropsychiatric effects of ketamine with lamotrigine. *Arch Gen Psychiatry* 2000;57:270–6.
- Azbill RD, Mu X, Springer JE. Riluzole increases high-affinity glutamate uptake in rat spinal cord synaptosomes. *Brain Res* 2000;871:175–80.
- Baron BM, Dudley MW, McCarty DR, Miller FP, Reynolds IJ, Schmidt CJ. Guanine nucleotides are competitive inhibitors of *N*-methyl-D-aspartate at its receptor site both *in vitro* and *in vivo*. *J Pharmacol Exp Ther* 1989;250:162–9.
- Bennett GJ. Update on the neurophysiology of pain transmission and modulation: focus on the NMDA-receptor. *J Pain Symptom Manage* 2000;19:S2–6.
- Burgos JS, Barat A, Souza DO, Ramirez G. Guanine nucleotides protect against kainate toxicity in an *ex vivo* chick retinal preparation. *FEBS Lett* 1998;430:176–80.
- Caciagli F, Di Iorio P, Giuliani P, Middlemiss MP, Rathbone MP. The neuroprotective activity of guanosine involves the production of trophic factors and the outflow of purines from astrocytes. *Drug Dev Res* 2000;50:32.
- Calcagnetti DJ, Fleetwood SW, Holtzman SG. Pharmacological profile of the potentiation of opioid analgesia by restraint stress. *Pharmacol Biochem Behav* 1990;37:193–9.
- Ciccarelli R, Ballerini P, Sabatino G, Rathbone MP, D'Onofrio M, Caciagli M, et al. Involvement of astrocytes in purine-mediated reparative processes in the brain. *Int J Dev Neurosci* 2001;19:395–414.
- Dall'Igna OP, Silva AL, Dietrich MO, Hoffmann A, Oliveira RV, Souza DO, et al. Chronic treatment with caffeine blunts the hyperlocomotor but not cognitive effects of the *N*-methyl-D-aspartate receptor antagonist MK-801 in mice. *Psychopharmacology* 2003;166:258–63.
- D'Amour FE, Smith DL. A method for determining loss of pain sensation. *J Pharmacol Exp Ther* 1941;72:74–9.
- Deutsch SI, Rosse RB, Schwartz BL, Mastropaolo J. A revised excitotoxic hypothesis of schizophrenia: therapeutic implications. *Clin Neuropharmacol* 2001;24:43–9.
- Deutsch SI, Rosse RB, Long KD, Gaskins BL, Mastropaolo J. Guanosine possesses specific modulatory effects on NMDA receptor-mediated neurotransmission in intact mice. *Eur Neuropsychopharmacol* 2008;18:299–302.
- Fisher K, Coderre TJ, Hagen NA. Targeting the *N*-methyl-D-aspartate receptor for chronic pain management: preclinical animal studies, recent clinical experience and future research directions. *J Pain Symptom Manage* 2000;20:358–73.
- Frizzo MES, Lara DR, Dahm KCS, Prokopiuk AS, Swanson R, Souza DO. Activation of glutamate uptake by guanosine in primary astrocyte cultures. *NeuroReport* 2001;12:879–81.
- Frizzo MES, Lara DR, Prokopiuk AS, Vargas CR, Salbego CG, Wajner M, et al. Guanosine enhances glutamate uptake in brain cortical slices at normal and excitotoxic conditions. *Cell Mol Neurobiol* 2002;22:353–63.
- Frizzo MES, Soares FA, Dall'Onder LP, Lara DR, Swanson RA, Souza DO. Extracellular conversion of guanine-based purines to guanosine specifically enhances astrocyte glutamate uptake. *Brain Res* 2003;972:84–9.
- Frizzo ME, Dall'Onder LP, Dalcin KB, Souza DO. Riluzole enhances glutamate uptake in rat astrocyte cultures. *Cell Mol Neurobiol* 2004;24:123–8.
- Joseph MH, Marsden CA. Amino acids and small peptides. In: Lim CK, editor. HPLC of small peptides. Oxford: IRL Press; 1986. p. 13–27.
- Lara DR, Schmidt AP, Frizzo MES, Burgos JS, Ramirez G, Souza DO. Effect of orally administered guanosine on seizures and death induced by glutamatergic agents. *Brain Res* 2001;912:176–80.
- Longuemare MC, Keung EC, Chun S, Sharp FR, Chan PH, Swanson RA. MK-801 reduces uptake and stimulates efflux of excitatory amino acids via membrane depolarization. *Am J Physiol* 1996;270:C1398–404.
- Lutty K, Cai SX, Woodward RM, Weber E. Antinociceptive effects of NMDA and non-NMDA receptor antagonists in the tail flick test in mice. *Pain* 1997;70:31–40.
- Malcon C, Achaval M, Komlos F, Partata W, Sauregg M, Ramirez G, et al. GMP protects against quinolinic acid-induced loss of NADPH-diaphorase-positive cells in the rat striatum. *Neurosci Lett* 1997;225:145–8.
- Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* 1997;17:2921–7.
- Niederberger E, Schmidtko A, Rothstein JD, Geisslinger G, Tegeder I. Modulation of spinal nociceptive processing through the glutamate transporter GLT-1. *Neuroscience* 2003;116:81–7.
- Portela LV, Oses JP, Silveira AL, Schmidt AP, Lara DR, Battastini AM, et al. Guanine and adenine nucleotidase activities in rat cerebrospinal fluid. *Brain Res* 2002;950:74–8.
- Regner A, Ramirez G, Belló-Klein A, Souza DO. Effects of guanine nucleotides on glutamate-induced chemiluminescence in rat hippocampal slices submitted to hypoxia. *Neurochem Res* 1998;23:519–24.
- Sawynok J, Liu XJ. Adenosine in the spinal cord and periphery: release and regulation of pain. *Prog Neurobiol* 2003;69:313–40.
- Schmidt AP, Lara DR, Maraschin JF, Perla AS, Souza DO. Guanosine and GMP prevent seizures induced by quinolinic acid in mice. *Brain Res* 2000;864:40–3.
- Schmidt AP, Schmidt SR. How effective are opioids in relieving neuropathic pain? *Pain Clin* 2002;14:183–93.
- Schmidt AP, Ávila TT, Souza DO. Intracerebroventricular guanine-based purines protect against seizures induced by quinolinic acid in mice. *Neurochem Res* 2005;30:69–73.
- Schmidt AP, Lara DR, Souza DO. Proposal of a guanine-based purinergic system in the mammalian central nervous system. *Pharmacol Ther* 2007;116:401–16.
- Schmidt AP, Tort AB, Lara DR, Souza DO. Guanosine and its modulatory effects on the glutamatergic system. *Eur Neuropsychopharmacol* 2008;18:620–2.
- Soares FA, Schmidt AP, Farina M, Frizzo ME, Tavares RG, Portela LV, et al. Anticonvulsant effect of GMP depends on its conversion to guanosine. *Brain Res* 2004;1005:182–6.
- Souza DO, Ramirez G. Effects of guanine nucleotides on KA binding and on adenylate cyclase activity in optic tectum and cerebellum of chicken. *J Mol Neurosci* 1991;3:39–46.
- Suh H, Song D, Huh S, Kim YH. Differential potentiative effects of glutamate receptor antagonists in the production of antinociception induced by opioids administered intrathecally in the mouse. *Brain Res Bull* 2000;52:143–50.
- Tort AB, Mantese CE, Anjos GM, Dietrich MO, Dall'Igna OP, Souza DO, et al. Guanosine selectively inhibits locomotor stimulation induced by the NMDA antagonist dizocilpine. *Behav Brain Res* 2004;154:417–22.
- Vinadé ER, Schmidt AP, Frizzo MES, Izquierdo I, Elizabetsky E, Souza DO. Chronically administered guanosine is anticonvulsant, amnesic and anxiolytic in mice. *Brain Res* 2003;977:97–102.
- Vinadé ER, Schmidt AP, Frizzo ME, Portela LV, Soares FA, Schwalm FD, et al. Effects of chronic administered guanosine on behavioral parameters and brain glutamate uptake in rats. *J Neurosci Res* 2005;79:248–53.
- Yamamoto S, Nakanishi O, Matsui T, Shinohara N, Kinoshita H, Lambert C, et al. Intrathecal adenosine A1 receptor agonist attenuates hyperalgesia without inhibiting spinal glutamate release in the rat. *Cell Mol Neurobiol* 2003;23:175–85.
- Zhao GM, Kamei J. Effect of antagonism of the NMDA receptor on tolerance to [D-Pen₂, D-Pen₅]enkephalin, a delta 1-opioid receptor agonist. *Peptides* 1996;17:233–6.
- Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983;16:109–10.