The Role of the Guanine-Based Purinergic System in Seizures and Epilepsy

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Abstract: Guanine-based purines have been traditionally studied as modulators of intracellular processes, mainly G-protein activity. However, more recently, several studies have shown that they exert a variety of extracellular effects not related to G-proteins, including trophic effects on neural cells, modulation of glutamatergic activity, behavioral effects and anticonvulsant activity. In this article, the putative effects of the guanine-based purines against seizures and neurotoxicity are reviewed. Current evidence suggests that guanine-based purines, especially guanosine, seem to be endogenous anticonvulsant substances, perhaps in a similar way to the adenine-based purines. Although studies addressing the mechanism of action of guanine-based purines are still lacking, their anticonvulsant activity is probably related to the modulation of several glutamatergic parameters, especially the astrocytic glutamate uptake. These findings point to the guanine-based purines as potential new targets for the development of novel drugs for neuroprotection and management of epilepsy.

Keywords: Guanine-based purines, seizures, epilepsy, adenine-based purines, guanosine, purines, neuroprotection, glutamate.

1. INTRODUCTION

The purinergic system usually relates to the adenine-based purines, including the nucleotides adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP) and adenosine 5'-monophoshate (AMP), and the nucleoside adenosine. Adenine-based purines exert several biological roles, including the pivotal role on energy metabolism. Extracellular adenosine and ATP are usually considered the major endogenous effectors of the purinergic system, acting on P1 and P2 receptors, respectively [1]. However, guanine-based purines, namely the nucleotides guanosine 5'-triphosphate (GTP), guanosine 5'-diphosphate (GDP) and guanosine 5'-monophosphate (GMP) and the nucleoside guanosine also are a relevant component of the purinergic system.

Traditionally, guanine-based purines have been studied as intracellular modulators of signal transduction processes, modulating the activity of G-proteins [2]. Nevertheless, more recently, guanine-based purines have been shown to exert relevant extracellular effects, including those related to the modulation of the glutamatergic system [3-12]. Although the exact mechanism underlying these extracellular effects remains unclear, it does not seem to involve a direct modulation of G-proteins [12]. Guanine-based purines were shown to inhibit the binding of glutamate and analogs [3,6,7,13], to be neuroprotective under excitotoxic conditions [14-16], as well as anticonvulsant against seizures induced by glutamatergic agents [10,11,17-20]. Notably, the effects of guanine-based purines on animal models of seizures were one of the first direct and reliable evidences of an *in vivo* modulation of the glutamatergic system by those substances [6,11].

In this article, the putative effects of the guanine-based purines in seizures and neurotoxicity are briefly reviewed, with emphasis on their potential role in neuroprotection and epilepsy management.

2. GUANINE-BASED PURINERGIC SYSTEM

2.1. Historical Overview

In 1971, the first evidence for a more complex class of signaling pathway emerged establishing that the sensor and intracellular effector are separate proteins that communicate through proteins called guanine nucleotide-dependent regulatory proteins, GTP binding proteins or G-proteins [21]. G-proteins alternate between active GTP-bound and inactive GDP-bound forms. Activation is catalyzed by receptors and deactivation by an intrinsic property of G-proteins, its GTPase activity. G-proteins couple cell surface receptors to cellular effectors, modulating cell responses to external stimuli: the interaction of agonists with their receptors triggers the binding of GTP to G-proteins, forming an active complex G-protein/GTP, which simultaneously modulates the activity of effector systems and decreases the agonist binding to specific receptors [2].

Recently, it has become increasingly recognized that guanine-based purines have also important extracellular signalling effects, including *in vitro* inhibitory effects on the activity of the glutamatergic system, trophic effects on neural cells, neuroprotection against ischemic insults, effects on learning and memory, modulation of pain pathways, anticonvulsant effects and other behavioral alterations [reviewed in ref. 12]. Before reviewing the putative aspects of guanine-based purines on seizures and epilepsy animal models, we

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will summarize some aspects related to the synthesis and metabolism of the main constituents of the guanine-based purinergic system.

2.2. Synthesis and Metabolism of Guanine-Based Purines

Extracellular purine nucleosides and nucleotides are ubiquitous intercellular messengers, which may affect several biological functions, largely acting as neurotransmitters or neuromodulators [1,16]. Several lines of evidence indicate that guanine-based purines, mainly the nucleotide GTP and the nucleoside guanosine, act as intercellular signalling molecules, as well as their counterparts ATP and adenosine [16,22]. In the nervous system guanine-based purines mediate both immediate effects, such as neurotransmission, and trophic effects which induce changes in cell metabolism, structure and function.

Adenine- and guanine-based purines share some metabolic steps (i.e., nucleoside transporters and ecto-nucleotidases). Therefore, it is tempting to propose that guanine- and adenine-based purines may respond similarly in certain conditions. Astrocytes are the main sources of extracellular adenine- and guanine-based purines in the central nervous system (CNS) [22]. They are largely involved in several brain functions in physiological conditions, participating in neuronal development, synaptic activity, homeostatic control of the extracellular environment and also in processes related to brain injuries, by arresting and repairing further neural damage [23,24]. Additionally, growing evidence indicates that purines are widely involved in the molecular mechanisms underlying the multiple functions of astrocytes, either exerting their influence on key intracellular activities (energy metabolism and nucleic acid synthesis) or activating a variety of membrane receptors [25]. Cultured astrocytes release guanine-based purines, a process that is importantly augmented after hypoxic and low glucose insults [22]. Interestingly, the release of guanine-based purines seems to be larger than that observed for adenine-based purines [22]. Actually, guanine-based purines are released in amounts about 3-fold greater that their adenine-based counterparts and the amounts of guanine-based purines, especially guanosine, is further augmented when the cells are exposed to a brief period of hypoxia/hypoglycemia [22]. Notably, purine nucleosides and nucleotides are extensively released from degenerating cells, particularly under hypoxic and ischemic conditions. This latter is particularly relevant in the CNS, where purines may depress neurotransmission, thus reducing excitotoxic neuronal damages [26], and may regulate the responses of nervous tissue to injury.

The presence of adenine- and guanine-based purines and their metabolites in human and animal cerebrospinal fluid (CSF) has been well described [27-30]. Astrocytes, as well as neurons, are responsible for both nucleoside metabolism and uptake of the nucleosides adenosine and guanosine [31]. Following their release, essentially from astrocytes, the extracellular levels of the various purine nucleotides and nucleosides are regulated by the activities of cell surface-located enzymes, which have the same function of the corresponding intracellular enzymes [32]. The enzymes involved in extracellular nucleotide hydrolysis include membrane-bound ecto-nucleotidases, ecto-nucleotidases released from membranes and the naturally occurring soluble nucleotidases. These enzymes, in association with ecto-5'-nucleotidase, hydrolyze extracellular nucleotides in a stepwise fashion down to nucleosides and are crucial for physiological modulation of CNS functions, as well as for the purine-induced neuroprotection [33]. Several such enzymes (ectonucleotidases) comprise the ecto-nucleoside triphosphatase (E-NTPase) family. E-NTPases include the ecto-ATPase that preferentially converts ATP into ADP; the ecto-ATP diphosphohydrolase (ectoapyrase) that hydrolyses either ATP or ADP, and the ecto-5'-nucleotidase that catalyses the hydrolysis of AMP to adenosine [34,35]. Nevertheless, the selectivity for adenine-based purines is not complete, as these enzymes also hydrolyse all purine and pyrimidine nucleotides, including guanine-based purines. In regard to the nucleosides, adenosine is deaminated by adenosine deaminase (ADA) and guanosine is converted to guanine by guanase. These soluble nucleotidases are also present and active in rat CSF [36,37], where they hydrolyze all guanine and adenine nucleotides with the following order of catalytic efficiency: GDP > ADP = ATP = GTP > AMP = GMP [30]. Interestingly, at high concentrations, GDP hydrolysis rate is greater than that of ADP, perhaps favoring the accumulation of GMP and guanosine. In fact, these enzymes can be released to the extracellular space (CSF) from choroid plexus, endothelial cells or even microglia and play an important regulatory role of the purinergic system under physiological and pathological conditions [34,35]. Notably, in cultured astrocytes, inhibition of ecto-5'-nucleotidase activity significantly impaired accumulation of extracellular guanosine, indicating that, similar to extracellular adenosine, it is to some extent derived from the extracellular metabolism of guanine nucleotides [38].

Nucleosides are also removed from the extracellular space into neurons and glia by transporter systems. Uptake of purine and pyrimidine nucleosides by astrocytes is also important for nucleic acid synthesis and synthesis of AMP, ADP, and ATP from adenosine and GTP from guanosine [39]. Peng *et al.* [40] has identified two equilibrative nucleoside transporters in astrocytes (ENT_1 and ENT_2), together with the concentrative nucleoside transporter (CNT_2) responsible for nucleoside uptake. Interestingly, an equilibrative nucleoside transporter (ENT_1) was also recently identified in the rat brain endothelial cells and choroid plexus epithelial cells, indicating a more ubiquitous distribution of the purine nucleoside transmembrane transport system [41].

Growing evidence suggests that guanine-based purines interact at the level of signal-transduction pathways with other transmitters, for example, glutamate and GABA [42]. It is commonly accepted that purines production in the brain is closely related to the release of neurotransmitters and that K^+-induced depolarization evokes the release of endogenous neurotransmitters which, in turn, promote purine outflow [43,44]. Released nucleotides and nucleosides are considered to act as retrograde synaptic transmitters, modulating the release of several putative neurotransmitters, including glutamate and GABA. However, to date, little is known about the potential influence of guanine-based purines on neuronal function and synaptic plasticity and new studies are warranted.

Regarding the CNS guanosine bioavailability, a single intracerebroventricular (i.c.v.) administration of GMP in mice

causes a large increase in the CSF levels of GMP, guanosine and oxypurines (hypoxanthine, xanthine and uric acid) [45]. We also observed that intraperitoneal (i.p.) administration of GMP in anticonvulsant doses produced a 3-fold increase of CSF levels of guanosine in rats, not affecting GMP levels [46]. Intracerebroventricular administration of guanosine did not affect GMP and hypoxanthine CSF levels, in spite of causing a significant increase in guanosine, xanthine and uric acid CSF levels [45]. Intrathecal (i.t.) administration of guanosine produced a significant increase in guanosine, inosine, xanthine and uric acid CSF levels [47]. Notably, the significant increase of CSF concentration of oxypurines after i.c.v. or i.t. injection of guanosine probably indicates an *in vivo* degradation. Additionally, animals treated with oral guanosine in anticonvulsant doses presented a 2-fold increase in CSF concentration of guanosine as compared to control [19]. Intraperitoneal or oral administration of high-dose guanosine (up to 120 $mg.kg^{-1}$) produced a 6.8 or 7.8-fold increase in guanosine CSF levels, respectively [48]. Importantly, guanosine CSF levels remained increased up to 360 min after a single i.p. administration of guanosine in rats [49]. However, systemic guanosine did not affect the CSF levels of inosine, oxypurines and adenine-based purines [48]. Notably, a previous study has demonstrated that an i.p. administration of guanosine increased the amounts of both guanosine and guanine in the spinal cord, with a peak around 30 min [50]. Considering that extracellular guanine also exerts several biological effects [51,52], it is tempting to propose that some biological effects of guanosine may be regulated by its conversion to guanine by a membrane located purine nucleoside phosphorylase.

2.3. Effects of Guanine-Based Purines Against Seizures and Toxicity in Animals

Glutamate is the major excitatory neurotransmitter in the mammalian CNS, participating in plastic processes involved in learning and memory [53], development and aging [54] and environmental adaptation [55]. However, glutamate may also be a potent neurotoxin and overstimulation of the glutamatergic system (by exogenous or endogenous stimuli), which occurs when extracellular glutamate levels increase over the physiological range, has been implicated in the pathogenesis of various acute and chronic CNS disorders [56-58]. Consequently, the equilibrium between the physiological and pathological glutamatergic tonus is essential for brain function and its disruption is related with the pathogenesis of various CNS disorders including the epilepsies [56,59-61].

It is now clearly shown that glutamatergic excitotoxicity is prevented by astrocytic glutamate uptake, a process responsible for maintaining the extracellular glutamate levels below toxic levels [23,58,61]. Since adenosine decreases glutamate release and guanosine increases glutamate uptake (and persists for longer periods of time extracellularly), both purine nucleosides may act in concert to reduce the impact of glutamate-induced excitability. This issue might be especially important in the endogenous and exogenous modulation of glutamate-related seizures. Glutamate undoubtedly plays a pivotal role on epilepsy and probably in other CNS disorders precipitating seizure activity [56-58,62,63]. However, the cellular and molecular mechanisms involved in the generation and maintenance of seizures and toxicity are not fully understood.

Guanine nucleotides, intracerebroventricularly administered, had long been shown to prevent seizures induced by quinolinic acid, a toxin that overstimulates the glutamatergic neurotransmission [6]. This effect was compatible with the antagonistic properties of guanine nucleotides on glutamate receptors being studied in our group [3]. However, after further exploring the interaction of guanine nucleotides with glutamate, we observed that besides the administration of

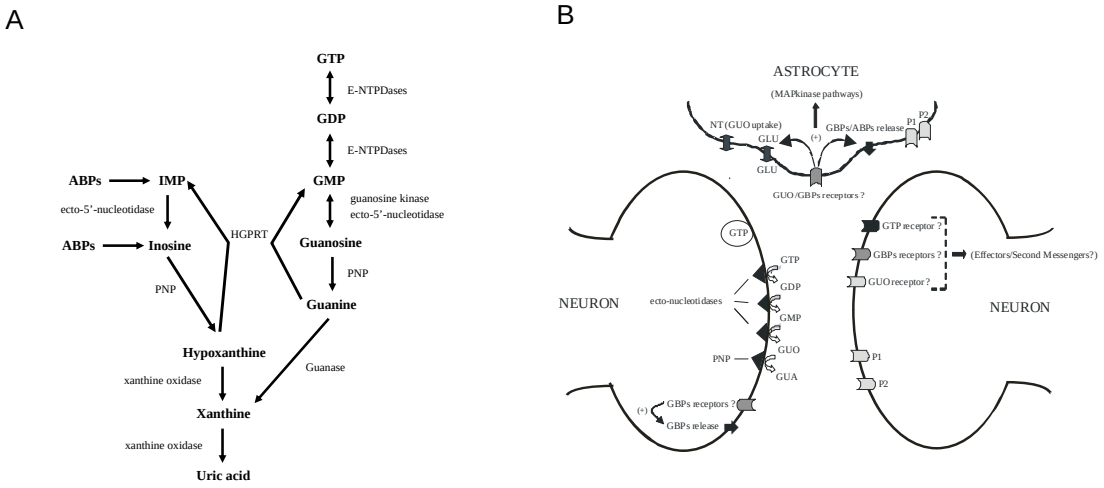

Fig. (1). Panel **A**: Schematic model of the sources and different enzymes pathways of extracellular guanine-based purines. ABPs = adenine-based purines; E-NTPDases = ecto-nucleotide-diphosphohydrolase; PNP = purine nucleoside phosphorylase; HGPRT = hypoxanthine-guanine phosphoribosyltransferase. Panel **B**: Schematic representation of the guanine-based purinergic system. Once released from astrocytes, guanine-based purines may interact with its specific receptors on neurons and astrocytes, exerting trophic effects and modulating neurotransmitters release and uptake. "?" is referred to the potential existence of specific receptors for guanine-based purines; MAPkinase = Mitogen-activated protein kinase; NT = nucleoside transporters; GUO = guanosine; GUA = guanine; GLU = glutamate; GBPs = guanine-based purines.

GMP, guanosine also prevented seizures induced by quinolinic acid [11]. Later we showed that a single i.c.v. administration of GTP and GDP was also protective against seizures induced by quinolinic acid in mice [18]. Notably, we also observed that single and chronic oral administrations of guanosine were also effective in the same seizure model [10]. Importantly, quinolinic acid is a direct NMDA agonist, but also stimulates glutamate release and inhibits its uptake [64]. Additional studies also provided evidence that guanosine and GMP administered intracerebroventricularly, intraperitonially or orally dose-dependently protected against seizures induced by the other glutamatergic agents such as kainic acid and α-dendrotoxin in adult and young rodents [17,19,20,46,65,66]. The fact that chronic oral exposure to guanine-based purines produced anticonvulsant effects indicates that these substances are orally active in the long term and points to a future target for new antiepileptic drugs in adults and children. Of note, chronically administered GMP had also been shown to be neuroprotective against quinolinic acid-induced striatal neuronal cell death in rats [15], strengthening the notion that the guanine-based purinergic system may be a valuable target for the treatment of neurodegenerative disorders. Table **1** summarizes the main studies addressing the effects of guanine-based purines on seizures and toxicity induced by glutamate and its analogs *in vitro* and *in vivo*.

Guanine-based purines, mainly GMP and guanosine, have usually presented similar neuroprotective profile in several *in vivo* and *in vitro* protocols [10,11,18-20,64]. However, most effects of nucleotides (mainly GMP) seemed to be due to their conversion to guanosine. Specifically for seizures, an acute i.c.v. administration of the ecto-5'-nucleotidase (enzyme that converts GMP to guanosine) inhibitor AOPCP prevented the anticonvulsant effects of GMP against quinolinic acid in rats, without affecting the effect of guanosine [46]. Moreover, we demonstrated that anticonvulsant effects of i.c.v. GTP and GDP seemed to be mediated by their conversion to guanosine, since their poorly hydrolysable analogs GTPγS, GppNHp and GDPβS were not capable of preventing seizures induced by quinolinic acid in mice [18]. Of note, a recent study demonstrated that GMP-induced antinociception was also prevented by AOPCP, corroborating with anticonvulsant effects [45].

Although the behavioral effects of guanosine in preventing quinolinic acid-induced seizures have been well described [10,11,17,18], little is known about its electrophysiological effects in the brain. Recently, we performed the first study addressing this issue based on epidural electroencephalogram (EEG) recordings of rats [67]. In addition to clear EEG changes occurring during the seizure events, we found that quinolinic acid disrupted a prominent basal theta (4-10 Hz) activity during peri-ictal periods and promoted a relative increase in the power level at the gamma band; guanosine, when successfully preventing seizures, counteracted this effect following quinolinic acid administration. Interestingly, we observed that MK-801, a known NMDA-antagonist used as a positive control, presented different spectral effects when compared to guanosine in rats protected against quinolinic acid-induced seizures, producing large gamma oscillations following quinolinic acid administration [67]. Additionally, the combined pre-treatment with both guanosine and MK-801 in this model led to qualitatively different results than the observed when each drug was administered alone [67]. Considering these recent evidences, we suggest that a diverse mechanism of action between both drugs (guanosine versus MK-801 or perhaps other NMDA antagonists) exists and that guanosine might be related to a lower incidence of cognitive side effects than NMDA antagonists in the clinical setting.

It is well known that guanine-based purines, mainly guanosine, are protective against quinolinic acid-induced seizures in a dose-dependent manner [11,18]. However, it still remains puzzling why these substances are not effective, even in high doses, in preventing quinolinic acid-induced seizures in 100% of the cases. In previous studies, we have found that the most effective doses of guanine-based purines accounts for nearly 50% of protection in the quinolinic acid-induced seizures model [10,11,18,67]. Importantly, Torres *et al*. [67] observed that this somewhat partial anticonvulsant effect does not seem to be related to individual differences among animals, suggesting that dynamic variables within rats are probably determining whether guanine-based purines will successfully prevent seizures or not. Importantly, this study has demonstrated that protected and non-protected animals under guanosine treatment can be distinguished electrophysiologically, even before the beginning of the motor seizures. Accordingly, we found that the level of theta power greatly decreased in animals displaying seizures under guanosine treatment, both before and after the seizure event, similarly to what we observed in vehicle pretreated rats. On the other hand, animals successfully protected by guanosine exhibited a higher level of theta power than animals displaying seizures. It is possible that these variables are related to pharmacokinetic and pharmacodynamic factors, although it could also be related to the current internal state of the brain.

Besides epilepsy and general seizure behavior, brain ischemia is responsible for significant morbidity and mortality and significant resources have also been dedicated to developing new neuroprotective strategies. Since adenine-based purines have been demonstrated to play a role in endogenous neurodegenerative and neuroprotective processes [68], guanine-based purines could also be investigated for possible therapeutic manipulations. As stated above (section 2.2), using primary cultures of astrocytes prepared from the rodent cerebral hemispheres, it was shown that they spontaneously release guanine-based purines even in basal conditions. Interestingly, the amount of guanine-based purines (especially guanosine) released over a 3-hour period was greater than that of adenine-based purines [22]. Moreover, the exposure of these cultures to hypoxia/low glucose levels resulted in sustained increase in the release of guanine- and adenine-based purines over basal values up to 90 minutes after the insult. Notably, the release of purines was not related to an artifact of diminished cell viability [22]. These effects in an *in vitro* ischemia/stroke model are consistent with the hypothesis that these compounds may exert pivotal modulatory effects on synaptic transmission and more sustained trophic effects.

Guanine-based purines have also recently been shown to play a role in the Lesch-Nyhan syndrome [69] and perhaps in several other neurodegenerative diseases [70,71]. The rationale for this hypothesis is based on clinical and biochemical characterization of some neurological disorders such as

Table 1. Summary of Main Experimental Studies Investigating the Guanine-Based Purines on Seizures and Glutamatergic Toxicity

***In Vitro* Studies**	
Inhibit kainic acid binding	Souza and Ramirez, 1991[3]; Paz *et al*., 1994[7]; Ramos *et al*., 1997[9]
Prevent cell responses to excitatory amino acids	Burgos *et al*., 1998[4]; 2000[5]
Inhibit glutamate and its analogs binding	Rubin *et al*., 1997[52]; Baron *et al*., 1989[6]
Prevent NMDA-induced excitotoxicity	Baron *et al*., 1989[6]; Molz *et al*., 2008[100]; Caciagli *et al*., 2000[38]
Stimulate glutamate uptake by astrocytes	Frizzo *et al*., 2001[107]; 2003[102]; Gottfried *et al*., 2002[112]
Stimulate the glutamate uptake by synaptic vesicles	Tasca *et al*., 2004[8]
Stimulate glutamate uptake in brain slices	Frizzo *et al*., 2002[14]; 2005[110]; Thomazi *et al*., 2004[113]
Prevent the decrease of glutamate uptake induced by hypoxic-ischemic insult	Moretto *et al*., 2005[114]
Prevent quinolinic acid-induced release of glutamate on synaptosomes	Tavares *et al*., 2005[65]
Prevent neural apoptosis	Di Iorio *et al*., 2004[70]; Pettifer *et al*., 2004[71]
Preserve neural viability in mouse spinal cord cultures during chemical hypoxia	Litsky *et al*., 1999[123]
Protect glial cells against glucose deprivation and mitochondrial inhibition	Jurkowitz *et al*., 1998[124]
Reduce apoptosis and stimulates neurogenesis in rats with parkinsonism	Su *et al*., 2009[52]
Reduce apoptosis and inflammation in rats with acute spinal cord injury	Jiang *et al*., 2007[134]
***In Vivo* Studies**	
Prevent quinolinic acid-induced seizures in mice	Schmidt *et al*., 2000[11]; 2005[18]; Lara *et al*., 2001[10]
Prevent quinolinic acid-induced seizures in young rats	de Oliveira *et al*., 2004[17]
Prevent quinolinic acid-induced seizures in adult rats	Soares *et al*., 2004[46]
Prevent quinolinic acid-induced neural death	Malcon *et al*., 1997[15]
Prevent seizures induced by several glutamatergic agents in rodents	Lara *et al*., 2001[10]; Vinadé *et al*., 2003[20]
Electrophysiological effects of guanosine against quinolinic acid-induced seizures in rats	Torres *et al*., 2010[67]
Induce transitory amnesia in rats	Roesler *et al*., 2000[77]; Saute *et al*., 2006[79]
Induce transitory amnesia in mice	Vinadé *et al*., 2003[10]; Vinadé *et al*., 2004[78]
Prevent the facilitatory effect of glutamate on memory in rats	Rubin *et al*., 1996[76]
Chronic treatment is anxiolytic in mice	Vinadé *et al*., 2003[20]
Attenuate hyperlocomotion induced by MK-801	Tort *et al*., 2004[80]
Prevent MK-801-induced hyperalgesia	Schmidt *et al*., 2009[83]
Prevent in vivo decrease of astrocytic glutamate uptake induced by quinolinic acid	Vinadé *et al*., 2005[19]
Improve locomotor function and remyelination in rats submitted to a spinal cord injury model	Jiang *et al*., 2003[135]; 2007[134]
Produce antinociceptive effects against glutamatergic pain models in rodents	Schmidt *et al*., 2008[45]; 2009[47,49]; 2010[48]
Improve motor behavior in rats with parkinsonism	Su *et al*., 2009[52]
Neuroprotective effects against stroke	Chang *et al*., 2008[136]

Lesch-Nyhan syndrome and the neurobiological consequences of the hypoxanthine phosphoribosyltransferase (HPRT) deficiency. Conceivably, diminished reutilization of free guanine bases due to absent or reduced HPRT activity and relatively high guanase activity in the brain could lead to deficient endogenous pools of guanosine associated with

glutamatergic synapses. These issues demonstrated the potential roles that guanine-based purines play in neurodevelopment and as neuromodulators. Nevertheless, these findings remain to be further investigated but strongly suggest guanine-based purines as potential drug targets in the experimental therapy of neuroinflammatory and neurodegenerative diseases.

Considering that purines, their metabolites and the soluble nucleotidases responsible for their hydrolysis are detected in the human and animal CSF and blood serum [27,29,36,72,73], and their potential role on an "endogenous neuroprotection system", it is possible that these parameters may be new putative markers of CNS injury. We have demonstrated that pentylenetetrazol-induced seizures promote an increase in CSF nucleotidases activity represented by further hydrolysis of GDP and ADP and an increase in concentration of guanosine and inosine (probably related to quick degradation of adenosine to inosine) 30 minutes after the insult [74]. Increases of GDP/ADP hydrolysis and levels of nucleosides guanosine/inosine after pentylenetetrazol-induced seizures presented a somewhat similar profile to other well-known brain injury markers (S100β-protein and neuron-specific enolase - NSE). This temporal similarity suggests that those compounds could become biochemical brain markers to evaluate neural injury.

2.4. Behavioral Effects of Guanine-Based Purines

It has been well demonstrated for several glutamate antagonists, mainly NMDA-receptor antagonists, that they may induce amnesia and severe locomotor deficits in animals [75]. It is well documented that glutamate plays a key role on memory mechanisms [53], and previous studies demonstrated that GMP was able to reverse the facilitatory effect of post-training intra-hippocampal glutamate administration on inhibitory avoidance task performance in rats [76]. Further studies demonstrated that GMP and guanosine are capable to modulate memory processes since pretraining administration of both guanine-based purines impaired retention of inhibitory avoidance responses in rats [20,77]. The guanine-based purine effects on memory were reproduced with anticonvulsant doses after acute/chronic i.p./oral administration and adenosine-receptor antagonists failed to prevent these effects [78]. Furthermore, the amnesic effect related to the pretreatment with GMP also depended on its conversion to guanosine [79]. These findings suggest an amnesic effect of guanosine on inhibitory avoidance in rodents, in a pattern compatible with inhibition of glutamatergic activity and independent of adenosine A_1 and A_{2A} receptors.

Most studies have indicated that guanosine *per se* does not affect spontaneous locomotion in rodents [9,20,80]. Additionally, no obvious motor disturbance or sedative effects were observed since acute or chronic administration of guanine-based purines did not alter rotarod and open field performance, as evidenced with other glutamate antagonists such as MK-801 [9,20]. Interestingly, NMDA-receptor antagonists have been related to significant locomotor disturbances [81]. Recent evidence suggest that NMDA receptor antagonism may be associated with glutamatergic activation of non-NMDA glutamatergic receptors induced by increased glutamate release, which appears to be closely related to those behavioral alterations [82]. Therefore, despite of reducing glutamatergic effects at NMDA receptors, NMDA-receptor antagonists may stimulate non-NMDA receptors by increasing the release of glutamate. Interestingly, guanosine produced an approximately 60% attenuation of hyperlocomotion induced by MK-801 (a non-competitive NMDA-receptor antagonist), whereas it did not affect the hyperlocomotion induced by the indirect dopamine agonist amphetamine or by the non-selective adenosine-receptor antagonist caffeine [80]. Additionally, we have recently observed that MK-801 induces paradoxical hyperalgesia in the rat tail-flick paradigm, a behavior effect significantly correlated with increased CSF levels of the excitatory amino acids glutamate and aspartate [83]. Notably, guanosine prevented both neurochemical and behavioral effects induced by MK-801 [83]. The attenuation of some behavioral effects of MK-801 by guanosine (locomotor and nociceptive effects) may be related to an increase of glutamate uptake by astrocytes promoted by guanosine, reducing glutamate levels at the synaptic cleft and leading to less activation of non-NMDA receptors [83,84].

More recently, our group has demonstrated that guanine-based purines produce consistent antinociceptive effects against several pain models, including those based on thermal or chemical stimuli [45,47-49,84,85]. These antinociceptive effects were investigated by using the nucleotide GMP and the nucleoside guanosine, but other guanine-based purines might cause such effects as well. Interestingly, we also demonstrated that GMP-induced antinociception was prevented by the ecto-5'-nucleotidase inhibitor α,β-methyleneadenosine 5'-diphosphate (AOPCP), suggesting that its effects result from conversion to guanosine [45]. Our studies clearly demonstrated that i.c.v., i.t. or systemically (i.p.) administered guanosine produces significant inhibition of pain-related behavior induced by several algogens in mice [45,47-49,84,85]. Additionally, i.t. or i.p. guanosine prevents biting behavior induced by i.t. administration of glutamate and non-NMDA agonists, but it was not effective against NMDA [47,48]. We also demonstrate that these antinociceptive effects may involve some adenosine receptors (A_1 and A_{2A}) and spinal cord glutamate uptake [48].

The contribution of adenosine A_1 and A_{2A} receptors to the effects of guanosine has also been ruled out in some behavioral studies. The adenosine antagonist caffeine failed to inhibit the anticonvulsant effect of an acute orally administration of guanosine on quinolinic acid-induced seizures in mice [10] or the amnesic effect of guanosine in rats [77,78]. Conversely, more recently, we demonstrated that a pretreatment with non-selective (Caffeine) and selective A_1/A_{2A} receptor antagonists (DPCPX and SCH58261) significantly affected guanosine-induced nociception [48]. Allopurinol-induced antinociception, an event related to the accumulation of guanosine and adenosine in the CSF, was prevented by caffeine and DPCPX as well [85]. Therefore, at least for antinociception, adenosine receptors seem to be relevant to guanine-based purine effects, but further work is warranted.

2.5. Insights into the Mechanism of Action of Guanine-Based Purines

It has been classically demonstrated that by acting *via* G-proteins, GTP is able to simultaneously inhibit binding of neurotransmitters (and their agonists) to metabotropic recep-

tors and modulate adenylate cyclase activity [2,86]. However, we have demonstrated that the effects of guanine nucleotides on kainic acid binding site and on adenylate cyclase activity could be dissociated [3]. In lysed membrane preparations, the guanine nucleotides GMP, GDP and GTP were able to inhibit the binding of kainic acid with the same efficiency, whereas only GTP was able to stimulate cell membrane adenylate cyclase activity. However, in vesicular preparations, all guanine nucleotides were still able to inhibit binding of kainic acid, whereas GTP lost the ability to stimulate adenylate cyclase activity. These findings strongly suggested that the inhibition of kainic acid binding by guanine nucleotides was not dependent on a G-protein-mediated system. This result corroborated studies from other groups, which had previously shown that the inhibitory effects of guanine nucleotides on the binding of glutamate or ionotropic glutamatergic ligands presented several inconsistencies, when compared with studies on receptors known to be coupled to their second messengers through a G- protein [6,87-90]. Subsequent studies from our group supported the hypothesis that guanine nucleotides could antagonize the glutamatergic transmission by acting at extracellular sites located on the membrane surface [91-94].

Searching for a relevance of the inhibitory action of extracellular guanine nucleotides on glutamate binding, several studies further investigated their putative effects on neural cell responses to glutamate and/or analogs [7,95-101]. It was observed that guanine nucleotides inhibited glutamate-stimulated GFAP (glial fibrillary acidic protein) phosphorylation [96], glutamate (and analogs)-induced modulation of intracellular cAMP levels [97,98], kainate-stimulated lactate dehydrogenase (LDH) release [4], kainate-activated currents [94,101] and kainate-stimulated increase in Ca^{2+} influx [93]. Since most excitatory synapses in the CNS have glutamate as neurotransmitter, the potential modulatory action of guanine nucleotides on the glutamatergic neurotransmission claimed attention to new investigations on their extracellular roles.

Several studies have indicated that guanine-based purines, especially guanosine, may be neuroprotective endogenous compounds released under excitotoxic conditions, preventing further toxicity to neural cells [reviewed in ref. 12]. Considering that guanine-based purines seem to be especially effective after conversion to guanosine [45,65,79,102] and the fact that this nucleoside probably exerts only weak glutamate receptor antagonism, the hypothesis of direct receptor interaction as the mechanism of neuroprotection and anticonvulsant action of guanine-based purines is unlikely, although this issue deserves further investigation [103,104].

Guanosine occurs naturally in the brain and has been reported to present numerous biological effects when administered extracellularly, including trophic effects on neural cells (mainly astrocytes) [16,39] and modulation of glutamatergic activity [12]. Guanosine stimulates the release of adenine-based purines from astrocytes, which may be responsible for some effects of guanine-based purines [105]. For example, the ability of guanine-based purines to stimulate proliferation of rat brain microglia in a concentration-dependent manner appears to be mediated by specific purinergic receptors that recognize adenine-based purines [105]. But this explanation is also incomplete, since many of the effects of guanine-based purines persist in the presence of P_1 and/or P_2 purine receptor antagonists [45,99,106,107]. An alternative hypothesis is that there are distinct receptors for guanine-based purines. Although those effects might be related to guanosine uptake into the intracellular compartment, a consensus has emerged that at least some effects of guanosine involve its binding to a specific membrane protein [108,109], postulated to be a G protein-coupled receptor [16,108,109]. Moreover, several of the effects of guanosine may be mediated through G-protein dependent signalling pathways involving changes in the intracellular levels of cyclic nucleotides or mitogen-activated protein kinase (MAPK) pathway raising the possibility that some of the effects of guanine-based purines, particularly guanosine, involve activation of cell-surface receptors [26,106]. Indeed, the actual existence of its putative specific receptor has yet to be demonstrated. More certain is the fact that guanosine presents clear anti-glutamatergic properties, as demonstrated in several *in vivo* and *in vitro* approaches [reviewed in ref. 12], which places it as a new potential neuroprotective strategy against glutamatergic excitotoxicity.

The mechanism of action underlying the modulation of glutamatergic activity by guanosine, and perhaps other guanine-based purines, is currently under research in our and other laboratories. It has been suggested that astrocytes are importantly involved, since guanosine has been shown to stimulate glutamate uptake by cultured astrocytes and brain slices [14,102,107,110]. Astrocytic glutamate uptake is a crucial process for the maintenance of extracellular glutamate concentrations below toxic levels in physiological conditions and under brain stress, thus supporting synapse homeostasis (glutamate-glutamine cycle) [23]. Actually, this is the main mechanism of glutamate removal from the synaptic cleft [61]. Notably, both neuronal and astrocytic cell cultures are able to release guanosine under basal or ischemic conditions [16,22,111] and kainate stimulates the release of guanosine [111]. In physiological conditions, the effects of guanosine on glutamate uptake in brain slices seem to be age (more in young animals) and structure (more in cortex) dependent but, in excitotoxic conditions, guanosine seems to be more widely involved in modulating glutamate uptake [14,110,112,113]. In cultured primary astrocytes from cortices of 1-day-old and adult rat brain cortical slices, guanosine was shown to increase the sodium-dependent uptake of glutamate in a dose-dependent manner [107]. Importantly, adenosine affected neither the basal uptake nor the stimulatory effect of guanosine. Theophylline, a nonspecific P_1 (A_1/A_{2A}) adenosine-receptor antagonist, stimulated basal uptake of glutamate without affecting the stimulatory effect of guanosine. Finally, dipyridamole, a nucleoside transport inhibitor, also stimulated basal glutamate uptake, and this stimulatory effect was additive with that of guanosine. Thus, these findings suggest that the guanosine stimulatory effect on astrocytic uptake of glutamate is exerted from the extracellular side and is, at least partially, independent of the adenosinergic system [107].

GMP and GTP mimicked the stimulatory effect of guanosine on glutamate uptake by astrocytic cells in culture [102]. However, a significant additive effect on uptake was not observed with the simultaneous addition of guanosine, GMP and GTP to the culture medium, compared with the

effect of each compound alone. These data were consistent with the possibility that only one compound was mediating the stimulatory effect on uptake or the three compounds were metabolically interconvertible with each other. Importantly, a poorly hydrolysable analogue of GTP did not stimulate the uptake of glutamate by cultured astrocytes and the effect of GMP was abolished when cultures were pretreated with AOPCP. Finally, guanosine failed to affect the astrocytic uptake of GABA [102]. Therefore, guanosine seems to be mediator of the stimulatory effect of guanine-based purines on the astrocytic uptake of glutamate, and this process was independent of adenosine and relatively specific for glutamate [102]. As astrocytic uptake of glutamate is the most important mechanism for terminating its actions within the synapse, the stimulation of uptake by guanosine may be a relevant process in regulating glutamatergic neurotransmission, especially under excitotoxic conditions [23,58,61].

Oral administration of guanosine prevented the decrease of glutamate uptake by brain slices of rats submitted to quinolinic acid seizure model [17,20]. Additionally, guanosine has been shown to prevent the decrease of glutamate uptake by hippocampal slices of neonatal rats exposed to a hypoxic-ischemic insult *in vivo* [114]. Moreover, we demonstrated that *in vitro* and *in vivo* quinolinic acid stimulated synaptosomal glutamate release and inhibited glutamate uptake by astrocytes, which could lead to increased extracellular glutamate levels and seizures [64,115]. However, this neurochemical effect was prevented by *in vivo* pretreatment with systemic guanosine or GMP [65]. Additionally, quinolinic acid stimulates glutamate uptake by synaptic vesicles, an effect prevented by glutamate antagonists and the guanine-based purines guanosine and GMP [66]. Altogether these findings indicate that glutamate uptake modulation induced by guanosine might play a crucial role in the underlying mechanisms involved in the anticonvulsant effects of guanine-based purines.

In addition to their effects on neurotransmission, guanine-based purines also have important trophic functions, affecting the development, structure or maintenance of neural cells [39]. Of note, guanosine has been shown to stimulate the release of neurotrophic factors, an event largely related to cell proliferation [16]. Some trophic effects of purines seem to be mediated *via* purinergic cell surface receptors, whereas others require uptake of purines by the target cells [39]. Guanosine and GTP, apparently through different mechanisms, are related to several trophic events, including stimulation of astroblast growth [116], *in vitro* axonal growth and proliferation of a wide range of cell types [117,118], and trophic effects on the CNS, including stimulation of astrocyte proliferation, induction of synaptogenesis [119], synthesis and release of trophic factors such as nerve growth factor from astrocyte cultures, and differentiation of PC12 cells and hippocampal neurons *in vitro* [38,39,105,106,116,120,121]. Some of the trophic actions of guanine-based purines may be indirect, occurring as a result of stimulating the synthesis and release of trophic factors and/or enhancing the effects of these specific trophic factors. Another possibility is that some actions of guanosine could be mediated intracellularly after its uptake. However, with respect to a specific neurotrophic role for guanosine, its extracellular levels remained elevated for up to a week after focal brain injury [122]. Additionally, many trophic effects of guanine-based purines were not affected by the nucleoside uptake inhibitors, such as dipyridamole, indicating that they are triggered extracellularly [120].

The potential ability of exogenously administered guanine-based purines to provide an alternative source of energy to ATP has been suggested as an explanatory hypothesis for their neuroprotective effects in the context of oxidative stress and cell damage [16,123]. For example, after exposure to rotenone, an inhibitor of the mitochondrial respiratory chain, and the induction of chemical hypoxia, guanosine was shown to preserve the viability of cultured astrocytes and neurons [123,124]. The ability of guanosine to maintain cellular levels of ATP above a critical threshold under hypoxia may provide an explanation of the mechanism of their cell damage prevention. Indeed, the addition of a purine nucleoside phosphorylase inhibitor to the cultures, which would interfere with a pathway for the participation of purine nucleosides in the production of ATP under anaerobic conditions, attenuated their protective effect; this effect of purine nucleosides to preserve cell viability was especially dramatic with neurons. The data also suggested that neuronal protection by purine nucleosides is either dependent on or enhanced by the presence of glia [123].

3. CONCLUSIONS AND PERSPECTIVES

This article reviews the evidences about the potential anticonvulsant activity displayed by some components of the guanine-based purinergic system, perhaps providing new targets for neuroprotection and epilepsy treatment in children and adults. Guanine-based purines seem to modify the homeostasis of the glutamatergic system, modulating some glutamatergic parameters such as glutamate uptake by astrocytes and seizures induced by glutamatergic agents. Furthermore, the profile of extracellular activity of the guanine-based purines (endogenous compounds, orally active and no obvious CNS side effects except for transitory memory impairment) makes this system a very interesting object for discovery of new pharmacological options to treat diseases related to overstimulation of glutamatergic system such as epilepsy and other neurodegenerative diseases.

More specifically, to advance in guanine-based purine research, further studies are necessary on their mechanisms of action, cloning of putative selective receptors and characterization of second messengers related to their extracellular effects. In fact, the investigation of the extracellular effects of guanine-based purines and their underlying mechanisms of action is still in its infancy and advance is urgently warranted. This is especially true for the so called guanosine receptor characterization, which is pivotal for the proposal of a new neuromodulation pathway. Additional elucidation of the mechanism of action of guanosine and its membrane-binding site is under current investigation in our laboratory.

Furthermore, little information about potential side effects and systemic toxicity of these compounds is available. A recent study showed a minor toxic potential of guanosine in mice, displaying an absent mortality index and lack of changes in weight body gain or core temperature up to 72 h after guanosine systemic administration, even in high doses [48]. However, this study has demonstrated some indication of liver toxicity (elevated liver enzymes) induced by guanosine in doses higher than 240 $mg.kg^{-1}$ [48]. 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. In fact, specific studies about the safety profile of these compounds are pivotal for their future use in a clinical basis.

Although it is early to propose the use of guanine-based purines for clinical research, an interesting approach to investigate their role clinically is the investigation of purine derivatives already used in humans. For example, we have demonstrated that allopurinol, a xanthine oxidase inhibitor, was an effective and well-tolerated adjuvant treatment for poorly responsive schizophrenia, refractory aggressive behavior and mania [125-129]. These results were confirmed by an independent group [130,131] and are hypothesized to be due to an indirect increase in extracellular purine levels (adenosine and guanosine) [12]. Notably, refractory epilepsy may also respond to allopurinol [132,133]. More recently, we have demonstrated that allopurinol produced dose-dependent antinociceptive effects in several animal pain models [85]. The non-selective adenosine-receptor antagonist caffeine and the selective A_1 adenosine-receptor antagonist, DPCPX, but not the selective A_{2A} adenosine-receptor antagonist, SCH58261, completely prevented allopurinol-induced antinociception. Allopurinol also caused an increase in CSF levels of purines, including the nucleosides adenosine and guanosine, and decreased CSF levels of uric acid. Allopurinol-induced antinociception may be related to adenosine, and perhaps guanosine, accumulation. Considering that allopurinol is an old and extensively used compound and seems to be well tolerated with no obvious CNS toxic effects, allopurinol may be the first commercially available effective drug enhancing the effects of the purinergic systems for the treatment of human brain diseases, including chronic pain, psychiatric disorders and epilepsy. These findings indicate that new studies addressing more selective xanthine oxidase inhibitors in neuroprotection could represent a fine approach to investigate the therapeutic potential of purine in a clinical setting.

In conclusion, the role of the guanine-based purines as new targets for brain protection remains to be fully characterized, but current evidence strongly suggests their potential for the treatment of brain diseases such as epilepsy.

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