Guanine-based purines have been traditionally studied as modulators of intracellular processes, mainly G-protein activity. However, they also exert several extracellular effects not related to G proteins, including modulation of glutamatergic activity, trophic effects on neural cells, and behavioral effects. In this article, the putative roles of guanine-based purines on the nervous system are reviewed, and we propose a specific guanine-based purinergic system in addition to the well-characterized adenine-based purinergic system. Current evidence suggest that guanine-based purines modulate glutamatergic parameters, such as glutamate uptake by astrocytes and synaptic vesicles, seizures induced by glutamatergic agents, response to ischemia and excitotoxicity, and are able to affect learning, memory and anxiety. Additionally, guanine-based purines have important trophic functions affecting the development, structure, or maintenance of neural cells. Although studies addressing the mechanism of action (receptors and second messenger systems) of guanine-based purines are still insufficient, these findings point to the guanine-based purines (nucleotides and guanosine) as potential new targets for neuroprotection and neuromodulation.

Keywords: Guanine-based purines; Adenine-based purines; Guanosine; Purinergic neurotransmission; Neuroprotection; Glutamatergic excitotoxicity

Abbreviations: ADA, adenosine deaminase; ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; cAMP, adenosine 3′,5′ cyclic monophosphate; cGMP, guanosine 3′,5′ cyclic monophosphate; CNS, central nervous system; CSF, cerebrospinal fluid; DNA, deoxyribonucleic acid; ERK, extracellular regulated kinases; GDP, guanosine 5′-diphosphate; GMP, guanosine 5′-monophosphate; GTP, guanosine 5′-triphosphate; KA, kainic acid; MK-801, dizocilpine; NGF, neural growth factor; NMDA, N-methyl-D-aspartate.

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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′-monophosphate (AMP) and the nucleoside adenosine. Adenine-based purines exert several biological roles, including the pivotal role on energy metabolism. However, guanine-based purines, namely the nucleotides guanosine 5′-triphosphate (GTP), guanosine 5′-diphosphate (GDP), and guanosine 5′-monophosphate (GMP) and the nucleoside guanosine can also be considered part of the purinergic system. Finally, the metabolites xanthine, hypoxanthine, uric acid, the nucleoside inosine, as well as receptors, transporters, and enzymes complete the purinergic system.

Traditionally, guanine-based purines have been studied as modulators of intracellular processes, especially regarding the activity of G proteins for signal transduction. Nonetheless, guanine-based purines have been shown to exert extracellular effects not related to their direct modulation of G proteins, including in vitro (Baron et al., 1989; Burgos et al., 1998, 2000a, 2000b, 2000c; Paz et al., 1994; Ramos et al., 1997; Souza & Ramirez, 1991; Tasca et al., 2005) and in vivo (Lara et al., 2001; Schmidt et al., 2000) modulation of the glutamatergic activity, behavioral effects (Roesler et al., 2000; Vinadé et al., 2005), and trophic effects on neural cells (Ciccirelli et al., 2001).

In this article, the putative roles of guanine-based purines in the nervous system are reviewed, with emphasis on their extracellular effects with potential role in neuroprotection. Similarly to the well-characterized adenine-based purinergic system, we propose a specific guanine-based purinergic system with relevant physiological and pathological implications.

1.1. Historical overview of the purinergic system

Purine bases, such as adenine and guanine, and their corresponding nucleosides and nucleotides are ubiquitous molecules found within and outside the cells of animals and plants. Among their several important biological roles, purine bases and their pyrimidine counterparts (thymine, cytosine, and uracil) are the building blocks of DNA and RNA. Purine nucleotides, mainly ATP, are involved in biochemical pathways and energy transfer within the cell. Moreover, cyclic nucleotides, such as adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) act as intracellular second messenger molecules during signal transduction (Barnstable et al., 2004; Bourne et al., 1990).

The molecule of ATP was discovered in 1929 (Fiske & SubbaRow, 1929; Lohman, 1929), and shortly the role of ATP as a universal source of chemical energy in biological systems was fully appreciated (Lippman, 1941). Although purines had been traditionally viewed as having mostly intracellular roles, it was discovered that extracellular adenosine is released by the heart during ischemia, triggers negative chronotropic effect on the heart, mediates dilatation of coronary vessels, and inhibits intestinal smooth muscle (Drury & Szent-Györgyi, 1929). It also became apparent that ATP was responsible for many purine-mediated physiological reactions (Drury, 1936).

Thirty years later, it was observed that ATP could be released from nerves upon stimulation (Holton, 1959). Through a firefly luminescence method for ATP detection, electrical stimulation of the rabbit great auricular nerve resulted in a transient elevation of extracellular ATP, establishing a foundation for the theory of purinergic neurotransmission.

In 1970, the first direct evidence that ATP may act as a transmitter in “nonadrenergic, noncholinergic” nerves in the gut and bladder was presented (Burnstock et al., 1970), and the concept of “purinergic nerves” and “purinergic neurotransmission” was introduced (Burnstock, 1972). Subsequently, Burnstock developed the concept of purinergic transmission in the peripheral nervous system, demonstrating that ATP fully conforms to the criteria for the definition of a neurotransmitter: (i) ATP is synthesized and stored in presynaptic terminals; (ii) ATP is released upon nerve stimulation; (iii) extracellular ATP can be rapidly degraded by coenzymes; and (iv) pharmacological agents that inhibited the effects of endogenous ATP also suppressed the effects of nerve stimulation. Finally, Burnstock postulated that ATP might be coreleased with other neurotransmitters, such as noradrenaline or acetylcholine (Burnstock, 1972).

Resistance to this concept remained for many years because ATP was recognized firstly for its intracellular roles in many biochemical processes, such as intracellular energy source, linking various metabolic cycles, and the intuitive feeling was that such a ubiquitous and simple compound was unlikely to be an extracellular messenger, although powerful extracellular enzymes involved in its breakdown had already been characterized (Burnstock, 2006a, 2006b).

The concept was eventually accepted and expanded, as purines are also important extracellular messengers to non-neuronal cells (Burnstock & Knight, 2004). Over the last 30 years, the roles of adenine-based purines, mainly the nucleoside adenosine and the nucleotide ATP, as neurotransmitters and neuromodulators in the central and peripheral nervous systems have been extensively elucidated (Burnstock, 2006a, 2006b). For this reason, the nucleotide ATP and the
nucleoside adenosine are usually considered the main effectors of the purinergic system (Ralevic & Burnstock, 1998).

Perhaps a similar story has been taking place with the extracellular roles of guanine-based purines, which may have been overshadowed by their well-known modulation of G protein-mediated signal transduction and by the abundance of information on adenine-based purines.

We will briefly review the adenine-based purinergic system in the nervous system, as it has been an obvious model for this putative guanine-based purinergic system.

1.2. Adenine-based purines

The role of ATP as an excitatory neurotransmitter, both centrally and in the periphery, is now well documented and accepted (Burnstock, 2006a, 2006b; Ralevic & Burnstock, 1998). ATP is stored in and released from neuronal presynaptic terminals, acting via specific P2 receptors, described in detail elsewhere (Abbracchio & Burnstock, 1994; Ralevic & Burnstock, 1998; Burnstock, 2007).

The neuromodulatory effects and sources of adenosine have been well characterized. Extracellular adenosine is enzymatically formed from extracellular nucleotides or comes from the release of intracellular adenosine (Brunedge & Dunwoodie, 1997). Intracellular adenosine is formed from the cleavage of S-adenosylhomocysteine by S-adenosylhomocysteine hydrolase or from the metabolization of 5′-AMP by an intracellular 5′-nucleotidase and can diffuse through bidirectional nucleoside transporters to the extracellular space. Released ATP and cAMP are sources for the production of AMP via ectonucleotidases or ectophosphodiesterase, respectively, and finally, an extracellular ecto-5′-nucleotidase hydrolyses AMP to adenosine (see Fig. 1). Extracellular adenosine can be taken up through nucleoside transporters and phosphorylated to AMP by adenosine kinase or deaminated to inosine by adenosine deaminase (ADA). These processes are mostly intracellular, but ADA is also associated with cell membranes (Brunedge & Dunwiddie, 1997; Ralevic & Burnstock, 1998).

Brain extracellular adenosine and ATP act not only as neurotransmitters and neuromodulators, but also as trophic factors involved in plastic processes, such as memory and learning, collateral sprouting of nerve processes, neuroprotection against noxious stimuli, and regulation of cell number through induction of apoptosis (programmed cell death; Ciccarelli et al., 1999a, 2001).

1.3. Receptors for adenine-based purines

Signaling via extracellular adenine-based purines is very complex (see Fig. 2). An extensive review of the purinergic receptors is found elsewhere (Burnstock, 2007; Zimmermann, 2006a, 2006b). In 1978, 2 types of purinoceptors, identified as P1 (for adenosine) and P2 (for ATP/ADP) were proposed (Burnstock, 1978). Simultaneously, 2 subtypes of the P1 receptors were firstly recognized (Londos et al., 1980; Van Caalker et al., 1979), but it was not until 1985 that a proposal suggesting a pharmacological basis for distinguishing 2 types of P2 receptors (P2X and P2Y) was made (Burnstock & Kennedy, 1985). Later, 2 further P2 receptor subtypes were proposed, P2T on platelets and P2Z on macrophages, and receptors that

---

Fig. 1. Schematic model of the sources of extracellular adeno- and guanine-based purines. E-NTPDases, ectonucleotide-diphosphohydrolase; ADA, adenosine deaminase; SHMT, serine hydroxymethyltransferase; SAHH, S-adenosylhomocysteine hydrolase; PD, ectophosphodiesterase; PNP, purine nucleoside phosphorylase; HGPRT, hypoxanthine-guanine phosphoribosyltransferase.
responded to pyrimidines and to purines were named P2U receptors (Gordon, 1986). In 1993, the first G protein-coupled P2 receptors were cloned (Lustig et al., 1993; Webb et al., 1993), and 1 year later 2 ion-gated receptors were also cloned (Brake et al., 1994; Valera et al., 1994).

Concerning ATP receptors, on the basis of molecular structure and transduction mechanisms, it was proposed that P2 should belong to 2 major families: a P2X family of ligand-gated ion channel receptors and a P2Y family of G protein-coupled receptors (Abbracchio & Burnstock, 1994). This nomenclature has been widely accepted, and currently 7 P2X subtypes and 8 P2Y receptor subtypes are recognized, including receptors that are sensitive to pyrimidines as well as purines (Burnstock, 2007). P2X receptors mediate the flow of Ca2+, Na+, and K+, whereas P2Y metabotropic receptors, via G proteins, activate second messenger systems, such as phospholipase C (PLC) and phospholipase A2 (PLA2). ADP and pyrimidine nucleotides (UTP and UDP) also activate some subtypes of P2 receptors.

Concerning adenosine receptors, to date 4 different adenosine P1 receptor subtypes, which are classified as A1, A2A, A2B, and A3 have been cloned and characterized (Brundege & Dunwiddie, 1997; Cunha, 2005; Fredholm et al., 2001, 2005; Ralevic & Burnstock, 1998). A1 and A3 receptors inhibit, whereas A2a and A2b receptors stimulate, adenylate cyclase. Both A1 and A2 receptors also increase inositol-3-phosphate (IP3) formation. The human A2B receptor has also been found to regulate PLC activity (Burnstock, 2007). Adenosine A1 receptors are widely distributed in the central nervous system (CNS) and have been shown to decrease neuronal excitability and synaptic activity and to inhibit the release of several neurotransmitters, such as glutamate, dopamine, serotonin, noradrenaline, and acetylcholine. A2A receptors are concentrated in dopamine-rich areas, modulating dopaminergic activity, but are also present in the hippocampus and cerebral cortex. A2B receptors are less well characterized and have been suggested to interact with inflammatory mediators. Similarly, A3 receptors have also been related to inflammation, especially in lungs (see Burnstock, 2007, for review). A number of P1 subtype-selective agonists and antagonists have been identified. All of the known P1 receptor agonists are closely related to adenosine in structure. Methylxanthines, such as caffeine and theophylline, are the classical nonselective A1–A2 adenosine antagonists.

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 (Rodbell et al., 1971). G proteins alternate between inactive GDP-bound and active GTP-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 (Gudermann et al., 1997; Johnston & Siderovski, 2007; Taylor, 1990). Recently, guanine-based purines, including the nucleotides GTP, GDP, and GMP, and the nucleoside guanosine have also been shown to exert extracellular effects, based upon studies that could be subdivided into 3 approaches: (i) inhibitory effects on the activity of the glutamatergic system in physiological and pathological conditions; (ii) effects on memory and behavior; (iii) trophic effects on neural cells. Before reviewing these aspects, we will summarize the findings regarding the characterization of the main constituents of the guanine-based purinergic system.

![Diagram of synaptic connections](image-url)
2.2. Metabolism, storage, and release of guanine-based purines

GTP may be stored in synaptic vesicles (Santos et al., 2006; Zimmermann, 1996) and indirect evidence indicated that guanosine could be released from synaptosomes (Fredholm & Vernet, 1979). Cultured astrocytes may release guanine-based purines (Ciccarelli et al., 1999b), a process that increased after hypoxia/hypoglycemia. In cultured astrocytes, inhibition of ecto-5′-nucleotidase activity significantly reduced accumulation of extracellular guanosine, indicating that, like extracellular adenosine, it is to some extent derived from the extracellular metabolism of guanine nucleotides (Caciagli et al., 2000).

The presence of adenine- and guanine-based purines and their metabolites in human and animal cerebrospinal fluid (CSF) has been described (Castro-Gago et al., 1992; Regner et al., 1997). The enzymes involved in extracellular nucleotide hydrolysis include membrane-bound ectonucleotidases, ectonucleotidases released from membranes, and the naturally occurring soluble nucleotidases (see Fig. 1). 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-dependent neuroprotective activities against brain insults (Sebastião et al., 1999). These soluble nucleotidases are also present and active in rat CSF (Portela et al., 2002), wherein they hydrolyze all guanine and adenine nucleotides with the following order of catalytic efficiency: GDP > ADP = ATP = GTP > AMP = GMP. Interestingly, at high concentrations, GDP hydrolysis rate is greater than that of ADP, perhaps favoring the accumulation of GMP and consequently guanosine. In fact, these enzymes can be released to the extracellular space (CSF) from choroid plexus, endothelial cells, or even microglia (Zimmermann, 2001, 2006a, 2007b) and play an important regulatory role of the purinergic system under physiological and pathological conditions.

Astrocytes, the main source of cerebral purines (Ciccarelli et al., 1999b), are involved in multiple 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 brain damage (Chen & Swanson, 2003). Astrocytes, as well as neurons, are also responsible for both nucleoside metabolism and uptake of adenosine and guanosine (Parkinson et al., 2005). 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 (Rathbone et al., 1999b). A recent study (Peng et al., 2005) identified 2 equilibrative nucleoside transporters in astrocytes (ENT1 and ENT2), together with the concentrative nucleoside transporter (CNT2) responsible for nucleoside uptake (see Fig. 2).

In regard to guanosine bioavailability, animals treated with oral guanosine presented a 2-fold increase in CSF concentration of guanosine as compared to vehicle group (Vinadé et al., 2005). We also observed that i.p. administration of GMP in anticonvulsant doses produced a 3-fold increase of cerebrospinal fluid levels of guanosine in rats, not affecting GMP levels (Soares et al., 2004).

2.3. Modulation of the glutamatergic system: Neuroprotective effects 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 receptors and modulate adenylate cyclase activity (Gudermann et al., 1997). However, we demonstrated that the effects of guanine nucleotides on kainic acid (a glutamatergic ligand to receptors not coupled to G proteins) binding site and on adenylate cyclase activity could be dissociated (Souza & Ramirez, 1991). In lysed membrane preparations (G proteins and receptors exposed to the incubation medium) from chicken brain, the guanine nucleotides GMP (which does not bind to G proteins), GDP and GTP were able to inhibit the binding of kainic acid with the same efficiency, whereas only GTP was able to stimulate adenylate cyclase. However, in vesicular preparations (G proteins not exposed to the incubation medium), 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 (Baron et al., 1989; Butcher et al., 1986; Hood et al., 1990; Monahan et al., 1988; Paas et al. 1996; Sharif & Roberts, 1981). 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. By using a poorly hydrolyzed GTP analogue (GMP-PNP) we were able to observe some distinctions between 2 groups of binding sites for guanine-based purines, such as stability to washing procedures of the intracellular (G proteins) but not of the extracellular (receptors) [3H]GMP-PNP binding. Dealing separately with each of these 2 groups of sites, we were able to discriminate some properties of extracellular and intracellular guanine-based purine binding sites in rat CNS (Paz et al., 1994; Ramos et al., 1997; Rotta et al., 2004; Rubin et al., 1997). This receptor antagonism was shown to be competitive in moderate to high micromolar concentrations of guanine nucleotides (Baron et al., 1989). In all these studies, the nucleoside guanosine had no (or very little) effect on the binding of glutamate and analogs to glutamate receptors (Porciuncula et al., 2002; Souza & Ramirez, 1991).

Searching for a relevance of the inhibitory action of extracellular guanine nucleotides on glutamate binding, our
group and Ramirez’s group investigated their putative effects on neural cell responses to glutamate and/or analogs (Aleu et al., 1999; Burgos et al., 1998; Burgos et al., 2000a, 2000b, 2000c; Burgos et al., 2003; Paz et al., 1994; Regner et al., 1998; Rubin et al., 1997; Tasca et al., 1995; Tasca et al., 1998; Tasca et al., 1999; Tasca & Souza, 2000). It was observed that guanine nucleotides inhibited glutamate-stimulated GFAP (astrocytic protein) phosphorylation (Tasca et al., 1995), glutamate (and analogs)-induced increase in intracellular cAMP levels (Tasca et al., 1998), glutamate-induced luminescence (Regner et al., 1998), kainate-stimulated LDH release (Burgos et al., 1998), kainate-activated currents (Aleu et al., 1999; Burgos et al., 2003), and kainate-stimulated increase in Ca\(^{2+}\) influx (Burgos et al., 2000a, 2000b, 2000c). Since most excitatory synapses in the CNS have glutamate as neurotransmitter, which participates in several physiological and pathological processes, the potential modulatory action of guanine nucleotides on the glutamatergic neurotransmission claimed attention to new investigations on their extracellular effects.

Guanine nucleotides administered intracerebroventricularly had long been shown (Baron et al., 1989) to prevent seizures induced by quinolinic acid, a toxin that overstimulates the glutamatergic neurotransmission (Stone, 2001). This effect was compatible with the antagonistic properties of guanine nucleotides on glutamate receptors being studied in our group. However, after further exploring the interaction of guanine nucleotides with glutamate, we observed that intraperitoneal administration of not only GMP, but also guanosine (that does not inhibit the binding of glutamatergic ligands), was able to prevent seizures induced by quinolinic acid (Schmidt et al., 2000). Later we showed that oral administration of guanosine was also effective in this model (Lara et al., 2001). Additional studies also provided evidence that guanosine and GMP administered intracerebroventricularly (i.c.v.), intraperitonially or orally protected against seizures induced by the glutamatergic agents quinolinic acid, kainate and α-dendrotoxin in adult and young rodents (Oliveira et al., 2004; Schmidt et al., 2005; Soares et al., 2004; Vinadé et al., 2003; Vinadé et al., 2005). Acute i.c.v. administration of the nucleotides GTP and GDP was also protective against seizures induced by quinolinic acid in mice (Schmidt et al., 2005). Chronically, GMP had also been shown to be neuroprotective against quinolinic acid-induced striatal neuronal cell death in rats (Malcon et al., 1997).

Guanine-based purines, mainly GMP and guanosine, have usually presented similar neuroprotective profile in several in vivo and in vitro protocols (Lara et al., 2001; Schmidt et al., 2000; Schmidt et al., 2005; Vinadé et al., 2003; Vinadé et al., 2005). However, most effects of nucleotides (mainly GMP) seemed to be due to 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 (Schmidt et al., 2005).

Taken together, as guanosine does not exert glutamate receptor antagonism, and guanine-purines seemed to be effective after conversion to guanosine, the hypothesis of direct receptor interaction as the mechanism of neuroprotective action of guanine-based purines was weakened, although this issue (direct interaction with glutamatergic receptors) deserves further investigation (Mendieta et al., 2001, 2005).

2.4. Astrocytic glutamate uptake and guanine-based purines

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; Anderson & Swanson, 2000; Chen & Swanson, 2003; Matute et al., 2006; Schousboe & Waagepetersen, 2005). In the search for other mechanisms of action of extracellular guanine-based purines than antagonism of glutamatergic receptors, we found that guanosine was able to increase the glutamate uptake by cultured astrocytes and brain slices (Frizzo et al., 2001, 2002, 2003). In basal or physiological conditions, the effects of guanosine on glutamate uptake in brain slices seemed to be age (more in young animals)- and structure (more in cortex)-dependent but, in excitotoxic conditions, guanosine was more broadly involved in modulating glutamate uptake (Gottfried et al., 2002; Frizzo et al., 2005; Thomazi et al., 2004). In cultures of primary astrocyte from cortices of 1-day-old Wistar rats and in adult rat brain cortical slices, guanosine was shown to increase the sodium-dependent uptake of glutamate in a dose-dependent manner (Frizzo et al., 2001). Depending on the preparation, the minimum effective concentration of guanosine was 100 nM to 1 μM. The maximal stimulation of uptake by guanosine was around 80% over control values. Importantly, adenosine affected neither the basal uptake nor the stimulatory effect of guanosine. Theophylline, a nonspecific P1 (A1/A2A) adenosine receptor antagonist, stimulated basal uptake of glutamate without affecting the stimulatory effect of guanosine. Finally, dipridamole, a nucleoside transport inhibitor, also stimulated basal uptake, and this stimulatory effect was additive with that of guanosine. Thus, these data suggest that the guanosine stimulatory effect on astrocytic uptake of glutamate is exerted from the extracellular side and is independent of adenosine and its receptors (Frizzo et al., 2001).

GMP and GTP mimicked the stimulatory effect of guanosine on glutamate uptake by astrocytic cells in culture (Frizzo et al., 2003). 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 3 compounds were metabolically
interconvertible with each other. Importantly, a poorly hydrolysable analogue of GTP (GMP-PNP) was not able to 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. 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 (Frizzo et al., 2003). 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 (Chen & Swanson, 2003; Duan et al., 1999; Matute et al., 2006; Schousboe & Waagepetersen, 2005).

Oral administration of guanosine prevented the decrease of glutamate uptake by brain slices of rats submitted to quinolinic acid-induced seizures (Oliveira et al., 2004; Vinadé et al., 2005). Additionally, a recent study demonstrated that guanosine prevented the decrease of glutamate uptake by hippocampal slices of neonatal rats exposed to a hypoxic–ischemic insult in vivo (Moreto et al., 2005). Moreover, we demonstrated that in vitro and in vivo quinolinic acid stimulated synaptosomal glutamate release and inhibited glutamate uptake by astrocytes, which could induce an increase in extracellular glutamate levels and consequently seizure behavior (Tavares et al., 2000, 2002). However, this neurochemical effect was prevented by in vivo pretreatment with systemic guanosine or GMP (Tavares et al., 2005). Quinolinic acid also stimulates glutamate uptake by synaptic vesicles, an effect prevented by glutamate antagonists and the guanine-based purines guanosine and GMP (unpublished results).

Interestingly, we found that GTP, GDP, GMP, and guanosine inhibited glutamate uptake by synaptic vesicles in vitro (Tasca et al., 2004), pointing to an intracellular interaction between guanine-based purines and the glutamatergic neurotransmission. The physiological significance of this intracellular effect remains to be clarified.

2.5. In vitro neuroprotection by guanine-based purines

Several studies have indicated that guanosine may be a neuroprotective endogenous compound released under excitotoxic conditions, preventing further toxicity to neurons. Both neuronal and astrocytic cell cultures are able to release guanosine and adenosine under basal or toxic (ischemic) conditions (Ciccarelli et al., 1999b; Ciccarelli et al., 2001; Dobolyi et al., 2000) and kainate stimulates the release of guanosine (Dobolyi et al., 2000). Interestingly, guanosine protected brain slices exposed to hypoxia/hypoglycemia (Frizzo et al., 2002) and medium from astrocytes treated with guanosine prevented NMDA-induced toxicity in neurons (Caciagli et al., 2000).

In slices submitted to glucose deprivation, GMP prevented LDH leakage and the loss of cell viability induced by glutamate (Molz et al., 2005). In slices submitted to ischemic conditions, GMP partially prevented the decrease in cell viability induced by glucose and oxygen deprivation and the addition of kainate (Oliveira et al., 2002). However, in these studies the possible role of guanosine in mediating the effects of GMP was not addressed.

2.6. 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 (Chen & Lipton, 2006). It is well documented that glutamate plays a key role on memory mechanisms (Izquierdo et al., 2006), and previous studies demonstrated that GMP was able to reverse the facilitatory effect of posttraining intrahippocampal glutamate administration on inhibitory avoidance task performance in rats (Rubin et al., 1996). 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 (Roesler et al., 2000). Treated animals, when retrained 1 week later, showed normal learning ability and guanosine administration immediately after training or pretest had no effect (Roesler et al., 2000). The guanine-based purine effects on memory were reproduced with anticonvulsant doses after acute/chronic intraperitoneal/oral administration and adenosine-receptor antagonists failed to prevent these effects (Vinadé et al., 2003, 2004). Furthermore, the amnesic effect related to the pretreatment with GMP also depended on its conversion to guanosine (Sauté et al., 2006). 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 A1 and A2A receptors.

NMDA receptor antagonists induce locomotor effects caused at least in part by a paradoxical increase in glutamate release (Adams & Moghaddam, 2001). Interestingly, guanosine produced a ~60% attenuation of hyperlocomotion induced by dizocilpine (MK-801, a NMDA-receptor antagonist), whereas it did not affect the hyperlocomotion induced by the indirect dopamine agonist amphetamine or by the nonselective adenosine-receptor antagonist caffeine (Tort et al., 2004). However, most studies indicate that guanosine per se did not affect spontaneous locomotion rodents after systemic administration (Lara et al., 2001; Tort et al., 2004; Vinadé et al., 2003). 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 (Lara et al., 2001; Vinadé et al., 2003).

The contribution of adenosine A1 and A2A receptors to the effects of guanosine has also been ruled out in 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 (Lara et al., 2001) or the amnesic effect of guanosine in rats (Roesler et al., 2000; Vinadé et al., 2004).
2.7. Neurotrophic 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, as observed by Rathbone’s group (Rathbone et al., 1999b). Some trophic effects of purines are mediated via purinergic cell surface receptors, whereas others require uptake of purines by the target cells (Rathbone et al., 1999b). Both extracellular guanosine and GTP, apparently through different mechanisms (i) have mitogenic effects promoting astroblast growth (Kim et al., 1991), (ii) are potent stimulators of in vitro axonal growth and proliferation of a wide range of cell types (Rathbone et al., 1992a, 1992b), (iii) can exert trophic effects on the nervous system (Rathbone et al., 1998, 1999b), including stimulation of astrocyte proliferation (Ciccarelli et al., 2000; Kim et al., 1991), synthesis and release of trophic factors such as immunoreactive nerve growth factor from astrocyte cultures (Caciagli et al., 2000; Middlemiss et al., 1995), and (iv) can enhance the differentiation of PC12 cells and hippocampal neurons in vitro (Gysbers & Rathbone, 1992, 1996). The role of GTP as a trophic mediator received strong support from data confirming that specific binding sites for GTP are present on the plasma membrane of neuronal-like PC12 cells (Gysbers & Rathbone, 1996; Gysbers et al., 2000; Guarneri et al., 2004) and C2C12 mouse skeletal muscle cells (Pietrangelo et al., 2002) and that GTP is stored in synaptic vesicles (Santos et al., 2006; Zimmermann, 1996). The expression of GTP specific binding sites is directly correlated with the effects of this nucleotide in promoting neuronal differentiation (Gysbers et al., 2000). Extracellular GTP enhances the neurotrophic effects of nerve growth factor on PC12 cells, significantly increasing the proportion of cells that have neuritis (Gysbers & Rathbone, 1996; Gysbers et al., 2000; Guarneri et al., 2004). Although some extracellular effects of GTP might be related to its conversion to guanosine, other findings indicate that a different mechanism of action between them may be present, as in the case of neurite outgrowth stimulation. For example, GTP transduction mechanisms in PC12 cells probably involve intracellular calcium mobilization and enhancement of NGF-induced extracellular regulated kinases (ERK). This mobilization, due to the activation of voltage-sensitive and ryanodinedsensitived calcium channels, as well as pertussis toxin-sensitive purinoceptors, modulated Ca^{2+}-activated K^+ channels not involved in activation of ERK (Guarnieri et al., 2004).

Guanosine has also been shown to stimulate the output of adenine-based purines from astrocytes and triggered these cells to proliferate and to produce large amounts of neuroprotective factors (Ciccarelli et al., 2000).

2.8. Receptors and second messenger systems associated with guanine-based purines

Astrocytes are important targets for purines since they express several P1 and P2 types receptor subtypes (Burnstock, 2006b; Ciccarelli et al., 2001; Neary et al., 1996). ATP (via P2 receptors) and adenosine (via A2 receptors) stimulate astrocyte proliferation, and adenosine (via A1 and/or A3 receptors) inhibits astrocyte proliferation, thus controlling the excessive reactive astroglisis triggered by P2 receptors. The activation of A1 receptors also stimulates astrocytes to produce trophic factors, such as NGF, S100β protein, and transforming growth factor beta, which contribute to protect neurons against injuries. However, evidence for putative receptors or specific binding sites for either guanosine or GTP in astrocytes (Ciccarelli et al., 2001) or other cell types (Vuorinen et al., 1992) has been lacking, despite several documented biological effects of these compounds. Neither guanosine nor GTP binds with high-affinity to adenine-based purine receptors (Muller & Sciör, 1993), suggesting that guanine-based purines had distinct cellular targets from adenine-based purines.

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 (Uemura et al., 1991). Additionally, many trophic effects of guanine-based purines were not affected by the nucleoside uptake inhibitors, such as NBPT or dipyridamole (Gysbers & Rathbone, 1992), indicating that they are triggered extracellularly. Guanosine also stimulated the release of adenine-based purines from astrocytes, which may, in turn, be responsible for some other effects of guanosine (Ciccarelli et al., 2000). Possibly, guanine-based purines would increase extracellular levels of adenine-based purines by interfering with their uptake and metabolism as well as by stimulating their release. 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 (Ciccarelli et al., 2000). But this explanation is also incomplete, since many of the effects of guanine-based purines persist in the presence of P1 and/or P2 purine receptor antagonists (Frizzo et al., 2001; Gysbers & Rathbone, 1992; Tasca & Souza, 2000).

An alternative hypothesis is that there are distinct receptors for guanine-based purines. Moreover, several of the effects of guanosine may be mediated through G protein-dependent signaling pathways involving cyclic nucleotides or MAP kinase pathway (Caciagli et al., 2000; Gysbers & Rathbone, 1996), raising the possibility that some of the effects of guanine-based purines, particularly guanosine, involve activation of cell-surface receptors.

As commented above, there is data supporting the possible existence of specific binding sites for GTP in PC12 cells (Gysbers et al., 2000). In regard to guanosine, Traversa et al. showed that a specific binding site for guanosine was detected on membrane preparations from rat brain (Traversa et al., 2002, 2003). Kinetics of guanosine binding to membranes was described (Traversa et al., 2002) as a single high-affinity binding site for guanosine with a KD of 95.4±11.9 nM and Bmax of 0.57±0.03 pmol mg⁻¹ protein. The order of potency...
in displacing guanosine was: guanosine=6-thio-guanosine>inosine>6-thio-guanine>guanine. Other naturally occurring purines, such as adenosine, hypoxanthine, xanthine, caffeine, theophylline, GDP, GMP, and ATP were unable to significantly displace the radiolabelled guanosine. Thus, this binding site is distinct from the well-characterized receptors for adenine-based purines. The addition of GTP produced a small concentration-dependent decrease in guanosine binding, which could suggest that this site is linked to a G protein. These findings are consistent with the existence of a novel cell membrane receptor site(s), specific for guanosine. Through its specific binding site, guanosine may promote its extracellular effect by activating MAP kinase cascade in astrocytes (Traversa et al., 2002). However, genetic and biochemical characterization of this specific membrane site for guanosine, its related second messengers and mechanisms involved in guanosine signal transduction remain to be clarified in future research.

3. Potential physiological and pharmacological implications of a new guanine-based purinergic system

The neuromodulatory effects of guanosine on the glutamatergic system are potentially relevant. Glutamate is the main excitatory neurotransmitter in mammalian CNS and is essential for its normal functions (Ozawa et al., 1998; Sheng & Hoogenraad, 2006). Glutamate acts via ionotropic (ligand-gated ion channel; NMDA, KA, or AMPA receptors) or metabotropic (coupled to G proteins) receptors, modulating several plastic brain processes, such as learning and memory, pain, ontogeny, and development, and several brain responses to external stimuli (Beart & O’Shea, 2007; Izquierdo et al., 2006; Parsons et al., 1998; Segovia et al., 2001).

However, overstimulation of the glutamatergic system (by exogenous or endogenous stimuli), which occurs when extracellular glutamate levels increase over the physiological range, is involved in various acute and chronic brain diseases (excitotoxicity), including neurodegenerative diseases, traumatic brain injury, cerebral ischemia, and seizures (Allen et al., 2004; Castellano et al., 2001; Lipton & Rosenberg, 1994; Maragakis & Rothstein, 2004; Meldrum, 1994). 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 (Anderson & Swanson, 2000; Chen & Swanson, 2003; Hertz, 2006; Matute et al., 2006; Schousboe & Waagepetersen, 2005). 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.

Glutamate undoubtedly plays a pivotal role on epilepsy and probably in other CNS diseases accompanied by seizures (Allen et al., 2004; Castellano et al., 2001; Lipton & Rosenberg, 1994; Maragakis & Rothstein, 2004; Meldrum, 1994). However, cellular and molecular mechanisms involved in the generation and maintenance of seizures and toxicity are not fully understood. Several studies demonstrated that guanine-based purines, mainly guanosine, are anticonvulsant against glutamatergic agents in animals as discussed earlier (Lara et al., 2001; Oliveira et al., 2004; Schmidt et al., 2000; Schmidt et al., 2005; Vinadé et al., 2003; Vinadé et al., 2005). Quinolinic acid is a NMDA agonist, but also stimulates glutamate release and inhibits its uptake (Tavares et al., 2002). We demonstrated that systemic (intrapertionital or oral) administration of GMP or guanosine dose-dependently protected against seizures induced by quinolinic acid in mice and rats (Lara et al., 2001; Schmidt et al., 2000; Tavares et al., 2005). This same effect was observed with all guanine-based purines when administered i.c.v. (Schmidt et al., 2005). These effects were reproducible even after chronic oral systemic exposure to guanosine or GMP, indicating that these substances are orally active in the long term (Vinadé et al., 2003, 2005). Guanosine also protected against quinolinic acid-induced seizures and prevented quinolinic acid-induced decrease on glutamate uptake by brain slices in young (12–14 days old) (Oliveira et al., 2004) or adult (Vinadé et al., 2005) rats, pointing that guanosine could be considered for epilepsy treatment and possibly other neurological disorders in adults and children. Additionally, when injected into the rat striatum, quinolinic acid causes dose-dependent widespread cell death. All cell types, including the NADPH-diaphorase-positive neurons appear to be sensitive to the toxin. The NADPH-diaphorase-positive cells are chronically destroyed by in vivo quinolinic acid injections, but this effect was blocked by the concomitant administration of GMP (Malcon et al., 1997), strengthening the notion that the guanine-based purinergic system may be a valuable target for the treatment of neurodegenerative disorders.

Guanine-based purines have recently been enrolled in the pathogenesis of the Lesch-Nyhan syndrome (Deutsch et al., 2005). Investigation of this disorder and the neurobiological consequences of the hypoxanthine phosphoribosyltransferase (HPRT) deficiency demonstrated the potential roles that guanine-based purines play in neurodevelopment and as neuromodulators and neurotransmitters. Conceivably, diminished reutilization of free guanine bases due to absent or negligent HPRT activity and relatively high guanase activity in the brain could lead to deficient pools of guanosine associated with glutamatergic synapses in Lesch-Nyhan syndrome. Nonetheless, if a guanosine deficiency were to exist in Lesch-Nyhan syndrome, the administration of guanosine itself or its analogs that could promote glutamate uptake might be a useful pharmacological strategy to be considered in the treatment of this disorder.

Apoptosis is implicated in the pathophysiology of Alzheimer’s disease. Recently, it was shown that extracellular guanosine inhibited staurosporine-induced apoptosis in astrocytes (Di Iorio et al., 2004). Guanosine has also been shown to protect SH-SY5Y cells against beta-amyloid-induced apoptosis (Petitier et al., 2004). More recently, guanosine was shown to dose-dependently inhibit the CD40-induced expression in mouse microglia cells, as well as functional CD40 signaling by suppressing IL-6 production (D’Alimonte et al., 2007). The antiapoptotic effects of guanosine seemed to be mediated...
purines. Interestingly, the amount of guanine-based}

purines (mainly guanosine) released over a 3-hr period was

greater than that of adenine-based purines (Ciccarelli et al.,
1999b). Moreover, the exposure of these cultures to hypoxia/
hypoglycemia resulted in sustained increase in the release of

guanine- and adenine-based purines over basal values up to
90 min after the insult. Importantly, the release of purines
was not related to an artifact of diminished cell viability
(Ciccarelli et al., 1999b). These effects in an in vitro

ischemia/stroke model are consistent with the hypothesis

that these compounds, especially guanosine, may exert
pivotal modulatory effects on synaptic transmission and
more sustained trophic effects. 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 (Ciccarelli
et al., 2001; Litsky et al., 1999). For example, after exposure
to rotenone, an inhibitor of the mitochondrial respiratory
chain, and the induction of chemical hypoxia, inosine and

guanosine (250–500μM) were shown to preserve the viability
of cultured astrocytes and neurons (Jurkowitz et al., 1998;
Litsky et al., 1999). The ability of purine nucleosides (inosine
and 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
most dramatic with neurons. The data also suggested that
neuronal protection by purine nucleosides is either dependent
on or enhanced by the presence of glia (Litsky et al., 1999).

Adenine-based purines have been suggested to play a role in
psychiatric diseases, such as schizophrenia and bipolar
disorder (Lara & Souza, 2000; Lara et al., 2006; Machado-
Veira et al., 2002). Since guanine- and adenine-based

purines are closely related and share many in vitro and in
vivo extracellular effects and pathways, it is not surprising
that guanine-based purines also play a role in psychiatric
diseases, as well as adenine-based purines. Recently, we
showed that guanosine attenuates hyperlocomotion induced
by dizocilpine (a pharmacological model of schizophrenia) in
mice (Tort et al., 2004) and presented an anxiolytic profile
after chronic oral treatment (Vinadé et al., 2003). These
effects may be due to an increase in glutamate uptake by
astrocytes promoted by guanosine, reducing the neurotrans-
mmitter levels at the synaptic cleft, leading to less activation of
non-NMDA receptors, with subsequent less increase in the

efflux of dopamine in the prefrontal cortex (Tort et al.,
2004). These findings point to a potential antipsychotic
property of guanosine, as this model could predict compounds
that target psychotic symptoms that are not generally treated
with typical antipsychotics (Adams & Moghaddam, 2001).

Moreover, the neuroprotective and neurotrophic effects of
guanosine may also be advantageous for the treatment of
schizophrenia, which is associated with inadequate
neurodevelopment and increased brain loss after onset of the disorder (Lara et al., 2006).

Considering that purines, their metabolites, and the soluble nucleotidases responsible for their hydrolysis are detected in the human and animal CSF and blood serum (Castro-Gago et al., 1992; Oses et al., 2004a; Portela et al., 2002; Regner et al., 1997; Silva et al., 2004), their potential role on an "endogenous neuroprotection system," and the lack of precise and safe parameters to evaluate brain injury consequences, it is possible that these parameters may be new putative markers for CNS injury. We have demonstrated that PTZ-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 min after the insult (Oses et al., 2004b). Increases of GDP/ADP hydrolysis and levels of nucleosides guanosine/inosine after PTZ-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.

Altogether, these findings point to the influence of guanine-based purines in the homeostasis of the glutamatergic system, modulating some glutamatergic parameters such as glutamate uptake by astrocytes and synaptic vesicles, seizures induced by glutamatergic agents, learning and memory, anxiety, ischemia, and excitotoxicity. Since guanosine is an endogenous

<table>
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<th>Table 1</th>
<th>Summary of main extracellular effects of guanine-based purines</th>
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<td>In vitro studies</td>
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<td><strong>Guanine nucleotides</strong></td>
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<tr>
<td>Inhibit kainic acid binding</td>
<td>Souza &amp; Ramirez, 1991</td>
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<tr>
<td>Inhibit glutamate binding and its analogs</td>
<td>Rubin et al., 1997</td>
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<tr>
<td>Prevent cell responses to excitatory amino acids</td>
<td>Burgos et al., 1998</td>
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<tr>
<td>Stimulate glutamate uptake by astrocytes</td>
<td>Frizzo et al., 2003</td>
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<tr>
<td>Stimulate the glutamate uptake by synaptic vesicles</td>
<td>Tasca et al., 2004</td>
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<tr>
<td>GMP prevents loss of cell viability induced by glutamate</td>
<td>Molz et al., 2005</td>
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<tr>
<td>GMP prevents loss of cell viability induced by hypoxia</td>
<td>Oliveira et al., 2002</td>
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<tr>
<td>GTP induces astrocyte proliferation</td>
<td>Ciccarelli et al., 2000</td>
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<tr>
<td>GTP induces synthesis and release of trophic factors</td>
<td>Middlemiss et al., 1995</td>
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<tr>
<td>GTP stimulates proliferation of a wide range of cell types</td>
<td>Rathbone et al., 1992a</td>
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<tr>
<td><strong>Guanosine</strong></td>
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<tr>
<td>Increases glutamate uptake by astrocytes</td>
<td>Frizzo et al., 2001, 2002</td>
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<tr>
<td>Prevents the decrease of glutamate uptake induced by hypoxic-ischemic insult</td>
<td>Moretto et al., 2005</td>
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<tr>
<td>Protects brain slices exposed to hypoxia/hypoglycemia</td>
<td>Frizzo et al., 2002</td>
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<tr>
<td>Prevents NMDA-induced toxicity in neurons</td>
<td>Caciagli et al., 2000</td>
</tr>
<tr>
<td>Induces trophic effects on neural cells (astrocytes)</td>
<td>Rathbone et al., 1999a, 1999b</td>
</tr>
<tr>
<td>Prevents beta-amyloid-induced apoptosis</td>
<td>Pettifer et al., 2004</td>
</tr>
<tr>
<td>Inhibits staurosporine-induced apoptosis in astrocytes</td>
<td>Di Iorio et al., 2004</td>
</tr>
<tr>
<td>Increases the expression of ApoE in astrocytes</td>
<td>Ballerini et al., 2006</td>
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| In vivo studies |
| **Guanine nucleotides** |
| Prevent quinolinic acid-induced seizures in rodents | Schmidt et al., 2005 |
| GMP prevents quinolinic acid-induced neuronal death | Malcon et al., 1997 |
| GMP prevents quinolinic acid-induced release of glutamate on synaptosomes | Tavares et al., 2005 |
| GMP induces amnesia in rats | Saute et al., 2006 |
| GMP reverses the facilitatory effect of glutamate on memory in rats | Rubin et al., 1996 |
| **Guanosine** |
| Prevents seizures induced by several glutamatergic agents in rodents | Lara et al., 2001 |
| Chronic treatment is anxiolytic in mice | Vinadé et al., 2003 |
| Induces amnesia in rats | Roessler et al., 2000 |
| Attenuates hyperlocomotion induced by MK-801 | Tort et al., 2004 |
| Prevents in vivo decrease of astrocytic glutamate uptake induced by quinolinic acid | Vinadé et al., 2005 |
| Improves locomotor function and remyelination in rats submitted to a spinal cord injury model | Jiang et al., 2003 |

Table 2 | Research agenda for new studies related to the guanine-based purinergic system |
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<td>(1) Characterization of the guanosine and GTP receptors</td>
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<td>(2) Identification of specific pharmacological tools to manipulate this system</td>
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<td>(3) Elucidation of signal transduction mechanisms linked to guanosine and GTP receptors</td>
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<td>(4) Correlation between some purinergic parameters (extracellular levels of nucleosides and nucleotidases activity) and CNS events</td>
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<tr>
<td>(5) Investigation of the safety profile and toxicity of guanine-based purines after systemic and/or continuous administration</td>
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<tr>
<td>(6) Clinical trials in humans by using purine derivatives (guanosine, xantine oxidase inhibitors such as allopurinol, nucleoside transport inhibitors)</td>
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</table>
compound and seems to be well tolerated with no obvious CNS side effects except for memory impairment, it could be useful in the future to treat or prevent neurological diseases associated with overstimulation of the glutamatergic system. Additionally, these studies on the extracellular effects of guanine-based purines have strengthened the proposal for a specific guanine-based purinergic system in addition to the well-known adenine-based purinergic system.

4. Conclusions and perspectives for new guanine-based purine studies

This article reviews the evidence to hypothesize that a guanine-based purinergic system plays significant roles in the nervous system, providing new targets for neuroprotection and neuromodulation (Table 1). These guanine-based purines have been relatively neglected when compared to adenine-based purines and should be further investigated as a neurotransmission/neuromodulator system in terms of physiological, pharmacological, biochemical, and genetic parameters. Furthermore, the profile of guanine-based purines to modulate the glutamatergic system makes this system a very interesting object for discovery of new pharmacological options to treat diseases related to overstimulation of glutamatergic system (Table 2).

More specifically, to advance in guanine-based purine research, further work is necessary on the mechanisms of action of guanine-based purines, cloning of specific receptors, and characterization of second messengers related to their extracellular effects. Since the recent identification of a high-affinity binding site for guanosine in rat brain membranes (Traversa et al., 2002, 2003), a modest advance on guanosine receptor characterization and elucidation of its mechanism of action has emerged. New studies on guanosine binding are needed and should include a careful purification of cellular membranes by decreasing mitochondrial contamination. Although signal transduction mechanisms linked to guanosine receptor are also unknown, they may be linked via G proteins to the MAPK cascade since the ability of guanosine to enhance synthesis of trophic factors in astrocytes is associated with an increase in phosphorylation of ERK1 and ERK2 and is blocked by pretreatment with pertussis toxin (Caciagli et al., 2000). New studies regarding guanine-based purines receptors could further address this issue. Additional elucidation of the mechanism of action of guanosine and its membrane-binding site is under current investigation in our laboratory.

Guanine-based purines present several beneficial effects to CNS during excitotoxic conditions. However, little information about potential side effects of these compounds is available. Although no obvious disturbances regarding locomotion and behavior apart from amnesia were noted, specific studies about the safety profile of these compounds is pivotal for their future use in a clinical basis. These studies should include further investigation on absorption, metabolism, half-lives, storage, and elimination of these compounds (guanosine being the best candidate so far). Likewise, pharmacological determination of lethal doses and therapeutic index are also relevant and should be addressed in future studies.

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. These studies should include clinical trials designed to address neuroprotection measures. It may be a reality for memory-enhancing agents in Alzheimer’s or neurodegenerative diseases. Propentofylline and AIT-082 are purine derivatives that have been demonstrated to exert trophic effects in animals by increasing production of neurotrophic factors in brain and spinal cord (Rathbone et al., 1999a). Additionally, we have demonstrated that allopurinol, a xantine oxidase inhibitor, was an effective and well-tolerated adjuvant treatment for poorly responsive schizophrenia, refractory aggressive behavior, and mania (Brunstein et al., 2005; Lara et al., 2000; Lara et al., 2003; Machado-Vieira et al., 2001). These results were confirmed by an independent group (Akhondzadeh et al., 2005, 2006). Refractory epilepsy may also respond to allopurinol (Togha et al., 2007; Zagnoni et al., 1994). These effects may be due to an indirect increase in extracellular purine levels (adenosine and guanosine). Thus, allopurinol may be the first commercially available effective drug enhancing the effects of the purinergic systems for the treatment of human brain diseases. These findings, together with the evidence of purine modulation of CNS, indicate that new studies addressing xantine oxidase inhibitors in neuroprotection and psychiatric disorders could represent a fine approach to investigate the therapeutic potential of purine in a clinical basis.

In conclusion, the guanine-based purinergic system is yet to be fully characterized, but current evidence strongly suggests its functional roles in the mammalian nervous system, providing new targets and strategies for the treatment of brain diseases.

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