



● PERSPECTIVE

Current perspectives on the antidepressant-like effects of guanosine

Purines have a recognized importance as intercellular messengers. These molecules play an important role in the development and maintenance of the central nervous system (CNS), as well as in its response to pathological conditions. Both adenine and guanine-based purines can be released from astrocytes, where they play a relevant role as extracellular signaling molecules. Particularly, the nucleoside guanosine has been proposed as an extracellular molecule that regulates the response of CNS to damage. Following injury, this nucleoside attains high concentrations in the extracellular space, where it activates multiple signaling pathways, leading to neurotrophic and neuroprotective effects. Despite the fact that the role of guanosine in the CNS is just now being elucidated, several lines of evidence have suggested that this nucleoside protects neural tissue from damage by activating anti-apoptotic, anti-inflammatory and antioxidant mechanisms (Rathbone et al., 2008). Within this context, the protective effects of guanosine have been demonstrated against a plethora of different insults. These effects seem to be partially mediated by a specific G-protein coupled binding site, raising the possibility of existence of a putative guanosine receptor. Additionally, the trophic and neuroprotective effects of guanosine also appear to involve an interaction with the adenosinergic system, since blockage of adenosine A1 and A2A receptors can partially inhibit these pro-survival properties of guanosine (which does not act as a ligand for these receptors). The action of guanosine on both the specific G-protein coupled binding site and the adenosinergic receptors is associated with the activation of pro-survival signaling pathways such as those involving the phosphatidylinositol-3 kinase (PI3K)/Akt and mitogen-activated protein kinases (MAPKs) (Rathbone et al., 2008). Indeed, synchronization of distinct signaling pathways by this nucleoside seems to underlie most of its trophic and neuroprotective properties, including its ability to stimulate astrocytic glutamate uptake and the release of growth factors, as well as the induction of anti-inflammatory and antioxidant responses (Rathbone et al., 2008; Bettio et al., 2016a). The trophic response elicited by guanosine involves the astrocytic release of molecules with a crucial role in the reparative processes of CNS including nerve growth factor (NGF), transforming growth factor beta (TGF β) and fibroblast growth factor 2 (FGF-2) (Bau et al., 2005; Rathbone et al., 2008; Su et al., 2009). Furthermore, this nucleoside is able to induce neural stem cell proliferation by increasing the expression of brain-derived neurotrophic factor (BDNF) (Su et al., 2013).

Keeping in mind that the multiple mechanisms underlying guanosine activity are commonly dysregulated in most neuropathologies (*i.e.*, excitotoxicity, neuroinflammation and oxidative damage), several studies have evaluated the therapeutic potential of this molecule in different *in vitro*

and *in vivo* models of neurological conditions (for review see Bettio et al., 2016a). Within this context, the idea that guanosine may be an interesting target for future clinical investigations is supported not only by its protective effects, but also by its ability to stimulate regenerative processes in the CNS. For instance, the combination of neuroprotective and trophic properties of guanosine have an impact in the loss of dopaminergic neurons observed in Parkinson's disease, as evidenced by a study investigating the functional recovery induced by chronic administration of this nucleoside in rats with parkinsonism (Su et al., 2009). This symptomatic improvement was related to the ability of guanosine to reduce apoptosis, stimulate neurogenesis in the subventricular zone (SVZ) and increase the number of tyrosine hydroxylase positive cells in the substantia nigra. Moreover, the relevance of guanosine for neural regeneration is further reinforced by studies investigating its systemic administration in rodents with spinal cord injury (SCI). Similarly, the functional recovery elicited by this nucleoside in SCI models seems to occur both through prevention of damage during the acute phase (*i.e.*, inflammatory cascades and apoptosis) and stimulation of regenerative processes (*i.e.*, proliferation and maturation of progenitor cells involved in remyelination) (Jiang et al., 2008).

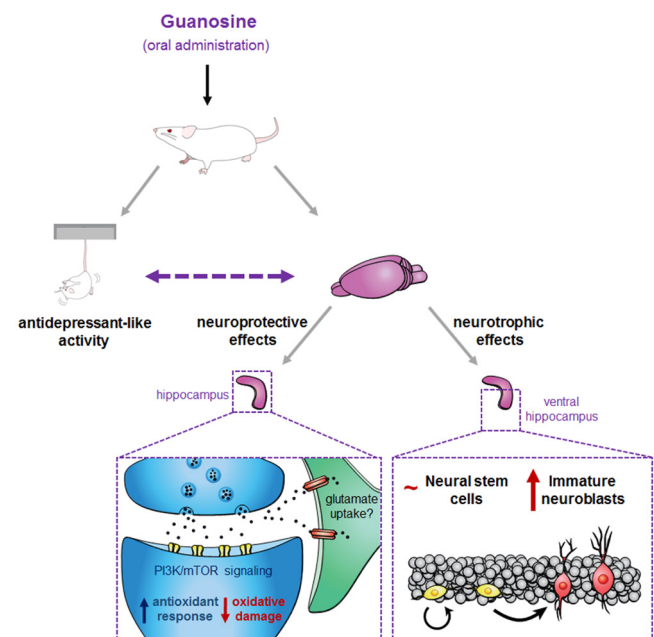


Figure 1 Mechanisms implicated in the antidepressant-like effect of guanosine.

The antidepressant-like properties of guanosine are associated with the activation of the phosphatidylinositol-3-kinase (PI3K) signaling pathway and its downstream target mammalian target of rapamycin (mTOR), as well as with its neuroprotective effects in the hippocampus, where this nucleoside reduces the occurrence of oxidative damage by stimulating the antioxidant response of neurons. In addition, the ability of this purine to stimulate glutamate uptake by neighboring astrocytes, may also contribute to its neuroprotective activity, particularly at hippocampal glutamatergic synapses. Furthermore, the antidepressant-like effects of guanosine are also correlated with the modulation of hippocampal structural plasticity (*i.e.*, adult hippocampal neurogenesis). In particular, guanosine can induce morphological alterations in the ventral portion of the hippocampal dentate gyrus (a region thought to play a role in affective behaviors and mood regulation), promoting a significant increase in the number of immature neurons without altering stem cell proliferation.

In line with this and given the similarities between the mechanisms underlying the neuroprotective effects of guanosine and those implicated in the activity of classic antidepressants (*i.e.*, release of trophic factors, activation of pro-survival signaling, reduction of excitotoxicity and inflammatory/oxidant parameters), our group has focused on investigating the antidepressant-like activity of this nucleoside. We found that the acute administration of guanosine causes an antidepressant-like effect in two predictive animal models: the forced swimming test (FST) and the tail suspension test (TST). In addition, we showed that these effects are dependent on the activation of the PI3K signaling pathway and its downstream target mammalian target of rapamycin (mTOR) (Bettio et al., 2012) (**Figure 1**).

Since stress is a major risk factor for depression and exposure to stressful events may stimulate a cascade of events that leads to hippocampal oxidative damage and behavioral alterations, we next investigated whether the neuroprotective properties of guanosine are related to its ability to prevent some of the deleterious effects induced by stress (Bettio et al., 2014). We focused on the hippocampus, as this structure plays a crucial role in the pathophysiology of depression and is particularly vulnerable to the effects of stress (due to its high concentration of glucocorticoid receptors). As expected, exposure to acute restraint stress caused an imbalance in the intracellular redox state in the hippocampus, namely an increase in lipid peroxidation and alterations in the activity of endogenous antioxidant enzymes. Guanosine pre-treatment was able to normalize the hippocampal redox status and increase the activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) (**Figure 1**). It is appealing to speculate that these effects are, at least partially, related to the activation of nuclear factor E2-related factor 2 (Nrf2), a major mechanism of cellular defense against oxidative stress that controls the expression of important endogenous antioxidant enzymes such as SOD, CAT, and heme oxygenase-1 [HO-1; an enzyme that has also been implicated in the neuroprotective effects of guanosine (Bau et al., 2005)]. Future studies are thus warranted to investigate the potential role of Nrf-2 activation in the antioxidant response induced by guanosine. In addition, the evaluation of the protective effects of guanosine against stress-induced mitochondrial dysfunction is also warranted, since it is known that guanosine is able to protect these organelles by maintaining redox homeostasis and inducing HO-1 (Dal-Cim et al., 2012).

Previous *in vitro* studies have also shown that guanosine can induce cell proliferation and neuronal differentiation, which may underlie its neurotrophic and regenerative properties in the CNS (Rathbone et al., 2008). Of note, regulation of proliferation, differentiation, and survival of neural stem cells is promoted by signaling events mediated by several neurotrophic factors, which are thus crucial for maintaining and restoring tissue functionality during regenerative processes. The release of trophic factors in the hippocampus and the consequent stimulation of neurogenesis (*i.e.*, the generation and functional integration of new neurons) and synaptogenesis (*i.e.*, the formation of new functional synapses) also appears to play a crucial role in the mechanisms triggered by many antidepressants. In support of its pro-neurogenic properties, guanosine has been shown to induce

cell proliferation in the SVZ (Su et al., 2009). Furthermore, we have recently investigated whether this nucleoside can influence hippocampal neurogenesis (Bettio et al., 2016b), a form of plasticity thought to play a role in certain aspects of cognition (*i.e.*, spatial learning and memory) as well as in mood regulation (and therefore antidepressant mechanisms). Our findings demonstrated that chronic guanosine treatment results in an antidepressant-like effect that is positively correlated with an increase in the number of immature neuroblasts in the sub-granular zone (SGZ) of the ventral hippocampal dentate gyrus (DG) (Bettio et al., 2016b) (**Figure 1**). Of note, the hippocampus is a functionally heterogeneous structure that has distinct patterns of gene expression and anatomical connections along its dorsal/ventral axis. Thus, while the dorsal hippocampus participates in learning and spatial memory processes, the ventral hippocampus is thought to primarily regulate emotional and motivational behaviors. Therefore, our results raise the possibility that guanosine may distinctly influence these two hippocampal sub-regions by preferentially stimulate neuronal differentiation in the ventral hippocampus, and thus resulting in changes in motivational but not cognitive (*i.e.*, learning and memory) aspects of behavior. In agreement, chronic guanosine treatment was shown to have neuroprotective effects in the hippocampus without causing alterations in the performance of animals in the Morris water maze (a behavioral test commonly used to assess hippocampal-dependent spatial learning and memory) (Ganzella et al., 2012).

Despite these compelling findings, the elucidation of the mechanisms underlying the antidepressant-like properties of guanosine is still in its early stages and numerous questions remain to be addressed. For example, it is well-known that a reduction in hippocampal stem cell proliferation is implicated in the pathophysiology of depression. Therefore, even though we did not observe alterations in hippocampal cell proliferation in naïve animals, it is possible that guanosine may indeed induce hippocampal cell proliferation in situations where this proliferative capacity is compromised (as seen with depression). Accordingly, current studies are underway to determine whether different regimes of guanosine administration may indeed affect hippocampal cell proliferation. In addition, future investigations are warranted to unravel the mechanisms underlying the guanosine-induced increase in neuronal commitment that we specifically observed in the ventral DG (Bettio et al., 2016b). It is possible that the well-known anti-apoptotic effects of guanosine have contributed to an increase in the survival of immature neurons in the ventral aspect of the hippocampal DG (Rathbone et al., 2008). In addition, since it is known that this nucleoside presents neurotogenic properties (Bau et al., 2005), a specific increase in neuronal commitment and differentiation (rather than solely stimulation of neuronal survival) is likely to underlie these findings. In agreement, the guanosine-induced enhancement in neurite outgrowth was shown to be accompanied by an increase in the expression of various differentiation markers (Bau et al., 2005).

Another important point that deserves further attention is the influence that guanosine may have on synaptic plasticity in the hippocampus. Mounting evidence has suggested that compounds (such as ketamine) with the ability to induce

the rapid release of growth factors and consequent increase in synaptogenesis may act as fast-acting antidepressants (Abdallah et al., 2015). Within this scenario, we are currently investigating whether the ability of guanosine to alleviate depressive-like phenotypes in models of chronic stress is indeed associated with an increase in the levels of multiple growth factors. This study will also be important since to date we have only evaluated the antidepressant-like effect of guanosine in predictive animal models and therefore, by using animal models of chronic stress, it will be possible to evaluate other endophenotypes that may mimic human depression, such as alterations in food consumption, as well as anhedonic and anxiety-like behaviors. Additionally, considering that neuritogenesis and synaptogenesis are both critical processes of neuronal differentiation, maturation, and ultimately synaptic integration, it is likely that guanosine may increase synaptic transmission in key brain regions, particularly the hippocampus and prefrontal cortex. Thus, investigating whether chronic guanosine treatment will affect the levels of specific synaptic proteins and the expression of various forms of synaptic plasticity (such as long-term potentiation and long-term depression) and determining the time-course of such changes will allow us to verify how fast this nucleoside may be able to reverse the chemical and functional alterations associated with depressive-like phenotypes.

Despite being a disorder with high prevalence and prominent social impact, the current pharmacological therapy for major depression is associated with several side effects and a delayed onset of action. Taking into account that depression is associated with neuronal atrophy and cell death (particularly in the hippocampus), the well-known neuroprotective properties of guanosine and its ability to stimulate the release of several neurotrophic factors, make this nucleoside an interesting therapeutic candidate for the treatment of this mood disorder. We have provided evidence that this nucleoside is able to modulate hippocampal processes, preventing the development of depressive-like phenotypes and exerting an antidepressant-like activity by mechanisms dependent on its neuroprotective and neurotrophic properties. Future studies will further elucidate the role of this nucleoside as an endogenous regulator of hippocampal plasticity, as well as its efficacy in reversing the deleterious effects associated not only with depression but also with a broad spectrum of neurological conditions characterized by hippocampal dysfunction.

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References

- Abdallah CG, Sanacora G, Duman RS, Krystal JH (2015) Ketamine and rapid-acting antidepressants: a window into a new neurobiology for mood disorder therapeutics. *Ann Rev Med* 66:509-523.
- Bau C, Middlemiss PJ, Hindley S, Jiang S, Ciccirelli R, Caciagli F, DiIorio P, Werstik ES, Rathbone MP (2005) Guanosine stimulates neurite outgrowth in PC12 cells via activation of heme oxygenase and cyclic GMP. *Purinergic Signal* 1:161-172.
- Bettio LEB, Cunha MP, Budni J, Pazini FL, Oliveira Á, Colla AR, Rodrigues ALS (2012) Guanosine produces an antidepressant-like effect through the modulation of NMDA receptors, nitric oxide-cGMP and PI3K/mTOR pathways. *Behav Brain Res* 234:137-148.
- Bettio LEB, Freitas AE, Neis VB, Santos DB, Ribeiro CM, Rosa PB, Farina M, Rodrigues ALS (2014) Guanosine prevents behavioral alterations in the forced swimming test and hippocampal oxidative damage induced by acute restraint stress. *Pharmacol Biochem Behav* 127:7-14.
- Bettio LEB, Gil-Mohapel J, Rodrigues ALS (2016a) Guanosine and its role in neuropathologies. *Purinergic Signal* doi:10.1007/s11302-016-9509-4.
- Bettio LEB, Neis VB, Pazini FL, Brocardo PS, Patten AR, Gil-Mohapel J, Christie BR, Rodrigues ALS (2016b) The antidepressant-like effect of chronic guanosine treatment is associated with increased hippocampal neuronal differentiation. *Eur J Neurosci* 43:1006-1015.
- Dal-Cim T, Molz S, Egea J, Parada E, Romero A, Budni J, Martín de Saavedra MD, del Barrio L, Tasca CI, López MG (2012) Guanosine protects human neuroblastoma SH-SY5Y cells against mitochondrial oxidative stress by inducing heme oxygenase-1 via PI3K/Akt/GSK-3beta pathway. *Neurochem Int* 61:397-404.
- Ganzella M, De Oliveira EDA, Comassetto DD, Cechetti F, Cereser VH, Moreira JD, Hansel G, Almeida RF, Ramos DB, Figueredo YN, Souza DG, Oses JP, Worm PV, Achaval M, Netto CA, Souza DO (2012) Effects of chronic guanosine treatment on hippocampal damage and cognitive impairment of rats submitted to chronic cerebral hypoperfusion. *Neurol Sci* 33:985-997.
- Jiang S, Ballerini P, Buccella S, Giuliani P, Jiang C, Huang X, Rathbone MP (2008) Remyelination after chronic spinal cord injury is associated with proliferation of endogenous adult progenitor cells after systemic administration of guanosine. *Purinergic Signal* 4:61-71.
- Rathbone M, Pilutti L, Caciagli F, Jiang S (2008) Neurotrophic effects of extracellular guanosine. *Nucleosides Nucleotides Nucleic Acids* 27:666-672.
- Su C, Elfeki N, Ballerini P, D'Alimonte I, Bau C, Ciccirelli R, Caciagli F, Gabriele J, Jiang S (2009) Guanosine improves motor behavior, reduces apoptosis, and stimulates neurogenesis in rats with parkinsonism. *J Neurosci Res* 87:617-625.
- Su C, Wang P, Jiang C, Ballerini P, Caciagli F, Rathbone MP, Jiang S (2013) Guanosine promotes proliferation of neural stem cells through cAMP-CREB pathway. *J Biol Regul Homeost Agents* 27:673-680.