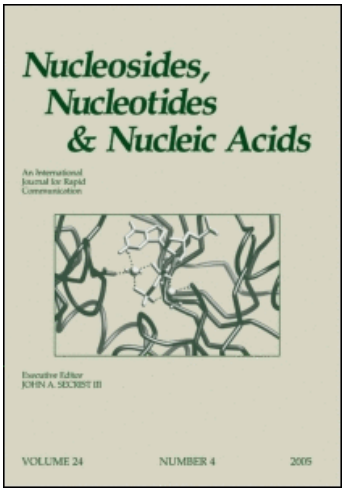

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Michel Rathbone[a]; Lara Pilutti[b]; Francesco Caciagli[c]; Shucui Jiang[d]
[a] Department of Medicine (Neurology), McMaster University, Hamilton, Canada [b] Department of Kinesiology, McMaster University, Hamilton, Canada [c] Department of Biomedical Sciences, University of Chieti, Chieti, Italy [d] Department of Surgery (Neurosurgery), McMaster University, Hamilton, Canada

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NEUROTROPHIC EFFECTS OF EXTRACELLULAR GUANOSINE

Michel Rathbone,[1] Lara Pilutti,[2] Francesco Caciagli,[4] and Shucui Jiang[3]

[1]*Department of Medicine (Neurology), McMaster University, Hamilton, Canada*
[2]*Department of Kinesiology, McMaster University, Hamilton, Canada*
[3]*Department of Surgery (Neurosurgery), McMaster University, Hamilton, Canada*
[4]*Department of Biomedical Sciences, University of Chieti, Chieti, Italy*

□ *Central nervous system (CNS) astrocytes release guanosine extracellularly, that exerts trophic effects. In CNS, extracellular guanosine (GUO) stimulates mitosis, synthesis of trophic factors, and cell differentiation, including neuritogenesis, is neuroprotective, and reduces apoptosis due to several stimuli. Specific receptor-like binding sites for eGUO in the nervous system may mediate its effects through both MAP kinase and PI3-kinase signalling pathways. Extracellular guanine (eGUA) also exerts several effects; the trophic effects of eGUO are likely regulated by conversion of eGUO to eGUA by a membrane located purine nucleoside phosphorylase (ecto-PNP) and by conversion of eGUA to xanthine by guanine deaminase.*

Keywords Guanosine; neurotrophic effects; MAP kinase; PI3 kinase; purine nucleoside phosphorylase

Guanosine (GUO) participates in structural and regulatory components of cells, but also exists extracellularly.[1] In brain, extracellular guanine-based purines are primarily released from glial cells, most likely as nucleotides that are metabolized by ecto-nucleotidases to extracellular GUO (eGUO).[2] In turn eGUO is converted to guanine (GUA) by the recently identified membrane located purine nucleoside phosphorylase (ecto-PNP) (F. Caciagli, unpublished observations). Stimulation of astrocyte cultures by, for example, combined oxygen-glucose deprivation or field electrical stimulation markedly increases eGUO,[1] and following focal brain injury in vivo, eGUO is elevated for up to one week.[3,4]

Less is known about extracellular GUA (eGUA). But it too, likely has an important role in the central nervous system (CNS). It is metabolized irreversibly to xanthine by guanine deaminase, an enzyme particularly located

Address correspondence to Michael Rathbone, Department of Medicine (Neurology), McMaster University, Health Sciences Centre, 4N71B, 1200 Main Street West, Hamilton, ON L8N 3Z5, Canada. E-mail: mrathbon@mcmaster.ca

in neurons of certain forebrain areas: cortical pyramidal neurons, hippocampal pyramidal neurons in CA3, CA1, and granule cells in the dentate gyrus, amygdala neurons and ventral striatal medium spiny neurons.[5] The probable physiological importance of eGUO and eGUA in the striatum is strengthened by the finding that increasing dopamine turnover in rabbit striatum increases GUA and reduces GUO,[6] compatible with GUA and GUO having an important role.

We are currently investigating the relationship of ecto-PNP located on glia, neuronally localized guanine deaminase, and the functions of eGUA and eGUO in brain. There is evidence that GUO may play an important role in CNS signalling and may exert neuroprotective and neurorestorative effects in response to physiological and pathological conditions. For example, eGUO exerts numerous neurotrophic effects including: causing the proliferation of glial cells;[7–10] neurite outgrowth;[11,12] synthesis and release of purines and trophic factors, such as nerve growth factor (NGF), from several cell types;[13–18] and anti-apoptotic effects.[19,20] The effects of GUO are likely mediated through putative G-protein-linked cell-surface receptors.[21,22]

Although the localization of guanine deaminase in various areas of the brain raises the possibility that eGUO and eGUA may also have a role in synaptic transmission, most of the effects of eGUO delineated in the CNS appear to be "trophic," that is, it regulates cell growth, differentiation and survival.

eGUO stimulates the proliferation of astrocytes in vitro,[8,10] although this effect may be indirect and attributable, at least in part, to small numbers of contaminating microglia in the cultures since the effects of GUO on proliferation increase in proportion to the number of microglia present.[17] Under these conditions, GUO stimulates the release of interleukin-1 (Il-1) by microglia, which in turn stimulates astrocyte proliferation. However, eGUO can also directly affect proliferation since it causes proliferation of a wide range of other cell types that do not contain microglia.[7]

In addition to its effects on microglia, eGUO also enhances the synthesis of NGF and basic fibroblast growth factor (bFGF) mRNA in cultured mouse astrocytes,[13,15,18] and NGF and transforming growth factor β1 (TGFβ1) in rat astrocytes.[16] Not only does eGUO promote synthesis and release of peptide trophic factors but it also stimulates release of adenine-based purines including adenosine. Exposure of astrocytes to 300 μM eGUO for 1 hour increased extracellular levels of endogenous adenosine in culture medium by 1.5-fold.[17] Thus, extracellular adenosine may contribute to the trophic effects of eGUO.

A further biological effect of GUO that has been studied extensively is its ability to stimulate outgrowth of nerve processes (neurites). eGUO promotes neurite outgrowth in PC12 cells and also synergistically enhances NGF-dependent neurite outgrowth.[11,23] PC12 cells treated with 300 μM

GUO for 48 hours demonstrated neurite extensions in 6% of PC12 cells, significantly more than control conditions.[11] The presence of 40 ng/ml of 2.5S NGF resulted in 20–35% of PC12 cells with neurite extensions, and the co-presence of 300 μM GUO with 40 ng/ml of 2.5S NGF resulted in 40–65% of PC12 cells with neurite extensions following 48 hours.[5] Primary cultures of fetal mouse neurons respond with neurite outgrowth to much lower concentrations of eGUO from 1 to 100 μM.[24]

Finally, eGUO has been shown to have neuroprotective effects in vitro and in vivo. Anti-apoptotic effects of eGUO have been observed in astrocytes; cultures of astrocytes exposed to eGUO 1 hour before exposure to staurosporine had significantly fewer apoptotic cells than those exposed to staurosporine alone—23 and 54%, respectively.[20] Apoptosis induced by β-amyloid (βA) in SH-SY5Y human neuroblastoma cells was also reduced by eGUO.[25] Importantly, apoptosis induced in SH-SY5Y cells by MPP+, a mitochondrial toxin that produces a Parkinson's disease-like condition in animals and humans, was also reduced by addition of eGUO to the cultures not only when it is administered simultaneously with the MPP+ but also up to 48 hours later (Pettifer et al.[25]).

In vivo, systemically administered GUO is neuroprotective when administered 4 h after acute spinal cord crush injury in rats (Jiang et al.[26]). Furthermore, in rats with longstanding, stable spinal cord injury, systemically administered GUO promotes recovery of function; the locomotor gains correlate with remyelination of surviving denuded nerve fibres in the penumbra of the injury. The mature remyelinating oligodendrocytes appear to arise from quiescent oligodendroglial progenitor cells around the injury site (S. Jiang, unpublished data). It is not yet clear how systemically administered GUO stimulates the proliferation of the progenitor cells.

Although these trophic effects of eGUO are well established, the way in which eGUO produces its effects is only now being elucidated. Initially, the mitogenic effects of eGUO on astrocytes appeared be mediated in part through adenosine receptors, since the effects are at least partly inhibited by theophylline, an adenosine receptor antagonist.[8] In a variety of primary and tumor cell lines from chicks, mice and humans the effects of eGUO are inhibited by 1,3-dipropyl-7-methylxanthine (DMPX), an adenosine A2 receptor antagonist, but not by 1,3-dipropyl-8-(2-amino-4-chorophenyl) xanthine (PACPX), an A1 antagonist.[8] The ability of eGUO to stimulate [^{3}H] thymidine incorporation into cultures of rat fetal astrocytes was also partially inhibited by A1 and $A2_B$ receptor antagonists.[17]

Although the effects of eGUO on neurite outgrowth are also found to have a component that could be explained by eGUO-induced increases in extracellular adenosine in the medium,[12] eGUO had distinct extracellular effects that are unrelated to adenosine. Indeed, the neuritogenic effects of GUO are not inhibited by nitrobenzylthioinosine (NBTI) or dipyridamole,

nucleoside transport inhibitors, suggesting GUO exerts its effects at the cell-surface.[11,23]

It has been found that GUO-induced effects on astrocytes are partially inhibited by purinergic receptor antagonists; however, GUO does not bind adenine purinoceptors with high affinity[27] and GUO does not act through an A1/A2 receptor, which implies that it may exert neuritogenic effects through its own cell-surface receptor.[11,23] These considerations prompted a successful search for distinct GUO binding sites on cultured astrocytes and in whole rat brains.[16,28,29] These binding sites have characteristics of G-protein coupled receptors.

It is apparent that eGUO activates intracellular signalling pathways that are apparently related to these presumptive GUO receptors. For example, the anti-apoptotic effects of eGUO on β-amyloid (βA)-induced apoptosis in SH-SY5Y cells are inhibited by pre-treatment with LY294002, a PI3K inhibitor, and PD98059, an MEK inhibitor.[25] GUO further increases phosphorylation of Akt/PKB, which is also abolished by LY294002 and PD98059 treatment.[25] Similarly, in astrocytes exposed to the apoptosis-inducing agent staurosporine, eGUO reduces apoptosis and this effect is antagonized by pre-treatment with pertussis toxin (PTX), while the eGUO effect was antagonized by SB202190 (an inhibitor of p38 MAPK pathway). eGUO also inhibits effects of glycogen synthase kinase-3β (GSK-3β), a pro-apoptotic enzyme that is a downstream target of the PI3K/Akt/PKB pathway.[20] Furthermore, GUO promotes up regulation of mRNA and protein expression of Bcl-2, an anti-apoptotic protein and another downstream target of the PI3K/Akt/PKB pathway.[20] Thus, eGUO appears to exert effects through both the p38 MAPK and the PI3K/Akt/PKB pathways.

Signalling pathways involved in promotion of neurite outgrowth by eGUO are similar to those implicated in its anti-apoptotic effects. GUO treatment has been shown to promote increases in intracellular cAMP in astrocytes[30] and PC12 cells.[12] Increases in cAMP are inhibited by SQ22536, an inhibitor of adenylate cyclase; however, neurotrophic effects of guanosine are only partly inhibited.[12,30] These findings suggest that GUO-induced effects are mediated by both cAMP-dependent and -independent mechanisms.[30] GUO activated cAMP-dependent mechanisms are thought to activate the MAPK cascade and potentially other protein kinases.[30] GUO-induced effects are abolished by PTX, wortmannin and PD98059, MAPK inhibitors, indicating that GUO acts on a G_i-protein coupled cell-surface receptor to activate the MAPK pathway. Within the MAPK pathway, GUO has been found to activate and stimulate phosphorylation of MAP kinases ERK-1 and -2 in cultured astrocytes that may act to promote NGF synthesis and release.[16,18,24,31] The MAPK cascade has been identified as the major pathway by which NGF induces growth and differentiation in PC12 cells[32] and may, therefore, represent a convergent mechanism for NGF and GUO.

An additional pathway by which eGUO may exert effects involves cGMP and hemoxygenase. GUO increases intracellular cGMP in PC12 cells.[33] Other compounds known to increase cGMP, such as cell-permeable cGMP analogs, have also been found to exert neuritogenic effects in PC12 cells.[29,34] Therefore, eGUO appears also to act through guanylyl cyclase to increase cGMP.[29,39] Mechanisms involving cGMP and nitric oxide (NO) were initially proposed;[35,36] however, more recent studies suggest that GUO-induced neurite outgrowth is stimulated by the activation of cGMP through a carbon monoxide (CO)-dependent mechanism.[36] GUO-enhanced NGF-dependent neurite outgrowth is abolished in the presence of 0.1–1 mM methylene blue, a soluble guanylate cyclase (sGC) inhibitor, whereas this treatment does not affect neurite outgrowth enhanced by NGF alone.[36] sGC can be activated by NO, CO or hydroxyradicals, therefore, any of these compounds could be responsible for guanosine-induced NGF-dependent neurite outgrowth (36). Zinc protoporphyrin-IX (ZnPP) an inhibitor of heme oxygenase (HO), an enzyme responsible for CO synthesis, reduces cGMP concentrations and GUO-induced neuritogenic effects, but does not alter NGF-dependent neurite growth.[36] Moreover, eGUO significantly increases the expression of HO-1.[37,38] It appears that eGUO enhances NGF-dependent neurite outgrowth by activating HO-2 and inducing the expression of HO-1, which in turn synthesizes CO and stimulates sGC thereby increasing intracellular concentrations of cGMP.[36] CO, which has substantial neuroprotective effects,[39] may well contribute to the neuroprotective effects of GUO.

The biological significance of eGUO in vivo is only just beginning to be understood and the field is fertile for further exploration. Increases in eGUO are likely a natural response to injury, since eGUO is elevated for prolonged periods after experimental brain injury.[3,4] But the new data indicating that it may also affect quiescent progenitor cells in the nervous system raise the possibility that the effects of eGUO potentially extend to more chronic situations as well as to acute injury. Moreover, manipulation of eGUO concentration may be of potential therapeutic value in certain pathological conditions.

Studies of the metabolism of eGUO will be of great importance. For example, the studies may elucidate the functional interrelationship of the extracellular guanine-based purine nucleotides, GUO, and GUA. Extracellular guanine nucleotides also have trophic effects on cell proliferation,[7–9,14] as well as on differentiation of neurons,[11,12,24] and myoblasts.[40] Therefore, their extracellular conversion to GUO may be one level at which their biological effects can be regulated. Moreover, the presence of high concentrations of guanine deaminase in certain discrete populations of neurons,[5] together with the presence of ectoPNP on astrocytes (F. Caciagli, unpublished observations) implies that GUO and GUA have important extracellular roles in brain that extend beyond the trophic effects that have been elucidated to

date. Our laboratories are attempting to unravel the roles of these intriguing non-adenine-based purines. Better comprehension of their biological effects, their metabolism and the mechanisms through which their effects are mediated may prove useful not only in understanding the physiological and pathological processes in the CNS, but also in targeting interventions for several pathological conditions.

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