Supplemental Tables and Figures

Supplemental Table 1: Publications that suggest physiological effects of extracellular cGMP. Please note that this table is not exhaustive and only comprises selected examples of literature about extracellular cGMP actions.

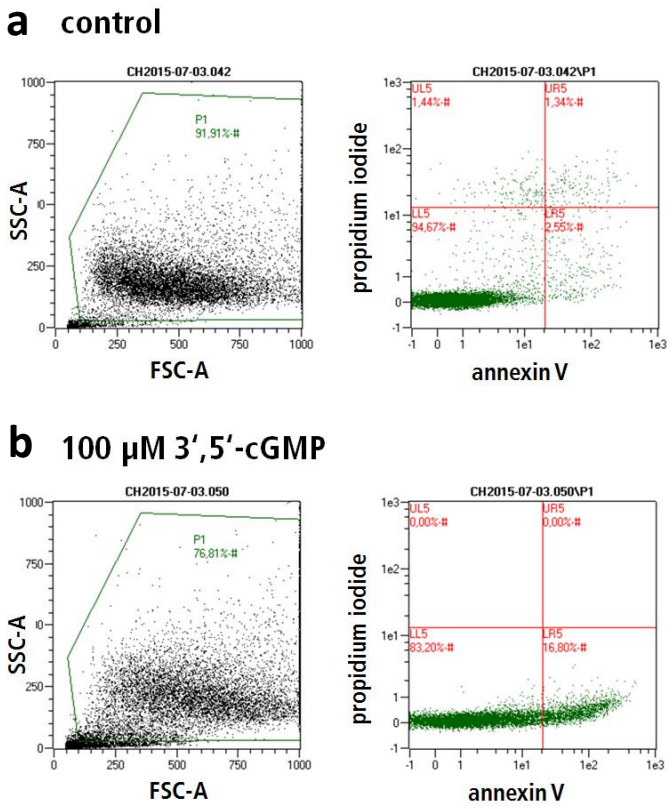
Renal effects of extracellular cGMP	
ANP stimulation of the basal pole of filter-grown human podocyte HGVEC.SV1A4 cells leads to release of cGMP into the apical medium.	(Ardaillou et al., 1992)
SNP inhibits sodium transport through porcine renal tubular LLC-PK1 cell monolayers, which depends on probenecid-sensitive cGMP extrusion.	(Chevalier et al., 1996)
NO stimulates cGMP generation and extrusion by human renal proximal tubule cells. Extracellular cGMP reduces cellular sodium uptake.	(Sasaki et al., 2004)
Extracellular cGMP (exported from its renal synthesizing cells) causes the natriuretic effect of the NO donor SNAP.	(Ahmed et al., 2007)
Src is an important downstream signaling molecule for extracellular cGMP-induced natriuresis.	(Nascimento et al., 2011)
Gastrointestinal effects of extracellular cGMP	
cGMP in the serosal part of the jejunum increases fluid absorption, but mucosal activation by heat-stable enterotoxin from *E. coli* increases loop cGMP and fluid secretion.	(Jin et al., 1999)
Tapeworms release cGMP at a constant rate to the intestinal lumen. [^{3}H]cGMP radioligand binding data suggest mucosal cGMP binding sites for extracellular cGMP.	(Zimmerman et al., 2008)
Uroguanylin is antihyperalgesic in a model of trinitrobenzene sulfonic acid (TNBS)-induced visceral hypersensitivity. Oral cGMP mimicks the antihyperalgesic effects of uroguanylin.	(Silos-Santiago et al., 2013)
The hardly absorbed guanylate cyclase-C agonist linaclotide reduces symptoms of irritable bowel syndrome with constipation. In the mouse model, this effect appears to be mediated by extracellular cGMP.	(Castro et al., 2013)
Effects of extracellular cGMP on platelet activation	
Extracellular cGMP analogs potently and rapidly inhibit thrombin-, thromboxane-, and VWF-induced human platelet signaling and activation by a cGK-independent mechanism.	(Gambaryan et al., 2004)
Effects of extracellular cGMP in the CNS	
Extracellular cGMP reduces intracellular pH in cultured rat astrocytes by inhibiting a novel type of astrocytic Na^+/H^+ exchanger.	(Touyz et al., 1997)
Extracellular cGMP directly inhibits kainate-activated responses in a subpopulation of cultured mouse cerebellar neurons.	(Poulopoulou and Nowak, 1998)
Intracellular cGMP is neurotoxic, but extracellular cGMP protects from glutamate neurotoxicity in cultured primary cerebellar neurons.	(Montoliu et al., 1999)
Rats with hyperammonemia show impaired learning ability and impaired function of the brain glutamate-NO-cGMP pathway. Oral administration of the phosphodiesterase 5 inhibitor sildenafil increases extracellular cGMP and restores learning ability in a conditional discrimination task.	(Erceg et al., 2005)

Learning ability was impaired in hyperammonemic rats, but restored by cGMP PDE inhibitors or continuous intracerebral cGMP administration.	(Rodrigo et al., 2006) [Review]
The neurotransmitter release evoked by activation of presynaptic kainate receptors is inhibited by extracellular cGMP. The AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) autoreceptor-mediated response, however, is not affected by cGMP.	(Cervetto et al., 2010)
Administration of extracellular cGMP normalizes spatial reference memory (probably by modulation of TNF-α and of membrane expression of AMPA receptor GluA1 and GluA2 subunits), but does not normalize impairment of working memory in hyperammonemic rats.	(Cabrera-Pastor et al., 2016a)
Extracellular cGMP reduces glycine receptor activation, which increases intracellular Ca^{2+} in Purkinje neurons via voltage-dependent Ca^{2+}-channels. Extracellular cGMP increases learning ability, when basal calcium concentration is low, but reduces learning ability, when basal calcium is normal.	(Cabrera-Pastor et al., 2016b)
Extracellular cGMP reduces glycine receptor activation and modulates membrane expression of AMPA receptors by increasing GluA1 and reducing GluA2 subunit expression.	(Cabrera-Pastor et al., 2017)
Chronic intracerebral administration of extracellular cGMP restores motor coordination in hyperammonemic rats by reducing microglia activation and neuroinflammation. Extracellular cerebellar glutamate and GABA levels are normalized by cGMP administration.	(Cabrera-Pastor et al., 2018)
Increasing extracellular cGMP normalizes expression of various transporters for glutamate, glutamine and GABA in the cerebellum of rats with chronic hyperammonemia.	(Cabrera-Pastor et al., 2019)

Supplemental Table 2: Physiological effects by guanosine formed as a metabolite of GMP or cGMP. Please note that this table is not exhaustive and only comprises selected examples of literature about actions of extracellular guanosine generated by GMP or cGMP metabolism.

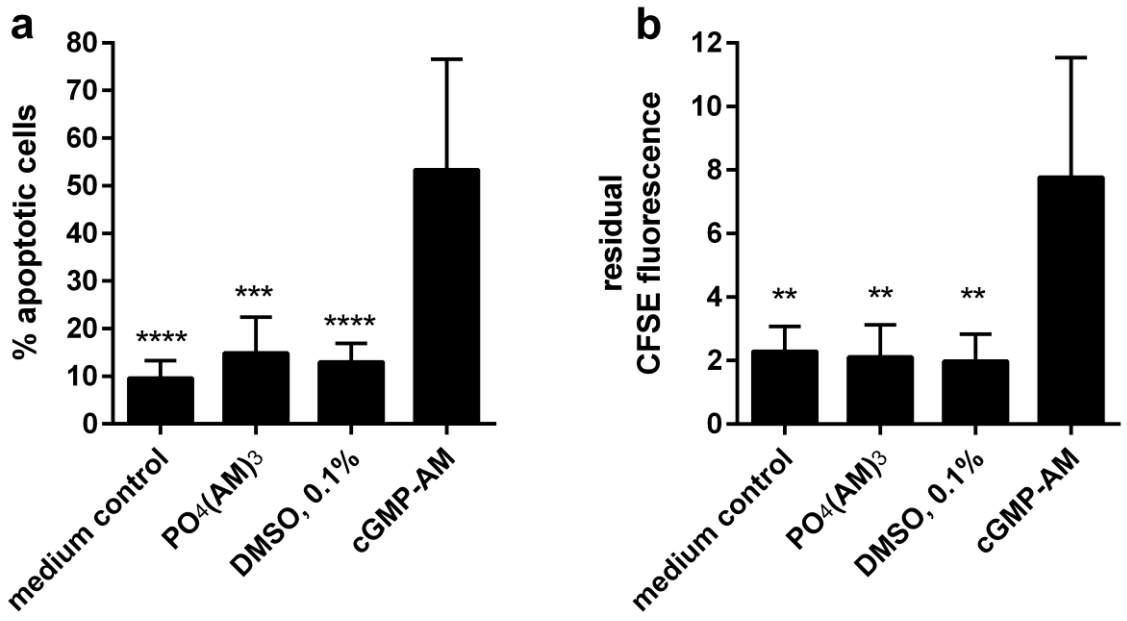
Effect	Reference
GMP exerts an anticonvulsant effect that appears to depend on its conversion to guanosine by ecto-5'-nucleotidase.	(Soares et al., 2004)
The amnesic effect of GMP is prevented by inhibiting its conversion to guanosine. This conversion occurs not only in the CNS, but also in the periphery.	(Saute et al., 2006)
In neuronal HT22 cells, extracellular glutamate causes glutathione depletion, ROS (reactive oxygen species) elevation and apoptosis (by cytotoxic Ca^{2+} influx). Extracellular cGMP as well as GMP was protective. The protective effect of extracellular GMP depended on its conversion to guanosine.	(Albrecht et al., 2013)

Suppl. Fig. 1



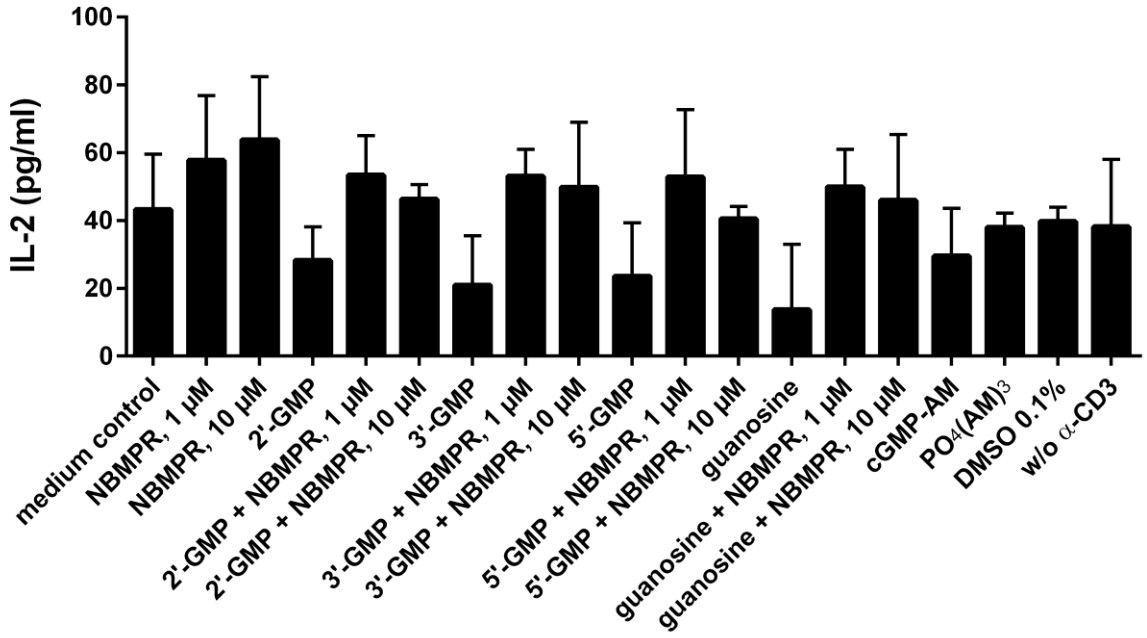
Suppl. Fig. 1: Quantification of 3',5'-cGMP-induced apoptosis of HuT-78 cells by flow cytometry (staining with propidium iodide + annexin V), representative measurement. (**a**) control; (**b**) after incubation with 100 µM of 3',5'-cGMP for 72 h; left side: gating of the cells in the FSC/SSC scattergram; right side: distribution with regard to propidium iodide and annexin V staining. The percentage of cells in the lower right (LR) and upper right (UR) quadrant represents the percentage of apoptotic cells.

Suppl. Fig. 2



Suppl. Fig. 2: Effect of 100 µM of cGMP-AM on apoptosis (**a**) and proliferation (**b**) in comparison to medium control, $PO_4(AM)_3$ and DMSO 0.1% as negative controls. Apoptosis **(a)** and proliferation **(b)** were determined by flow cytometry after 72 h of incubation (apoptosis: combined staining with APC-labeled annexin V and propidium iodide; proliferation: quantitation of residual CFSE fluorescence at the end of the incubation time, higher residual CFSE fluorescence means less proliferation). Data are means ± SD from n=6 (apoptosis) or n=5 (proliferation) independent experiments. Statistics: One way ANOVA and Dunnet's multiple comparison test, with "*" indicating significance in comparison to cGMP-AM; two, three and four symbols designate $p<0.01$, $p<0.001$ and $p<0.0001$, respectively.

Suppl. Fig. 3



Suppl. Fig. 3: Effect of potential products of 2',3'-/3',5'-cGMP metabolism (100 µM of 2'-GMP, 3'-GMP, 5'-GMP, guanosine) on IL-2 production of HuT-78 lymphoma cells in the absence and in the presence of NBMPR. cGMP-AM (100 µM) was included as a control for intracellularly delivered cGMP. Cells were incubated for 24 h in αCD3-antibody-coated plates with 100 µM of the corresponding guanosine-derived compounds in the absence and in the presence of NBMPR (1 µM and 10 µM). The "DMSO 0.1%" sample is a solvent control for the cGMP-AM sample. The "$PO_4(AM)_3$" is another control for the cGMP-AM sample, which imitates the intracellular formation of acetoxymethylester hydrolysis products. For unknown reasons, the HuT-78 cells only showed a low basal IL-2 production in these experiments that was not further enhanced by αCD3-antibody. Data are means ± SD from n=3 independent experiments. Statistics: One way ANOVA and Dunnet's multiple comparison test with medium control as control column (no significant difference detected).

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