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NO-GC in cells 'off the beaten track'

Andreas Friebe*, Barbara Voußen, Dieter Groneberg

Physiologisches Institut, Universität Würzburg, Würzburg, Germany

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ABSTRACT

Nitric oxide-sensitive guanylyl cyclase (NO-GC) has been shown to regulate a plethora of different functions in the body. These include, among many others, the fine-tuning of vascular tone, platelet reactivity and gastrointestinal motility. Evidence for the participation of NO-GC in these functions has been obtained from various species including humans, rodents, as well as insects. Clearly, individual cell types that express NO-GC contribute differentially to organ-specific NO/cGMP signaling in the body. Hence, identification of NO-GC-expressing cells and their individual involvement in NO/cGMP signaling constituted the focus of many studies over the last 40 years. Probably most information has been obtained from vascular smooth muscle cells and platelets, in which NO-GC is known to induce relaxation and inhibition of aggregation, respectively. Many other cell types that express the enzyme have been linked to certain functions, e.g. cardiomyocyte/inotropy or gastrointestinal smooth muscle cells/motility. However, in some cell types, e.g. myofibroblasts or pericytes, NO-GC expression is evident but individual functions of NO/cGMP signaling have yet to be assigned, whereas in other cell types, e.g. in erythrocytes, expression and role of NO-GC is still a matter of debate. This review discusses the current knowledge on 'less popular' cell types that express NO-GC (pericytes, myofibroblasts, cardiomyocytes, adipocytes, interstitial cells of Cajal, fibroblast-like cells and blood cells) and outlines possible further functions in cell types that have not gained strong attention so far.

1. The NO/cGMP pathway

NO-sensitive guanylyl cyclase (NO-GC) is functionally expressed in many mammalian organs. Expression levels have been found to vary depending on the type of tissue. Lung, brain, intestinal and kidney tissue as well as the vasculature contain high amounts of the enzyme whereas only small quantities of the enzyme are found in liver, spleen and skeletal muscle [78]. NO is accepted to be the main physiological activator of NO-GC in the body. Probably most cells that express NO-GC are neighbored by cells that contain either the endothelial or the neuronal form of NO synthase (NOS), or even both. Regulation of NOS activity, and thus NO synthesis, is the most critical factor that controls the activation of NO-GC. However, the cellular cGMP response does not only depend on NO production as it will also be critically influenced by cGMP degradation. Therefore, precise description of intracellular cGMP signaling will have to take into account the parallel regulation of NOS in the neighboring as well as the type(s) of cGMP-degrading phosphodiesterases present in the NO-GC-expressing cells.

NO-GC is a heterodimer made up of two different subunits α and β [31]. In general, two α subunits (α_1 or α_2) but only one β subunit (β_1) have been identified on the protein level. The β_1 subunit acts as dimerizing partner for both α subunits resulting in the formation of the two isoforms NO-GC1 ($\alpha_1\beta_1$) and NO-GC2 ($\alpha_2\beta_1$). So far, the $\alpha_1\beta_1$ dimer is thought to be the dominant NO-GC isoform in most tissues. Being much more restricted, the $\alpha_2\beta_1$ isoform occurs mainly in the neuronal system, and it is therefore thought to modulate synaptic transmission [17,78,83]. Knockout of the β_1 subunit in mice results in a global deletion of both NO-GC isoforms. As consequence, these GCKO animals suffer from a severe phenotype that affects both the cardiovascular and GI systems [32]. In contrast, only minor effects have been identified as consequence of the deletion of either α_1 or α_2 subunit. This is probably explained by the fact that the NO-GC isoforms can compensate for each other [77,105].

NO-GC has been identified in various cell types from many different species. Due to the lack of specific antibodies, very little is known on the expression of the NO-GC2 vs the NO-GC1 isoform. To the best of our knowledge, there are only two cell types that have been shown to express only one of the two GC isoforms. First, platelets are known to express exclusively NO-GC1 [32,77]. A second cell type with exclusive expression of NO-GC1 may be the erythrocyte, even though the expression of NO-GC1 in red blood cells in general is still a matter of dispute (see below).

All other cell types can be expected to express both isoforms, but the ratio of the isoforms in the individual cell type is not known. The only

* Corresponding author. Physiologisches Institut, Universität Würzburg, Röntgenring 9, 97070, Würzburg, Germany.
E-mail address: andreas.friebe@uni-wuerzburg.de (A. Friebe).

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exception to this are aortic SMC, in which, based on isoform-specific knockout studies, approx. 94% of the total NO-GC content is made up by NO-GC1, the remaining 6% being NO-GC2 [77].

The focus of this review will be to summarize information on a selection of cell types 1.) which have either not been known for a long time to express NO-GC or, 2.) in which the function of NO-GC is novel or not readily understood. Obviously, this review fails to be comprehensive, owing to the multitude of different cell types in the body. We will also not cover 'popular' cell types (i.e. vascular smooth muscle cells and platelets) that have been long known to express functional NO-GC. There is extensive literature on the expression of NO-GC and its function in these two cells, to which the reader is referred to (e.g., [16,24,31,36,56,75,80,114]. NO-GC has also been shown in various different neurons of the central and peripheral nervous system. However, due to the diversity of neuronal cell types and their functions, this area will also not be covered in this review. The reader is recommended to check excellent publications relating to NO/cGMP signaling in the central and peripheral nervous system regarding e.g., synaptic transmission [63], neurodegeneration [7] hearing [79] or pain [73,103].

2. Pericytes and myofibroblasts

Pericytes are mural cells of mesenchymal origin that are found mostly in capillaries but also in precapillary arterioles and postcapillary venules [4]. Generally, pericytes are assumed to control numerous functions. They participate in the regulation of the blood brain barrier [5], capillary flow [49], vascular permeability [40] and angiogenesis [38].

Myofibroblasts are thought to act as the primary fibrosis-promoting cells in many organs. Similar to fibroblasts, myofibroblasts are able to produce extracellular matrix but also reveal SMC-like characteristics such as contractility. The origin of myofibroblasts has been a debate over the last years. Potential precursor cells are tissue-resident fibroblasts, SMC, pericytes, endothelial cells, epithelial cells as well as bone-marrow derived fibrocytes. Recently, pericytes have emerged as an important source for myofibroblasts in several organs such as liver, lung and kidney [6,42,68].

NO-GC expression in pericytes has long been known but somehow has escaped the general attention. Indirect evidence for NO-GC in pericytes came from cGMP immunostaining [116] and NO-induced relaxation of bovine retinal pericytes cultivated on silicone sheets [48]. Direct proof of NO-GC in pericytes using immunohistochemistry and electron microscopy was first obtained from rat skeletal muscle [33]. Our group has identified NO-GC in pericytes of all so far investigated tissues: strong NO-GC immunostaining was detected in pericytes of lung (Fig. 1A), liver (hepatic stellate cells aka Ito cells; Fig. 1B) and kidney (mesangial cells; not shown). Of interest, all three NO-GC-expressing types of pericyets are known to be involved in organ fibrosis (see below). Other tissues with NO-GC in pericytes include Cremaster muscle, myocardium and brown adipose tissue (Fig. 1C–E). Although we have not quantitatively determined pericytic NO-GC expression, it appears to be rather high (based on the strength of the immunosignal) in comparison to most other cell types. The reason for this strong expression rate has yet to be determined.

The function of NO-GC in pericytes and myofibroblasts is still to be elucidated. Based on the tight association with capillary endothelium, regulation of diameter and permeability appear conceivable. This would allow for NO-mediated regulation of blood flow and pressure. The role of NO/cGMP signaling in fibrosis is currently a focus of several labs including ours. Involvement of NO-GC, PKG and other members of this signaling cascade have been shown to influence fibrotic processes in different organs such as skin [9,74], kidney [102,112], lung [27,90] and liver [62]. Inhibition of TGFβ-induced ERK activation by cGMP signaling has been proposed as potential anti-fibrotic mechanism [9,25]. Current preclinical evidence on the activation of NO/cGMP pathway through NO-GC have recently been reviewed by Ref. [99].

3. Cardiomyocytes

Our knowledge on the role of NO-GC in cardiomyocytes (CM) is still limited. Actually, NO-GC expression in cardiomyocytes is difficult to prove, and its subcellular location in the cell is still unknown. NO-GC has been indirectly shown in CM based on its involvement e.g., in the regulation of cardiac contractility and remodeling [20,50,51,104], although these data are partly contrasted by a report from Ref. [93] which showed that the increase in cGMP levels induced by NO-GC targeting drugs (cinaciguat and riociguat) is not associated with acute direct effects on CM contractility.

In general, various biochemical methods may be employed to demonstrate NO-GC in tissues/cell types. However, when expressed at low concentrations, this is not a trivial aspect. Immunohistochemistry, being a standard for cell-specific protein detection, may be hampered by the specificity of the antibody used and the concentration of the enzyme in a given cell. In fact, as shown in Fig. 1D, NO-GC is strongly expressed in pericytes whereas CM-specific staining is below the detection level. With this knowledge, Western blot or radioimmunoassay using cardiac tissue will clearly not provide unambiguous results. In addition, primary cultures of CM may contain contaminating amounts of pericytes (or SMC) which, based on the strong NO-GC expression, would confound results. Thus, highly purified preparations of CM are prerequisite.

Surprisingly, in the literature, direct evidence for NO-GC expression in CM by immunohistochemistry is scarce. Even when probing cardiac tissue or isolated cardiomyocytes from WT mice and mice that lack both GC isoforms (GCKO animals) with a specific antibody for NO-GCβ1 subunit (previously evaluated with KO tissues) we have been unable to unequivocally verify NO-GC expression in CM based on the weakness of the specific immunosignals (unpublished results). Very recent data indicate NO-GC to be located at the intercalated disc where it interacts with connexin 43 [23]. As gap junctions at the intercalated disc are formed by connexin 43, it is conceivable that the propagation of electrical currents in the myocardium are influenced by NO-GC.

Pharmacological effects of NO-GC stimulators/activators underline a role of NO/cGMP signaling in CM and heart function [15,39,41,106]. Compounds such as cinaciguat or vericiguat increase cGMP levels in isolated cardiac myocytes. This cGMP elevation was shown to improve cardiopulmonary hemodynamics, to protect against ischemia/reperfusion injury, to unload the heart in patients with acute decompensated heart failure and to prevent hypertrophy [29,67,84,93,96].

Mice lacking NO-GC might also help to show NO-GC expression in CM. However, employing an α_1-specific antibody on CM from mice lacking exon 6 of NO-GCα1 showed a staining similar to that in WT CM which is explained by unchanged expression the nonfunctional truncated form of the protein [20]. We have recently generated CM-specific KO mice for NO-GC using the aMHC-Cre-ER^{T2} strain. As immunohistochemistry was unable to prove CM-specific NO-GC deletion, we indirectly showed the activity of the CM-specific Cre recombinase by using a tomato reporter line in combination with PCR analysis of the truncated mRNA. To investigate the function of NO-GC in acute myocardial infarction, these mice were subjected to ischemia/reperfusion studies. Infarct size after I/R was unchanged compared to control animals; however, ischemic postconditioning as well as the NO-GC activator cinaciguat led to significant reduction of infarction in control mice, an effect not present in the absence of CM NO-GC. These data indicate a beneficial role of cardiomyocytic NO-GC regarding infarct size in mice (Frankenreiter et al., in revision).

In summary, direct proof for NO-GC expression in CM (the same holds true for several other cell types) is difficult based on the low expression level of the enzyme. Physiologically, these low amounts of NO-GC may still be sufficient to generate small, spatially confined cGMP pools [19,107] that are sufficient to mediate the indicated cardiac functions.