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Review

The function of NO-sensitive guanylyl cyclase: What we can learn from genetic mouse models

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ABSTRACT

The signaling molecule nitric oxide (NO) acts as physiological activator of NO-sensitive guanylyl cyclase (NO-GC) in the cardiovascular, gastrointestinal and nervous systems. Two isoforms of NO-GC are known to exist on the protein level. The enzyme is a heterodimer consisting of an alpha (α_1 or α_2) and a beta subunit (β_1). Strategies for the genomic deletion of either subunit have been developed in the recent years. Removal of one of the two isoforms by deletion of one of the α subunits allowed the investigation of the specific functions of the respective isoform. The deletion of the β_1 subunit led to complete knock-out thus completely disrupting the NO/cGMP signaling cascade. The phenotypes of these KO mice have corroborated the already known physiological importance of the NO/cGMP cascade e.g. in the regulation of blood pressure, platelet inhibition, interneuronal communication; yet, they have also given hints to novel functions and mechanisms. In addition, mice lacking both NO-GC isoforms permitted the investigation of possible cGMP-independent signaling pathways of NO. As cell- and tissue-specific knock-out models are beginning to emerge, a more detailed analysis of the importance of the NO receptor in specific tissues will become possible.

Introduction

In 1969, exactly 40 years ago, cGMP-forming activity was first described [27,69,81] and subsequently identified as the enzyme guanylyl cyclase. However, it took until the late seventies to find out that NO-containing compounds are potent activators of the enzyme [2,5]. Despite the stimulatory effect of NO[1]-releasing substances, the physiological significance of NO-induced activation of the enzyme did not become clear until the identification of endothelium-derived relaxing factor (EDRF; [20]) as NO [61,37]. Formation of EDRF had been shown to occur in endothelial cells in response to vasodilatory agonists such as acetylcholine, histamine or bradykinin, leading to vasodilation by smooth muscle cells. After the discovery of NO in the vascular system, NO formation was reported to occur throughout the body [54]. At the same time, the broad expression of the guanylyl cyclase that mediates the effects of NO was shown to occur.

In contrast to cAMP-forming enzymes which were found to occur only in the cell membrane, the enzymes responsible for cGMP production were found in cytosolic as well as membrane fractions. However, only the 'soluble' (or 'cytosolic') guanylyl cyclases (GC) were shown to mediate the effects of NO whereas the particulate GCs were identified as receptors for peptides such as the natriuretic peptides ANP or BNP. From today's standpoint, it appears more comprehensible to differentiate the two forms of GC by their mechanism of activation (NO vs. peptide) rather than by their cellular localization as one of the isoforms of the 'soluble' GC can be localized to plasma membranes [66]. Therefore, in this review, the term 'NO-sensitive guanylyl cyclase' (NO-GC) will be used instead of soluble/cytosolic GC.

NO-GC has long been known to regulate a plethora of physiological functions in the cardiovascular, neuronal and gastrointestinal systems (for review see [53,21,43,76]). The enzyme has been accepted to be the most important receptor for the signaling molecule NO. NO is synthesized by the family of NO synthases which exist in endothelial, neuronal and inducible forms. Stimulation of NO-GC by NO results in the production of the second messenger cGMP which exerts its effects via cGMP-dependent kinases, channels or phosphodiesterases [18,31,42,67]. Besides these cGMP-mediated effectors, NO has been proposed to mediate a variety of effects via cGMP-independent mechanisms (for review see [80]).

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[1] *Abbreviations:* NO, nitric oxide; NO-GC, NO-sensitive guanylyl cyclase; NOS, NO synthase; PKG, cGMP-dependent protein kinase; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; GSNO, S-nitrosoglutathione; DEA-NO, diethylamine nonoate; Proli-NO, proline nonoate; SNP, sodium nitroprusside; ICC, interstitial cells of Cajal; LTP, long-term potentiation; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; PSD-95, post-synaptic densitiy protein 95; SBP, systolic blood pressure; nNOS, neuronal NO synthase; eNOS, endothelial NO synthase.

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Isoforms of NO-sensitive guanylyl cyclase

NO-GC was first purified from rat and bovine lung [23,41]; for review see [79]. The enzyme was shown to contain heme as prosthetic group which is essential for NO stimulation: The heme group acts as NO receptor and formation of a NO–heme complex is thought to induce a conformational change in the protein leading to enzyme activation. Much effort has been made to elucidate how NO interacts and stimulates NO-GC, however the precise mechanism of activation is still unclear to date [12,64]. Purification of the enzyme allowed for the sequencing of the enzyme with subsequent cloning of three different subunits; based on sequence homology, these subunits were termed α_1, α_2 and β_1 [44,55,45,56,28]. NO-GC is a heterodimeric enzyme made up of one α and one β subunit (Fig. 1). Two isoforms of NO-GC are known to exist ($\alpha1\beta1$ and $\alpha2\beta1$; [65] in which the β_1 subunit acts as the dimerizing partner for either α subunit. In the absence of the β_1 subunit, the α subunits do not form dimers and are not catalytically active. A β_2 subunit was identified later by homology screening [84]. There is evidence, however, that this β_2 subunit is a pseudogene: It has never been identified in native tissues, mRNA expression was extremely low as determined by quantitative PCR, co-expression with any of the other subunits did not result in a functional enzyme and, in addition, the human gene for the β_2 subunit contains a frame shift mutation [52,3].

The physiological significance of two different isoforms still remains unclear. Biochemical and kinetic characterization of the two isoforms did not reveal any significant differences regarding sensitivity towards NO, catalytic activity, etc. [65]. However, the very C terminal amino acids of the α_2 subunit permit the interaction with PDZ domains of several proteins. In fact, localization of the $\alpha2\beta1$ isoform in the membranes of rat brain synaptosomes was shown to arise from the interaction of the PDZ domain-containing protein PSD-95 with the α_2 subunit [66]. Therefore, although having similar biochemical and kinetic properties, the two NO-GC isoforms may differ in their subcellular and tissue localization. In fact, expression studies revealed that the α_1 isoform is ubiquitously distributed with special emphasis in the cardiovascular tissues. The α_2-containing isoform shows highest expression in the brain, and it is likely that this isoform has an important role in the mediation of interneuronal communication. Clearly, subunit-specific deletion of NO-GC is an appropriate way to determine the individual contribution of each isoform.

In this review, the phenotypes of the three different published NO-GC KO lines will be summarized and discussed. The data obtained so far will in some cases be compared to those from other KO models that lack protein members of the NO/cGMP signaling cascade such as PKG- or NOS-deficient animals.

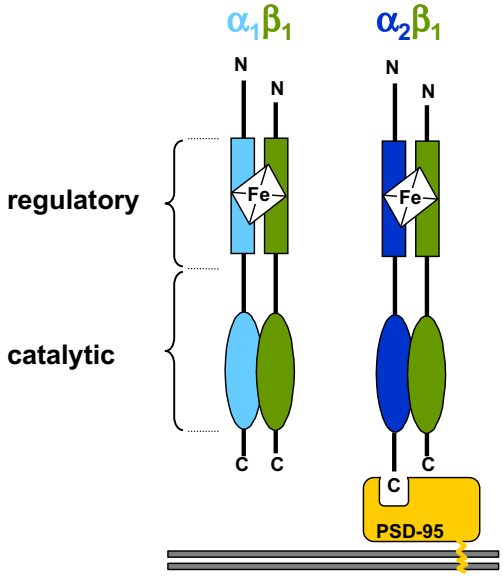

Fig. 1. Schematic illustration of the two isoforms of NO-GC. NO-GC is a heterodimeric enzyme made up of one α and one β subunit. As there are two a subunits known (α_1 and α_2), two dimers can be formed: $\alpha1\beta1$ and $\alpha2\beta1$. The N terminal part of NO-GC contains the regulatory prosthetic heme group which acts as NO receptor. Binding of NO results in a conformational change that leads to activation of the enzyme. The catalytic domain responsible for the conversion of GTP to cGMP is located in the C terminal part of the enzyme. The α_2 subunit can be bound by PDZ domain-containing proteins such as post-synaptic density protein 95 (PSD-95). This interaction allows the localization of the $\alpha2\beta1$ isoform to the plasma membrane.

Deletion of NO-GC subunits in the mouse

Nitric oxide is synthesized in nearly all cells and tissues. As receptor for NO, NO-GC has been shown to regulate a multitude of functions. As shown above, two different isoforms of NO-GC are known to exist with the β_1 subunit being the common dimerizing partner. Thus, deletion of the β_1 subunit should result in a total knock-out of the NO receptor whereas deletion of an α subunit should result in mice still expressing the other isoform, i.e. α_1-KO mice retaining the $\alpha2\beta1$ isoform and α_2-KO mice still expressing the $\alpha1\beta1$ dimer (Fig. 2). Therefore, β_1-KO mice may be useful to explore the functions of the NO receptor and identify possible NO targets besides NO-GC whereas the two α-KO mice will help to elucidate the particular roles of each isoform.

Mice strains lacking one of the three subunits of NO-GC (α_1, α_2 or β_1) have been generated [51,19,11]. Deletion of the β_1 subunit indeed resulted in a total knock-out of NO-GC. In β_1-KO mice, the expression of the dimerizing α subunits, α_1 and α_2, was greatly reduced or even not detectable in most tissues. This finding may be explained by a mechanism that synchronizes the expression of the β_1 with the α subunits. Although the genes of the β_1 and α_1 subunits are localized in line on chromosome 3 (*Mus musculus*) which may allow for a concerted regulation of transcription, the gene for the α_2 subunit lies on chromosome 9. Thus, co-transcriptional regulation of the β_1 with the two α subunits may be difficult to obtain. Alternatively, formation of functional $\alpha\beta$ dimers may be regulated by translational mechanisms. Conceivably, only co-translated subunits are folded correctly leading to functional enzyme molecules; single subunits may be degraded rapidly. This notion would explain not only the lack of α subunits in the β_1-KO mouse but also the partial reduction of β_1 in both α-KO strains: In these models, deletion of the α_1 subunit was accompanied by a strong reduction of the β_1 subunit and deletion of the α_2 subunit was paralleled by a

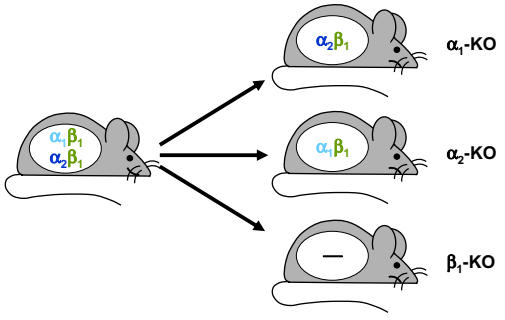

Fig. 2. Knock-out models for NO-GC. Three subunits of NO-GC have been successfully deleted in the mouse. Deletion of the α_1 subunit (α_1-KO) resulted in a mouse that still expresses the $\alpha2\beta1$ isoform. Lack of the α_2 subunit (α_2-KO) led to a mouse retaining the $\alpha1\beta1$ isoform. Genomic deletion of the β_1 subunit which is the common dimerizing partner for the two a subunits resulted in complete knock-out of NO-GC (β_1-KO).