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Review

GRK2 as negative modulator of NO bioavailability: Implications for cardiovascular disease

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ABSTRACT

Nitric oxide (NO), initially identified as endothelium-derived relaxing factor (EDRF), is a gaso-transmitter with important regulatory roles in the cardiovascular, nervous and immune systems. In the former, this diatomic molecule and free radical gas controls vascular tone and cardiac mechanics, among others. In the cardiovascular system, it is now understood that β-adrenergic receptor (βAR) activation is a key modulator of NO generation. Therefore, it is not surprising that the up-regulation of G protein-coupled receptor kinases (GRKs), in particular GRK2, that restrains βAR activity contributes to impaired cardiovascular functions via alteration of NO bioavailability. This review, will explore the specific interrelation between βARs, GRK2 and NO in the cardiovascular system and their inter-relationship for the pathogenesis of the onset of disease. Last, we will update the readers on the current status of GRK2 inhibitors as a potential therapeutic strategy for heart failure with an emphasis on their ability of rescuing NO bioavailability.

Contents

1. Introduction

Nitric oxide (NO) is a free radical and a major gaso-transmitter identified thanks to the efforts of several scientists, most notably, Drs. Murad, Furchgott and Ignarro, that were awarded the Nobel Prize in Medicine in 1998 for this discovery [1–4]. Their studies allowed the next generation scientists to demonstrated that NO is released endogenously in mammalian systems and that it represents a nodal regulator of different physiological processes [4].

In the cardiovascular system, NO is implicated in the functional regulation of many cell types, including vascular endothelial cells and cardiomyocytes [5]. Recent evidence highlights the importance of β-adrenergic receptor (βAR) activation as one of the main mechanisms upstream of NO generation [6–9]. βARs are members of the G protein-coupled receptor (GPCR) family, and it is now emerging clearly that these cell surface receptors trigger NO-dependent intracellular signaling not only in vascular endothelial cells and cardiomyocytes, but also in platelets and leukocytes [6,8–14]. Three isoforms of βARs exist: β_1, β_2 and β_3ARs. In 2012, the Nobel Prize in Chemistry was awarded to Drs.

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Lefkowitz and Kobilka for their work on βAR signaling including elucidating the structure of the β_2AR [15]. Overall, βARs have long been known to be key regulators of cardiovascular function, however more recent studies have found crucial links between the βAR and NO systems and importantly, studies have found that dysregulation of βAR signaling can negatively influence NO generation leading to the insurgence of pathological conditions that can affect the entire cardiovascular system [8,16–19]. Further, a critical role has been attributed to the GPCR kinase 2 (GRK2), a natural regulator of βARs via phosphorylation after receptor activation [9,20–23]. In particular, in certain pathological conditions, such as heart failure (HF), high levels of GRK2 can induce a dysfunction in βAR signaling pathway with a consequent impairment of NO bioavailability.

Here, we will first review the mechanisms by which βAR signals modulate NO generation in different cardiovascular compartments. Then we will focus on the pathophysiological role exerted by the up-regulation of GRK2 expression/activity on βAR-induced NO production, and on the functional repercussions of this negative modulation within the cardiovascular system. Finally, we will describe how GRK2 inhibitors can improve outcome in cardiac disease states such as HF, by improving signaling including NO bioavailability.

1.1. Nitric oxide: biochemistry, signaling pathways and effects in the cardiovascular system

NO is a free radical gas, whose production is catalyzed by a family of NO synthases (NOS's), which use as a substrate the terminal guanidine nitrogen of the amino acid L-arginine to form L-citrulline and the free radical NO [24]. Thus, the "little" NO is freely diffusible and able to cross biological membranes, rendering it an ideal biological messenger [24,25]. Currently, three isoforms of NOS have been cloned: the neuronal isoform (nNOS or NOS1), the inducible isoform (iNOS or NOS2) and the endothelial NOS (eNOS or NOS3) [26,27]. While eNOS and nNOS are constitutively expressed, and generate moderate amounts of NO, the expression of iNOS, is dependent on induction by cytokines or other stimuli that could lead to pathological disorders due to the high levels of NO produced (1000-fold more NO than from eNOS or nNOS) [26–29]. As reported by Thomas et al., the chemical biology of NO could lead to two different categories of reactions: direct and indirect [30]. The *direct effects* are those reactions that occur fast enough to allow NO to react directly with a biological target. Conversely, NO *indirect effects* require that NO reacts first with the oxygen of superoxide to generate reactive nitrogen species (RNS). These species will subsequently react with the biological targets [30,31]. In general, the direct effects of NO occur at low concentrations, whereas indirect effects require much higher NO concentrations. The latter can lead to either oxidative or nitrosative stress [24,30]. Oxidative chemistry can be defined as a process in which the oxidation state of the target molecule is increased. Instead, nitrosative stress is characterized by the addition of a nitrosonium cation (NO^+) equivalent to a thiol or secondary amine or hydroxy groups [30–33]. Reactive oxygen species (ROS) such as hydroxyl radical (OH•) or superoxide (O_2^-) are more frequently associated with oxidative stress. Peroxynitrite ($ONOO^-$) and nitrogen dioxide (NO_2) (both formed from the reaction of NO and O_2^-) are also potent oxidants and liable for nitrosative stress [30–33]. Conversely, N_2O_3 that is formed from the reaction of NO with O_2 (autoxidation) or from the NO/O_2^- reaction is a mild oxidant [24,30–33]. Oxidants are implicated in a number of reactions, spanning from tyrosine nitration to oxidation of sulfhydryl groups that could alter the normal structure and function of various proteins and kinases [34,35]. The vast majority of these reactions have been implicated in the negative modulation of cellular functions. However, it is now emerging that mild oxidizing agents can also signal through reversible modifications, particularly of cysteine residues [36,37].

Importantly, NO itself can undergo different redox states, from NO^+ (formed by loss of an electron from NO) to nitroxyl anion (NO^-), due to the addition of one electron. Redox siblings of NO such as NO^-/HNO are now known to have often divergent chemistry and cardiovascular effects when compared to NO. For instance, NO can be readily quenched without altering NOS activity [38], whereas HNO is rather insensitive to oxidative chemistry [39]. Interestingly, NO and its congeners can react with low molecular thiol groups to form *S*-nitrosothiols (SNOs) [34,35]. In the case of NO, the resulting reaction, termed *S*-nitrosylation, represents a dynamic cysteine-based modification that critically regulates many processes that govern cellular physiology, including protein stability and function, localization and interactome [40,41]. Interestingly, this process could be initiated also by the action of *trans-S*-nitrosylases, which directly transfer the *S*-nitrosylation moiety from a molecule to another recipient protein in a process called *trans-S*-nitrosylation (Fig. 1) [42]. Within cells, L-cysteine and glutathione are important sources of thiol groups that accept and transfer NO by *trans-S*-nitrosylation [43]. The formation of SNOs may attenuate NO toxicity because they are less reactive than NO, and less likely to generate harmful oxidizing species [32].

In biological systems, NO has been shown to reversibly bind with specific molecular targets with particular acceptor features, including not only thiols but also heme iron [44–47]. Accordingly, one of the most well-recognized NO partners is the soluble guanylyl cyclase (sGC) [47]. NO activates this enzyme though the binding to the heme moiety [47]. Once activated, the sGC generates guanosine 3′,5′ monophosphate (cGMP) from guanosine-5′-triphosphate (GTP) [48,49]. cGMP is a second messenger that produces its effects by interaction with intracellular receptor proteins. In several cell types such as smooth muscle cells and cardiomyocytes, one of the main receptors for cGMP is the serine/threonine protein kinase, cGMP-dependent protein kinase (PKG) [50–54]. PKG is a serine/threonine kinase that catalyzes the phosphorylation of proteins relevant, among other, to the regulation of the contractile activity of the smooth muscle cell and cardiac myocytes as well as the myocyte response to chronic hemodynamic stress, i.e. cardiac hypertrophy and apoptosis [19,51]. Therefore, when dysfunctional at any level, the NO/cGMP/PKG axis can factor in many cardiac diseases. Indeed, endothelial dysfunction due to reduced NO/cGMP signal and/or enhanced quenching from ROS can contribute to hypertension, both at the systemic and pulmonary circulatory level, and also atherosclerosis [21,55–59]. If vascular smooth muscle dysfunction takes place, this can lead to systemic and pulmonary hypertension as well as ischemic cardiac disease [21,57]. When this system is defective at the myocyte level, NO/cGMP signal can induce and/or propagate cardiac maladaptive hypertrophy and dysfunction in response to chronic hemodynamic stress, ultimately leading to HF [51,60,61].

1.2. βARs and NO signaling intersections: relevance to cardiovascular physiology

A complex, mutual relationship exists between βARs activity and NO bioavailability and cardiovascular effects has been documented by decades of existing literature [8,18,19]. In essence, not only does NO regulate cardiac and vascular response to βAR agonists, but it is also a direct signaling offspring after βAR stimulation [8,9,62]. Concerning the latter, there are different ways by which βARs can induce the activation of NOS's giving rise to NO. As mentioned above, β_1, β_2 and β_3ARs are the three βARs isoforms identified in mammals [63,64]. Alongside the classical pathway that involves stimulatory G (Gs) protein activation, βARs can also exert their effects through the coupling to the inhibitory G (Gi) proteins [19]. Importantly, while all the βARs isoforms have been shown to couple to Gs proteins, the β_2 and β_3AR can also significantly couple to Gi [19].

The Gs pathway is generally related to the activation of the adenylyl cyclase (AC), an enzyme that catalyze the conversion of adenosine triphosphate (ATP) to 3′,5′-cyclic AMP (cAMP) [4]. Of note, cAMP induces the activation the protein kinase A (PKA). PKA is a serine/threonine protein kinase that phosphorylates a number of proteins, eliciting specific cellular responses [4]. In the heart this kinase has been shown to

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