Published in final edited form as:

J Mol Cell Cardiol. 2013 September ; 62: 214–216. doi:10.1016/j.yjmcc.2013.06.006.

Moving into a new neighborhood: NOS goes nuclear

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Abstract

Regulation of heart function during physiological and/or pathological stresses occurs via numerous cellular signal transduction systems. Since there are a limited number of second messengers, these pathways are spatially and temporally compartmentalized resulting in tightly controlled localized signaling. In ventricular myocytes, prominent signaling cascades occur through G-protein coupled receptors (GPCRs) with the β -adrenergic (β -AR) and endothelin pathways being two important illustrations.

1. β -AR signaling cascade

Activation of the β -AR signaling cascade results in positive inotropy, positive lusitropy, and growth [1]. This cascade is initiated when an agonist (epinephrine, norepinephrine) binds to the β -AR receptor, activating the stimulatory G-protein (G α s) resulting in the activation of adenylate cyclase to produce cyclic AMP. Increased cAMP activates protein kinase A (PKA), which phosphorylates numerous excitation-contraction coupling proteins (e.g., L-type Ca²⁺ channel, phospholamban, ryanodine receptor, troponin I, etc.). This tightly coupled signaling cascade is highly localized by A-kinase anchoring proteins, G-protein coupling, phosphodiesterases, and phosphatases. Another component of spatial localization is receptor localization. Rybin et al. [2] demonstrated that β 1-AR receptors are located in non-caveolar sarcolemmal regions, whereas β 2-AR receptors are almost exclusively located in caveolae. Hence, the location (and sensitivity) of the receptor (along with its specific cascade components) determines the compartmentalized functional effects of β -AR signaling.

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Disclosures None.

2. Endothelin signaling cascade

Activation of the endothelin signaling cascade results in changes in contractility and growth [3,4]. However, the endothelin-induced modification of contractility is variable (minimal, positive or negative) mostly due to the variable expression of endothelin receptors. For example, the adult mouse ventricle exhibits a low receptor expression level and endothelin therefore invokes a nominal positive inotropic effect. Alternately, the neonatal mouse ventricle exhibits elevated receptor expression levels and endothelin-1 invokes a significant positive inotropic effect. Endothelin in the atria (which exhibits a very high receptor abundance) invokes a binary effect, an initial negative inotropic effect followed by a sustained positive inotropic effect. This signaling cascade is initiated when endothelin (a 21 amino acid peptide) binds to the endothelin receptor and activates an array of G-proteins (G_{α_q} , $G_{\alpha_{12}}$, or G_{α_i}) resulting in the production of inositol-(1,4,5)-triphosphate (IP_3) and diacylglycerol (DAG). Increases in these signaling molecules then function to activate protein kinase C, IP receptors, Na^+3/Ca^{2+} exchanger, increase myofilament Ca^{2+} sensitivity, mitogen-activated protein kinase, etc.. Contributing to the spatial localization of this pathway are the endothelin receptors themselves (ET_A and ET_B). ET_A , the major endothelin receptor in the heart, is predominately found on the plasma membrane (surface and T-tubules); while ET_B receptors localize primarily to the nucleus [5]. Thus, similar to the β -AR pathway, the endothelin signaling pathway is a tightly coupled cascade that is highly localized.

3. Nuclear GPCR

In line with localized signaling, recent studies demonstrate an increasing number of GPCRs located on the nuclear membrane, including β -AR (both β_1 -AR and β_3 -AR) and endothelin (ET_B) receptors [5,6]. As discussed above, the activation of surface (plasmalemmal) β -AR/endothelial receptors modulates contractility. Unlike surface receptors one would surmise that the activation of nuclear β -AR/endothelial receptors will modulate gene expression. It has been demonstrated that the nuclear β -AR cascade initiates transcription while nuclear ET_B receptor activation inhibits transcription [7]. However, the molecular pathway(s) of these nuclear receptors are not known. Interestingly, increased nitric oxide production has been shown with surface β -AR receptor and ET_B activation [8–10].

4. Nitric oxide signaling

Another important cardiovascular signaling pathway is nitric oxide (NO), which regulates multiple processes including vascular tone and myocardial contraction [11]. NO is generated via three enzymes termed NO synthase (NOS). Ventricular myocytes constitutively express two NOS isoforms: NOS1 (neuronal NOS) and NOS3 (endothelial NOS). NO produced via these NOSs is Ca^{2+} -calmodulin dependent, unlike NOS2 (inducible NOS), which is expressed in ventricular myocytes during inflammatory events. NOS1 and NOS3 exist as dimers, with each monomer having an oxygenase domain and a reductase domain. The production of NO from L-arginine by NOS requires a number of co-factors (BH_4 , FMN, FAD, and NADP). In this issue of *Journal of Molecular and Cellular Cardiology*, Vaniotis

et al., [12] have addressed whether NO/NOS is involved in the regulation of gene expression via the localized stimulation of the nuclear β -AR/ET_B receptors.

5. Nuclear β -AR/ET_B receptors activate NOS3

In initial studies, Vaniotis et al. [12] tested if these receptors regulate NO production in isolated cardiac nuclei. Both β 3-AR (isoproterenol or BRL37344) and ET_B (endothelin-1) receptor stimulation resulted in increased nuclear NO levels, which was prevented by non-specific NOS inhibition (L-NAME). The authors further investigated the NOS isoform involved and demonstrate (via Western blot) nuclei express NOS3 but not NOS1. This is the first report of NOS3 being localized to cardiac nuclear membranes. Thus, both β 3-AR and ET_B receptors increase NO production in the nucleus, likely via activation of NOS3.

6. Nuclear NOS signaling

Further work investigated the molecular pathways of how β -AR and ET_B receptor stimulation activates NOS3. Towards this end the authors investigated G_{qi}/PKB as both modulate NOS3 activity [13,14] and both receptors couple to this specific G-protein (see above). Interestingly, G_{qi} (pertussis toxin) and PKB (triciribine) inhibitions actually increased NO levels. These results are surprising since studies have shown that pertussis toxin and PKB inhibition (including with triciribine) decrease NO production via surface NOS3 in cardiac myocytes and other cell types [8,14–17]. These data are especially exciting because while it is clear that NOS3 has moved into a new neighborhood (i.e., nucleus), it appears that this is a completely different world. That is, depending on which neighborhood (nucleus or caveolae) NOS3 lives, its regulation seems to be drastically different. These results also highlight the importance of location as a determinant of these tightly controlled and localized signaling cascades.

7. Nuclear NOS regulates transcription

Since it is known that β -AR/ET_B can modulate transcription, the authors examined if NO was involved in this pathway. As previously shown, isoproterenol (β -AR) increased and endothelin-1 (ET_B) decreased transcription (measured via [α ³²P]UTP incorporation). Furthermore, NOS inhibition via L-NAME inhibited the effects of isoproterenol (but not endothelin-1) on transcription. Thus, while both pathways increase NO levels, only the β -AR pathway uses NO to modulate transcription. The purpose of endothelin-1 induced NO production within the nucleus remains unknown. Previous work has shown that NO is able to regulate gene transcription. For example, it is known that NO is able to regulate the transcription factor nuclear factor κ B [18]. However, this occurs via NOS2, which the authors have shown is not involved in the nuclear β -AR pathway. NOS3 has also been shown to modulate gene transcription. However, this occurs by diminishing the activation of detrimental Ca²⁺ signaling pathways (e.g., calcineurin/NFAT and CaMKII) [19,20]. Thus, these exciting results of Vaniotis et al., are the first to demonstrate that a GPCR activates NO production to regulate gene transcription at the level of the nucleus.

NO is able to modify cellular function via direct protein S-nitrosylation or through the cGMP/PKG pathway [21]. The authors found that similar to NOS inhibition, PKG inhibition

(KT5823 or Rp-8-Br-cGMP) decreased isoproterenol-induced transcription. These data imply that the NO-guanylate cyclase-PKG pathway is involved in gene transcription. While a study did show that PKG is localized to the nucleus [22], the expression and activity of guanylate cyclase, PKG, and phosphodiesterases in cardiac nuclei are unknown and warrant further investigation.

8. Nuclear NOS signaling in intact myocytes

Though the experiments described above were well-designed, they were performed in isolated nuclei. The authors performed additional, elegant experiments in intact ventricular adult cardiac myocytes. Cleverly, in intact myocytes surface β -AR receptors were irreversibly inactivated via an impermeable alkylating agent (EEDQ). In these myocytes, isoproterenol increased 18S rRNA, supporting that nuclear β -AR receptors are indeed functional and independent of surface receptors. Further experiments employed caged compounds to activate either nuclear β -AR receptors or ET_B . Using these caged compounds and simultaneously measuring NO production the authors show that stimulation of either nuclear β -AR or ET_B receptors increased NO levels that were blocked by L-NAME. Amazingly, there appeared to be compartmentalization of the NO produced by either β -AR receptors or ET_B . That is, activation of β -AR receptors increased nuclear NO levels, but its levels were more pronounced in extra-nuclear compartments. Alternately, the activation of ET_B receptors increased NO solely within the nucleus. These data suggest that NO signaling in the nucleus is localized, which may partly explain the distinct effects of nuclear β -AR receptors and ET_B . However, it should be noted that there are most likely additional components involved in each pathway contributing to the distinct functional effects of β -AR receptors and ET_B in the nucleus.

The localized NO signaling within the nucleus is similar to what is observed in the cytosol of the myocyte. That is, NO signaling is also localized resulting in disconnected functional effects. This localized NO signaling in the cytosol is the result of NO production via the two different isoforms—NOS1 and NOS3 [23–29]. While the localized signaling in the cytosol is due to the different NOS isoforms, this does not appear to be the case in the nucleus. Thus worthwhile experiments will be to identify the downstream signaling cascade components involved in localizing nuclear NO produced via β -AR receptors or ET_B .

The potential significance of these findings can be better appreciated by contemplating their application to understanding events that may occur during cardiac pathologies such as heart failure (HF). It has been well defined that in HF the proportion of surface β_1 -AR receptors is increased as a result of β_1 -AR receptor down regulation [30] and surface endothelin receptors are upregulated [31]. While the effects of HF on the nuclear populations of these receptors are currently unknown, nuclear signaling events are critical in the cardiomyocyte response to HF. The current findings by Vaniotis et al. therefore suggest an important role for similar alterations of nuclear GPCR, and localized nuclear NO signaling, to affect gene transcriptional regulation of the cellular program in response to HF. Thus, GPCR modulation in response to HF may serve a dual function to modulate localized surface GPCR regulated contraction and the nuclear GPCR regulated gene program. Future studies are clearly necessary to determine if altered nuclear receptor signaling is responsible for HF

induced β -AR or ET_B receptor modulation of gene transcription and the specific genes they may regulate in response to HF.

In summary, NO is an important cardiac signaling molecule involved in modulating a multitude of cellular processes. The current study by Vaniotis et al. expands the significance of localized GPCR regulated NO signaling by nicely demonstrating the nuclear involvement of NOS in the regulation of gene expression. These findings not only demonstrate a new neighborhood for NO signaling, but also open the door to a whole new world of localized GPCR regulated adaptation.

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