

Nuclear Compartmentalization of α 1-Adrenergic Receptor Signaling in Adult Cardiac Myocytes

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(See editorial: *A nuclear option? G-protein coupled receptors at the nucleus in cardiac myocytes* by Timothy D. O'Connell *Journal of Cardiovascular Pharmacology*, 2015 65:2;89–90)

Abstract: Although convention dictates that G protein-coupled receptors localize to and signal at the plasma membrane, accumulating evidence suggests that G protein-coupled receptors localize to and signal at intracellular membranes, most notably the nucleus. In fact, there is now significant evidence indicating that endogenous alpha-1 adrenergic receptors (α 1-ARs) localize to and signal at the nuclei in adult cardiac myocytes. Cumulatively, the data suggest that α 1-ARs localize to the inner nuclear membrane, activate intranuclear signaling, and regulate physiologic function in adult cardiac myocytes. Although α 1-ARs signal through G α q, unlike other Gq-coupled receptors, α 1-ARs mediate important cardioprotective functions including adaptive/physiologic hypertrophy, protection from cell death (survival signaling), positive inotropy, and preconditioning. Also unlike other Gq-coupled receptors, most, if not all, functional α 1-ARs localize to the nuclei in adult cardiac myocytes, as opposed to the sarcolemma. Together, α 1-AR nuclear localization and cardioprotection might suggest a novel model for compartmentalization of Gq-coupled receptor signaling in which nuclear Gq-coupled receptor signaling is cardioprotective.

Key Words: alpha-1-adrenergic receptor, cardiac myocyte

(*J Cardiovasc Pharmacol*™ 2015;65:91–100)

INTRODUCTION

Alpha-1 adrenergic receptors (α 1-ARs) are G protein-coupled receptors (GPCRs) that bind to and are activated by the endogenous catecholamines norepinephrine, and epinephrine. Classically, α 1-ARs signal through G α q/11 leading to the activation of phospholipase C β (PLC β) with the subsequent production of inositol phosphates (IP) and diacylglycerol

(DAG) leading to the release of calcium from IP-sensitive stores and the activation of protein kinase C (PKC), respectively.¹ The heart expresses all 3 α 1-AR subtype mRNAs, α 1A, α 1B, and α 1D,^{2–5} but at the protein level, cardiac myocytes express only α 1A- and α 1B-subtype,^{3,6} fibroblasts do not express α 1-ARs,^{5,7} and coronary vasculature express only the α 1D-subtype.^{6,8} Although α 1-ARs account for only 10% of the total adrenergic receptors (ARs) in cardiac myocytes (β -ARs account for 90%),¹ studies in cell and animal models indicate that α 1-ARs mediate important cardioprotective functions, including adaptive/physiologic hypertrophy, protection from cell death (survival signaling), positive inotropy, and preconditioning (Review: Ref. 1). Combined with the failure of α 1-AR antagonists in clinical trials (Review: Ref. 1), these recently identified cardioprotective functions of α 1-ARs suggest that α 1-AR agonist therapy could represent a novel therapeutic strategy for heart failure (HF).^{1,9} Although α 1-AR cardiac physiology is well studied, the identification of α 1-AR signaling mechanisms in cardiac myocytes continues to expand, and more than 70 molecules have been identified as targets of α 1-AR signaling thus far.⁹

Currently, the prevailing dogma is that GPCRs localize to and signal at the plasma membrane in cardiac myocytes (or any cell) to regulate many important processes in health and disease, including hypertrophy, contraction, and cell death. Compartmentalization of GPCR signaling is also important, and examples in cardiac myocytes include the regulation of 3'-5'-cyclic adenosine monophosphate signaling by β -ARs,¹⁰ or the influence of neuronal activity on the localization of β -ARs.¹¹ Interestingly, recent studies indicate that several GPCRs localize to intracellular membranes, including the nuclear membrane.^{12–14} Examples of nuclear GPCRs in adult cardiac myocytes include α 1-ARs (reviewed here), as well as β -ARs, endothelin receptors (ET-Rs), and angiotensin receptors (AT-Rs).^{14a} These findings suggest that the nuclei in adult cardiac myocytes represent a novel site for compartmentalized GPCR signaling.

In this review, we delineate recent evidence primarily derived from cultured adult mouse cardiac myocytes indicating that α 1-AR signaling is compartmentalized to the nuclei and describe how nuclear α 1-AR signaling regulates physiologic function. We propose 3 important criteria that must be met to establish nuclear α 1-AR signaling in adult cardiac myocytes: (1) Localization of endogenous α 1-ARs to the nuclei, (2) Activation of α 1-AR signaling at the nuclei, and (3) Physiologic significance of nuclear α 1-AR signaling. Finally, based on our findings that α 1-ARs are cardioprotective,¹ we propose a novel model for nuclear Gq-coupled GPCR (Gq-receptor) cardioprotective signaling in adult cardiac myocytes.

Received for publication July 31, 2014; accepted September 9, 2014.

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Supported by the National Institute of Health (grant P20 RR-017662), and The American Heart Association, Greater Midwest Affiliate.

The authors reports no conflicts of interest.

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LOCALIZATION OF ENDOGENOUS α 1-ARs TO THE NUCLEI IN ADULT CARDIAC MYOCYTES

Four lines of evidence suggest that endogenous α 1-ARs localize to the nuclei in adult cardiac myocytes. First, radioligand-binding assays on isolated and fractionated adult cardiac myocytes detect 80% of endogenous α 1-ARs in the nuclear fraction defined by the nuclear membrane protein lamina-associated polypeptide 2.¹⁵ Second, a fluorescent α 1-AR ligand, boron-dipyrromethene (BODIPY)-prazosin, labels endogenous α 1-ARs at the nuclei, but not the sarcolemma, in cultured adult cardiac myocytes.^{15,16} Third, in nuclei isolated from adult cardiac myocytes, BODIPY-prazosin labels the nuclear membrane.¹⁷ Fourth, reconstitution of α 1-ARs in cardiac myocytes lacking endogenous α 1A- and α 1B-subtypes (α 1ABKO) using α 1-AR–green fluorescent protein fusion constructs (α 1-GFP) recapitulates the nuclear localization of the endogenous α 1-ARs.^{15,17}

The finding that α 1-ARs localize to the nuclei in adult cardiac myocytes might seem to conflict with previous studies that examined α 1-AR localization. For example, α 1-AR antibodies (Santa Cruz Biotechnology) detect receptors on the T-tubules and sarcolemma in cultured adult rat cardiac myocytes by immunocytochemistry.^{17a} In contrast, a study performed in 2009 indicates that among 10 commercially available antibodies, none detects a specific signal in tissues of wild-type mice that was absent in α 1-AR triple knockout mice.¹⁸ A more recent study reports the same result for 3 additional commercially available α 1-AR antibodies.¹⁹ The lack of specificity of α 1-AR antibodies is not unique, and a recent survey shows that among 49 commercially available antibodies against 19 different GPCRs, none are specific.²⁰ In short, these findings raise concern about the use of antibodies to detect GPCR expression and localization.

Avoiding antibodies, other studies find that in cultured neonatal rat ventricular myocytes (NRVM), α 1-ARs localize to caveolae.^{21–23} However, radioligand binding on caveolar fractions isolated from NRVM detect only 27% of total α 1-ARs in the caveolar fraction,²² suggesting the majority of α 1-ARs in NRVM are in other fractions. This result seems consistent with the finding that 80% of endogenous α 1-ARs are at the nuclei in adult cardiac myocytes.¹⁵ It is worth noting that NRVM are not phenotypically mature adult cardiac myocytes. Moreover, rat cardiac myocytes express 10-fold more α 1-ARs than other species,²⁴ (rat \sim 114 fmole/mg heart protein^{5,24–26}; mouse: \sim 12 fmole/mg heart protein^{2,3,27–29}). Furthermore, in mice with 170-fold overexpression of an epitope tagged- α 1A-subtype in the heart, the α 1A-subtype localizes to the sarcolemma in ventricular tissue sections based on immunocytochemistry with an antibody to the epitope tag.²⁷ However, in mice with 5-fold overexpression of an α 1A-GFP fusion protein, the α 1A-subtype localizes to cardiac myocyte nuclei based on immunocytochemistry with a GFP antibody,¹⁵ perhaps suggesting that expression level affects localization.

α 1-AR Nuclear Localization Is Dictated by Nuclear Localization Signals in the C-terminus of Each Receptor Subtype

Targeting proteins to the nucleus requires a nuclear localization signal (NLS) encoded within the protein. These

NLS are short single stretches (monopartite) of basic amino acids, usually lysines and arginines or glycine–arginine repeats or 2 short stretches of basic amino acids separated by a short random stretch of amino acids (bipartite).^{30–32} Importins, a family of proteins that recognize and bind NLS, facilitate transport of proteins through the nuclear pore complex, and proteins targeting the nucleoplasm and the inner nuclear membrane (INM) use this mechanism.^{33–35} Examples of GPCRs that use an NLS/importin-based mechanism to localize receptors to the nucleus include the parathyroid hormone receptor and the gonadotropin-releasing hormone receptor.^{36–38}

Analysis of the sequence of GPCRs previously localized to the nucleus in cardiac myocytes, including α 1-ARs, ET-Rs, AT-Rs, and β -ARs, identifies at least 1 NLS in each receptor (Table 1). Importantly, mutation of NLS in the α 1A- or α 1B-subtypes (Table 1) results in loss of nuclear localization, although neither receptor redistributes to the sarcolemma.¹⁷ Functional NLS in α 1-ARs suggest a mechanism for nuclear localization in adult cardiac myocytes, but α 1-ARs do not localize exclusively to the nucleus in NRVM^{21–23} or in other cell types including smooth muscle cells.³⁹ This indicates that an NLS is required but not sufficient for nuclear localization of α 1-ARs. However, additional mechanisms required for nuclear localization remain to be determined. The difference in cell-specific localization of α 1-ARs could have implications for pharmacologic targeting of receptors on the plasma membrane in smooth muscle cells or at the nuclei in adult cardiac myocytes.

α 1-ARs Localize to the Inner Nuclear Membrane Oriented With the C-terminus Facing Inside the Nucleus

The nuclear envelope consists of INM and outer nuclear membranes (ONM) separated by the perinuclear space. The nuclear pore complex forms a junction between the INM and ONM and regulates transport between the nucleus and cytosol. The INM contains functional integral

TABLE 1. Nuclear Localization Sequences of GPCRs

Receptor	NLS Sequence	Location	Type
α 1A-AR	KKAFQNVLRIQCLCRK	C-terminal tail	B
α 1B-AR	RGRGRRRRRRRR	C-terminal tail	B
β 1-AR	RAGKRRPSRLVALREQKALKT	Third intracellular loop	B
β 3-AR	RRLLCRCGRRLPPEPCAAA RPALFPGVPAARRSSP	C-terminal tail	B
ET-A	RRNGSLRIALSEHLKQRR	Third intracellular loop	B
ET-B	KKFK RKKSGMQIALNDHLKQRR	C-terminal tail Third intracellular loop	B B
AT-1	KKFK KRYFLQLLKYIPPKAKSH	C-terminal tail	M B
AT-1	KKFKR	C-terminal tail	M

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membrane proteins, whereas the ONM is contiguous with the endoplasmic/sarcoplasmic reticulum (Review: Ref. 40). Conceivably, α 1-ARs could reside in the INM or ONM, oriented in either direction, which would have a significant impact on α 1-AR signaling.

Indirect evidence indicates that some GPCRs likely localize to the INM oriented with the C-terminus facing the nucleoplasm, thus inducing intranuclear signaling. In nuclei isolated from adult cardiac myocytes, α 1-ARs, β -ARs, ET-Rs, and AT-Rs activate intranuclear signaling.^{41–46} Furthermore, α 1-ARs and ET-Rs activate nuclear calcium transients, independent of changes in cytosolic calcium in cultured cardiac myocytes.^{46,47} Yet, without a suitable antibody, direct biochemical evidence for receptor localization and orientation is difficult to obtain. However, using a reconstitution system in α 1ABKO myocytes to express the α 1A-GFP, with the GFP on the C-terminus, fluorescent confocal microscopy and immunocytochemistry with a GFP antibody in differentially permeabilized myocytes detects α 1-ARs in the INM oriented with the C-terminus facing the nucleoplasm.⁴⁵ This is the first direct biochemical evidence for GPCR localization to the INM, oriented to induce intranuclear signaling in cardiac myocytes.

To our knowledge, biochemical evidence for GPCRs localization to the INM or ONM, aside from α 1-ARs, is lacking in adult cardiac myocytes. However, human gonadotropin-releasing hormone receptor type I (GnRH-R1) colocalizes with the inner nuclear membrane protein lamin A/C in HEK293 cells.³⁸ Receptor localization to the ONM or near the nucleus, as shown for the insulin growth factor receptor,⁴⁸ is entirely possible and cannot be excluded. In adult cardiac myocytes, the insulin growth factor receptor localizes to perinuclear t-tubule invaginations of the plasma membrane and induces nuclear calcium transients.⁴⁸ Furthermore, ET-Rs activate PLC ϵ -mediated signaling at the ONM in adult cardiac myocytes.⁴⁹ Therefore, indirect evidence also indicates that some GPCRs could localize to the ONM, and GPCR localization and orientation in the nuclear membrane remains a largely unanswered question.

Localization of α 1-AR Signaling Partners at the Nucleus in Adult Cardiac Myocytes

In cardiac myocytes, α 1-ARs signal primarily through $G\alpha_q$ and activate PLC as described above. For α 1-ARs to signal at the nuclei, the receptors must colocalize with their downstream signaling partners. Nuclei isolated from adult cardiac myocytes express $G\alpha_q$ and PLC β 1,¹⁵ and $G\alpha_q$ and PLC β 1 colocalize with α 1-ARs only at the nuclei in adult cardiac myocytes based on immunochemical detection of $G\alpha_q$ and PLC β 1 in α 1ABKO cardiac myocytes expressing α 1-GFP fusion proteins.¹⁵ Functionally, α 1-ARs induce nuclear calcium transients⁴⁷ and activation of PKC⁴⁵ providing indirect evidence for PLC activity at the nuclei (described in detail below). However, direct biochemical evidence for PLC β activity in the nuclei in adult cardiac myocytes is lacking, although PLC β activity is found in the nucleus in other cell types (Review: Ref. 50). However, the nuclear membrane lacks phosphatidylinositol

4,5-bisphosphate, the substrate for PLC β .⁴⁹ Interestingly, PLC ϵ is bound to muscle-specific A-kinase anchoring protein at the ONM and phosphatidylinositol 4-phosphate, the substrate for PLC ϵ , are both found in the Golgi membrane, which surrounds the nuclei in adult cardiac myocytes, and activation of PLC ϵ generates DAG and activates protein kinase D (PKD).^{49,51} Furthermore, PLC ϵ knockdown blocks α 1-induced hypertrophy, without altering inositol phosphate signaling, and cardiac myocyte-specific knockout of PLC ϵ prevents pressure overload-induced hypertrophy.⁴⁹ However, PLC ϵ is activated by G12/13, small GTPases, and G $\beta\gamma$, but is not activated directly by $G\alpha_q$,⁵² so if and how α 1-ARs activate PLC ϵ is unclear at this time.

In addition to Gq and PLC isoforms, nuclei in adult cardiac myocytes express PKC α , δ , and ϵ , with PKC δ expression proportionally the highest and only trace amounts of PKC ϵ .⁴⁵ G protein-coupled receptor kinases (GRK) 2, 3, and 5 are expressed in the heart, but only GRK3 phosphorylates α 1-ARs.⁵³ Furthermore, HF does not induce GRK3 expression,^{54,55} and transgenic overexpression of GRK3ct, an inhibitor of GRK3, improves remodeling after transverse aortic constriction⁵⁶ consistent with a protective function of α 1-ARs. Whether cardiac myocyte nuclei express GRK3 is unknown, although prolonged incubation with an α 1-AR ligand induces receptor movement of the α 1A-subtype off the nuclei in adult cardiac myocytes, providing circumstantial evidence for receptor phosphorylation and desensitization.¹⁵ Interestingly, cardiac myocyte nuclei express GRK5, which surprisingly functions as a histone deacetylase (HDAC) kinase,^{56a} but whether it phosphorylates GPCRs at the INM is unclear.

Summary

In adult cardiac myocytes, α 1-ARs localize to the INM oriented with C-terminus facing the nucleoplasm such that activation of α 1-ARs would induce intranuclear signaling. Although binding assays on fractionated myocytes detect a small fraction of receptors in a nominal plasma membrane fraction, BODIPY-prazosin staining fails to detect receptors at the sarcolemma in cultured adult cardiac myocytes indicating the majority if not all α 1-ARs localize to the nuclei in adult cardiac myocytes. Finally, α 1-AR signaling partners including $G\alpha_q$, an yet to be defined PLC isozyme, PKC α , δ , and ϵ , and possibly GRK3 localize to the nuclei in adult cardiac myocytes, providing the necessary components for nuclear α 1-AR signaling.

ACTIVATION OF α 1-AR SIGNALING AT THE NUCLEI IN ADULT CARDIAC MYOCYTES

Although conventional models of GPCR signaling suggest activation of signaling at the plasma membrane and transduction of signaling inside the cell, a so-called “outside-in” model, significant evidence indicates that α 1-ARs induce intranuclear signaling that exits the nucleus to activate cytosolic targets or an “inside-out” model. Four lines of evidence support α 1-AR signaling at the nuclei in adult cardiac myocytes including: (1) activation of α 1-AR signaling at the nucleus in adult cardiac myocytes, (2) requirement

for $\alpha 1$ -AR nuclear localization to induce signaling, (3) failure to detect $\alpha 1$ -AR signaling at the sarcolemma, and (4) rapid catecholamine uptake in a time frame consistent with signaling.

Activation of $\alpha 1$ -AR and Ca^{2+} Signaling at the Nucleus in Adult Cardiac Myocytes

As mentioned above, nuclei from adult cardiac myocytes express PKC α , δ , and ϵ , and PKC δ is the most abundant isoform (relatively).⁴⁵ Interestingly, in nuclei isolated from adult cardiac myocytes, the $\alpha 1$ -AR agonist phenylephrine activates PKC δ , based on phosphorylation at the activation site Thr505 and increases total PKC activity, based on increased phosphorylation of the PKC target myristoylated alanine-rich PKC substrate.⁴⁵ This would also support the idea that PLC signaling is activated at the nucleus, which is required to produce DAG needed to activate PKC. Furthermore, in NRVM, phenylephrine activates nuclear calcium transients that have the ability to trigger cytosolic calcium transients,⁴⁷ consistent with the idea that $\alpha 1$ -ARs signal at the nucleus in cardiac myocytes. Other Gq-coupled receptors signal at the nucleus in adult cardiac myocytes, including ET-Rs and AT-Rs. For example, endothelin induces calcium transients through activation of the ETB-R subtype in nuclei isolated from adult cardiac myocytes⁴¹ and intact cultured adult cardiac myocytes.⁴⁶ Interestingly, endothelin-induced nuclear calcium transients arise from the nuclear-perinuclear space, which is contiguous with the sarcoplasmic reticulum. Moreover, these transients arise in the presence of ryanodine, suggesting either the presence of nuclear ryanodine receptors⁴⁶ or nuclear IP3 receptors.^{46,57} Similarly, angiotensin induces calcium transients through activation of AT1-R subtype in nuclei isolated from adult cardiac myocytes.⁴³ Collectively, these findings indicate that nuclei from adult cardiac myocytes contain machinery sufficient to induce $\alpha 1$ -AR signaling specifically and Gq-coupled GPCR signaling in general. Furthermore, activation of intranuclear GPCR signaling supports the localization of $\alpha 1$ -ARs to the INM with the C-terminus facing the nucleoplasm.⁴⁵

Requirement for Nuclear Localization to Induce $\alpha 1$ -AR Signaling in Adult Cardiac Myocytes

Mutation of putative NLS in the C-terminus of both the $\alpha 1A$ - and $\alpha 1B$ -subtypes leads to a loss of nuclear localization in adult cardiac myocytes.¹⁷ Importantly, neither NLS overlaps with sequences required for ligand binding in the third and fifth transmembrane domains or G-protein activation in the third intracellular loop.^{58–60} Furthermore, in HeLa cells, where $\alpha 1$ -ARs do not localize to the nucleus (despite the NLS), activation of $\alpha 1A$ - or $\alpha 1B$ -NLS mutant receptors induces phosphorylation of extracellular signal-regulated kinase (ERK), indicating that the $\alpha 1$ -NLS mutant receptors are functional.⁴⁵ However, neither $\alpha 1$ -NLS mutant receptor ($\alpha 1A$ or $\alpha 1B$) can reconstitute $\alpha 1$ -AR-induced phosphorylation of ERK¹⁷ or phosphorylation of cardiac troponin I (cTnI)⁴⁵ in $\alpha 1$ ABKO cardiac myocytes. This demonstrates a requirement for nuclear localization to

induce $\alpha 1$ -AR signaling and suggests that the $\alpha 1$ -NLS mutant receptors function as localization knockouts in adult cardiac myocytes.^{17,45}

Surprisingly, nuclear $\alpha 1$ -ARs induce phosphorylation of ERK in caveolae at the sarcolemma¹⁵ and cTnI at the sarcomere.⁴⁵ However, pretreatment of adult cardiac myocytes with the nuclear export inhibitor leptomycin B inhibits phenylephrine-mediated phosphorylation of ERK and cTnI.^{17,45} This indicates that $\alpha 1$ -AR signaling arises in the nucleus and exits to activate cytosolic targets, supporting localization of $\alpha 1$ -ARs in the INM, activation of intranuclear signaling, and providing evidence for an “inside-out” signaling mechanism.

Failure to Detect $\alpha 1$ -AR Signaling at the Sarcolemma in Adult Cardiac Myocytes

Although BODIPY-prazosin failed to detect $\alpha 1$ -ARs at the sarcolemma, binding assays of fractionated adult cardiac myocytes have detected a small portion of receptor (<20%) in the nominal plasma membrane fraction.¹⁵ Regardless, the implication is that some $\alpha 1$ -AR signaling could occur at the sarcolemma in adult cardiac myocytes. However, a membrane impermeant $\alpha 1$ -AR antagonist fails to block $\alpha 1$ -AR signaling in adult cardiac myocytes. Specifically, CGP-12177A, a membrane impermeant β -AR antagonist that can block $\alpha 1$ -ARs at higher concentrations, fails to block phenylephrine-induced phosphorylation of ERK at concentrations as high as 200 μM . In contrast, prazosin, a membrane permeable $\alpha 1$ -AR antagonist, does block phenylephrine-induced phosphorylation of ERK.¹⁵ This suggests that there might not be functional $\alpha 1$ -ARs at the sarcolemma in adult cardiac myocytes.

Rapid Catecholamine Uptake Mediated by Organic Cation Transporters 3 in Adult Cardiac Myocytes

To activate $\alpha 1$ -ARs at the nuclear membrane, endogenous catecholamines, norepinephrine, and epinephrine, must cross the sarcolemma and ONM to reach the perinuclear space to bind to and activate $\alpha 1$ -ARs in the INM in a time frame (seconds) consistent with signaling. The *slc22A* gene family of organic cation transporters (OCT1-3) mediates catecholamine uptake in cardiac myocytes and smooth muscle cells,^{61,62} a process termed norepinephrine uptake-2. Importantly, the heart expresses high levels of OCT3, with lower levels of OCT1,⁶³ and based on immunocytochemistry, adult cardiac myocytes express OCT3 on the sarcolemma and nuclear membrane.¹⁵

In uptake assays with a fluorescent catecholamine analog, OCT3-mediated catecholamine uptake occurs rapidly in adult cardiac myocytes, on the order of seconds, steadily increasing for several minutes, peaking by 30 minutes, and is inhibited by preincubation with unlabeled norepinephrine.¹⁵ The biophysical properties of OCT3 also support rapid catecholamine uptake. The uptake kinetics of recombinant OCT3 expressed in HEK293 cells indicate a V_{max} of $\sim 30,000$ pmole $\cdot\text{mg}^{-1}$ protein per minute and kilometer of ~ 900 μM for norepinephrine, and $\sim 13,000$ and ~ 500 for epinephrine respectively.⁶⁴ In this

system, OCT3 catecholamine uptake occurs in seconds, with a half maximal response of 2 minutes.⁶⁴ These biophysical properties are likely consistent between cells with expression level dictating the amount of uptake, and the heart expresses high levels of OCT3.⁶³

In mice, cardiac uptake of the cation methyl-4-phenylpyridinium acetate happens quickly (~4000 ng/g heart tissue in 5 minutes) but is reduced 75% in OCT3 knockout mice.⁶³ In addition, OCT3 knockout mice mimic α 1ABKO mice with a trend towards a small heart (OCT3 KO, 10% smaller heart, $n = 7$ NS, α 1ABKO, 17% smaller heart, $P < 0.05$, $n = 27-33$).^{3,63} In cultured adult cardiac myocytes, blockade of OCT3 uptake with corticosterone inhibits α 1-AR-mediated phosphorylation (activation) of ERK.¹⁵ Furthermore, unlike β -AR inotropic responses, which occur rapidly, α 1-AR inotropy and calcium transients are delayed, with a latency of 2–5 minutes in isolated myocytes,^{17a, 65-70} consistent with catecholamines having to cross the membrane and reach the nucleus to activate signaling.

In summary, the high level of OCT3 expression in the heart, the kinetics of catecholamine uptake in adult cardiac myocyte, the reduced cation uptake with the trend toward a small heart in OCT3 KO mice, the ability to block α 1-AR signaling by inhibiting OCT3 in adult cardiac myocytes, and the latency of α 1-AR contractile function all suggest that catecholamine uptake is required for α 1-AR signaling.

Summary

Evidence from studies with isolated nuclei and whole cells indicates that α 1-ARs induce intranuclear signaling in adult cardiac myocytes. In isolated nuclei, α 1-ARs activate PKC indicating that nuclei contain machinery sufficient to induce α 1-AR (GPCR) signaling and supporting the observation that α 1-ARs localize to the INM oriented to induce intranuclear signaling. Furthermore, the failure of α 1-AR localization mutants (α 1-NLS mutants) to reconstitute signaling in α 1ABKO cardiac myocytes demonstrates a requirement for nuclear localization. The inability of the membrane impermeant α 1-AR antagonist CGP-12177A to block α 1-AR signaling suggests little or no functional α 1-AR signaling at the sarcolemma in adult cardiac myocytes. Finally, consistent with nuclear α 1-AR signaling, OCT3 mediates rapid uptake of catecholamines in cardiac myocytes, which is required for signaling.

PHYSIOLOGIC SIGNIFICANCE OF NUCLEAR α 1-AR SIGNALING IN ADULT CARDIAC MYOCYTES

Establishing the physiologic significance of nuclear GPCR signaling is paramount to validating a model of nuclear α 1-AR signaling. In the heart, α 1-ARs mediate adaptive/physiologic hypertrophy, positive inotropic responses, prevent cell death, and induce preconditioning.^{1,9} A confounding factor for many nuclear GPCRs, for example ET-Rs, is that only a fraction of the total receptor population localizes to the nucleus (5% for ET-Rs).⁴¹ However, α 1-ARs localize primarily to the nuclei in adult cardiac myocytes, and the lack of functional α 1-ARs at the sarcolemma¹⁵ simplifies ascribing physiologic function of nuclear α 1-ARs.

Requirement for Nuclear Localization of α 1-ARs to Induce Inotropic Responses in Adult Cardiac Myocytes

In cardiac myocytes, α 1-ARs induce positive inotropic responses, but in the basal state, α 1-AR-mediated inotropic responses are relatively minor. In HF, where β -ARs are downregulated, α 1-AR inotropy becomes more significant (Review: Ref. 1). In fact, α 1-AR-mediated inotropy can equal β -AR-mediated inotropy in muscle strips isolated from failing human hearts.⁷¹ In mice, transgenic overexpression of the α 1A-subtype induces a basal hypercontractile phenotype that is protective against pathologic stress induced by ischemic injury or pressure overload.^{72,73}

α 1-ARs induce inotropic response through a variety of mechanisms including altering K^+ and Ca^{2+} currents, intracellular pH, and myofilament Ca^{2+} sensitivity (Review: Ref. 1). Interestingly, in adult cardiac myocytes, phenylephrine induces an inotropic response correlated with phosphorylation of cTnI at a putative PKC δ site, threonine 144 (T144). However, in α 1ABKO cardiac myocytes, both responses are lost. Reconstitution of the α 1A-subtype, but not the α 1B-subtype, restores phenylephrine-induced inotropy and phosphorylation of cTnI at T144, whereas the α 1A-NLS “localization mutant” fails to restore function.⁴⁵ This demonstrates that nuclear localization is required for α 1-AR-mediated inotropy in adult cardiac myocytes.⁴⁵

Requirement for Nuclear Localization of α 1-AR to Induce Survival Signaling in Adult Cardiac Myocytes

In addition to regulating inotropic signaling, the α 1A-subtype prevents cardiac myocyte death in response to pathologic stress. In adult cardiac myocytes, an α 1A-subtype ERK signaling pathway prevents cell death (survival signaling),¹⁶ and the absence of this survival signaling pathway might help explain the pathologic response to pressure overload in α 1ABKO mice.⁷⁴ In adult cardiac myocytes, phenylephrine induces phosphorylation of ERK, but not in α 1ABKO cardiac myocytes. However, reconstitution of the α 1A- or α 1B-subtypes restores phenylephrine-induced phosphorylation of ERK in α 1ABKO cardiac myocytes, whereas both α 1A-NLS “localization mutants” fail to restore signaling.¹⁷ This implies that nuclear localization is also required for α 1-AR-mediated survival signaling.

α 1-AR Signal Transduction Out of the Nucleus

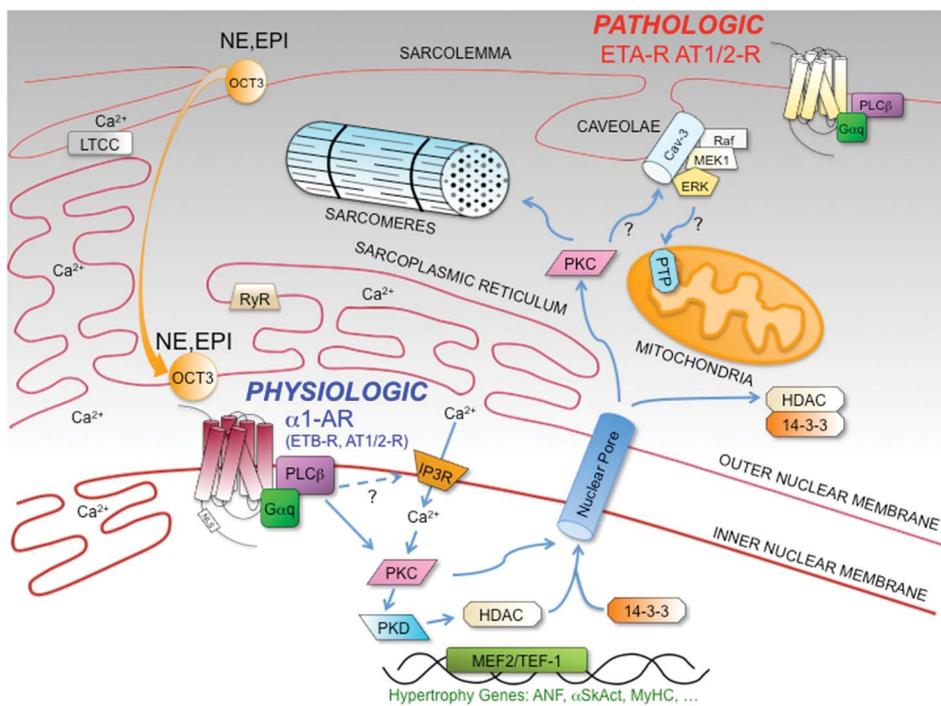
The most significant remaining question is how α 1-AR-mediated intranuclear signals exit the nuclei to reach cytosolic targets. Phenylephrine activates PKC δ in cardiac myocyte nuclei⁴⁵ and induces phosphorylation of cTnI at T144,⁴⁵ a putative PKC δ site,⁷⁵ together suggesting that PKC δ is exported to the sarcomere upon activation by α 1-ARs. Translocation of PKC from the cytoplasm to the plasma membrane is a hallmark of its activation in many cell types, but PKC translocation is more complex in cardiac myocytes.⁷⁶ In NRVM, PKC δ and PKC ϵ localize to the nucleus in the basal state, and α 1-AR stimulation induces PKC δ translocation to the perinuclear space and PKC ϵ translocation to the sarcomere,⁷⁷ consistent with α 1-AR-mediated activation and export of intranuclear

PKCδ in adult cardiac myocytes.⁴⁵ Mechanistically, PKC export could involve binding to a chaperone protein, such as 14-3-3. For example, α1-AR hypertrophic signaling involves PKD-mediated phosphorylation of HDAC5, binding of HDAC5 to 14-3-3 and nuclear export of the HDAC5: 14-3-3 complex, thereby inducing hypertrophic gene transcription.^{78,79} Whether 14-3-3 mediated export of signaling proteins represents a general mechanism whereby α1-ARs export signals from the nucleus remains to be determined.

Activation of Hypertrophic Genes by α1-ARs and “Inside-in” Signaling

In cardiac myocytes, α1-ARs induce the expression of hypertrophic genes, such as skeletal actin and α-myosin heavy chain.¹ Convention dictates that GPCR signaling initiates at the sarcolemma with second messengers and signaling molecules diffusing to the nucleus. However, the idea of “excitation–transcription coupling” could challenge this convention. Here, localized Ca²⁺ release from nuclear IP₃ receptors within the perinuclear space activates transcriptional regulators, such as HDAC5.⁵⁷ Potentially, this nuclear localized release of Ca²⁺ is mediated, at least in some cases, by nuclear GPCRs leading to transcriptional activation. In fact, in nuclei isolated from adult cardiac myocytes, β-ARs and ET-Rs induce gene transcription,^{44,57,80} suggesting that the nuclei might contain the machinery sufficient to induce a hypertrophic response. By extension, the localization of functional α1-ARs to the nucleus in adult cardiac myocytes could suggest an α1-AR “inside-in” hypertrophic signaling mechanism, whereby α1-AR hypertrophic signaling is regulated predominantly in the nucleus, an interesting, but as of yet unproven hypothesis.

FIGURE 1. Compartmentalization of Gq-receptor signaling in adult cardiac myocytes. In adult cardiac myocytes, Gq-receptors are compartmentalized. In this model, α1-ARs are the prototypical cardioprotective nuclear Gq-receptors, although the ETB-R and AT1/2-Rs are also at the nucleus. ETA-R and AT1/2-Rs are primarily at the sarcolemma and mediate pathologic remodeling in HF. α1-AR, α1-adrenergic receptor; ET-R, endothelin receptor; AT-R, angiotensin receptor; NE, norepinephrine; EPI, epinephrine; OCT3, organic cation transporter 3; PLCβ, phospholipase Cβ; IP3, inositol 1,4,5-trisphosphate; IP3R, IP3-receptor; PKC, protein kinase C; PKD, protein kinase D; HDAC, histone deacetylase; LTCC, L-type calcium channel; RYR, ryanodine receptor; PTP, mitochondrial permeability transition pore; Cav-3, caveolin-3; ERK, extracellular regulated kinase; MEK1, mitogen-activated protein kinase; raf1, MAP kinase; MEF2, myocyte enhancer factor-2; TEF-1; transcriptional enhancer factor-1.



Summary

The failure of α1-NLS “localization” mutants to reconstitute α1-AR inotropic function and survival signaling in adult cardiac myocytes demonstrates a requirement for nuclear localization to regulate physiologic function in adult cardiac myocytes. Although the requirement for nuclear localization appears clear, the exact mechanisms whereby α1-ARs regulate extranuclear signaling remain unclear, and how signals are exported from the nucleus remain to be determined.

POTENTIAL IMPLICATIONS OF NUCLEAR A1-AR SIGNALING: COMPARTMENTALIZATION OF GQ-COUPLED GPCR SIGNALING IN ADULT CARDIAC MYOCYTES

Convention indicates that GPCRs that signal through Gαq (Gq-receptors) worsen HF. Yet, accumulating evidence now indicates that not all Gq-receptors induce pathology, and in fact, α1-ARs are cardioprotective.^{1,9} Additionally, other studies now show that Gq-receptors localize to different sub-cellular compartments in cardiac myocytes. This could suggest a new model for compartmentalization of Gq-receptor signaling in adult cardiac myocytes (Fig. 1). In this model, Gq-receptor signaling at the nucleus is cardioprotective in adult cardiac myocytes, with α1-ARs as the prototypical cardioprotective nuclear Gq-receptors, as alluded to in previous reports.^{17,50,81}

Gq-receptor Pathophysiology in HF

HF is a clinical syndrome and the common end stage of many cardiovascular diseases, including ischemic heart disease and hypertension. The structural and functional changes in the heart during HF, a process termed remodeling,

include hypertrophy, contractile dysfunction (systolic and/or diastolic dysfunction), cell death, and fibrosis (interstitial and/or replacement fibrosis). Previous models suggest that Gq-receptors, including α 1-ARs, ET-R, and AT-R, activate important physiologic responses that worsen remodeling. Several lines of evidence support this idea including (1) Gq-receptors in NRVM induce hypertrophy with re-expression of the fetal gene program, a marker of pathologic remodeling,⁸² (2) overexpression of G α q in NRVM induces hypertrophy and a constitutively active mutant of G α q induces apoptosis,⁸³ (3) increasing levels of cardiac-specific overexpression of G α q produces progressively more pathologic phenotypes in mice,^{83,84} (4) cardiac-specific and conditional knockout of G α q and G α 11 prevents pathologic remodeling in mice,⁸⁵ and (5) G α q-inhibitor peptides block pathologic remodeling.⁸⁶ Therapeutically, this suggests that inhibiting Gq-receptor signaling in HF would improve outcomes. Although AT-R antagonists are now used in human HF (Review: Ref. 87), ET-R antagonists show no benefit (Review: Ref. 88) and α 1-AR antagonists in hypertension double the risk of HF and increase mortality (Review: Ref. 1), suggesting that cardiac Gq-receptors are functionally unique.

Gq-receptor Function: α 1-ARs Are Cardioprotective, Whereas ET-Rs and AT-Rs Worsen Pathologic Remodeling in HF

As mentioned above, studies in cell and animal models indicate that α 1-ARs mediate cardioprotective functions including an adaptive/physiologic hypertrophy, protection from cell death, positive inotropy, and preconditioning (Review: Refs. 1 and 9). Furthermore, α 1-ARs levels are increased approximately 2-fold in HF, suggesting a compensatory role.¹ Clinically, α 1-AR antagonists for HF (prazosin, V-HeFT) or hypertension (doxazosin, ALLHAT trial) fail to show efficacy (Review: Ref. 1), leading to termination of the ALLHAT trial due to increased mortality and a doubling in the risk of HF.^{89,90}

Both ET-R subtypes, ETA and ETB, are expressed in cardiac myocytes and fibroblasts (Review: Ref. 91). Mouse models indicate that ET overexpression induces HF,⁹² but gene-deletion models show less consistent results with global knockouts inducing embryonic lethality,^{93–95} and cardiac-specific knockouts suggesting direct and indirect effects to induce HF.⁹⁶ Clinically, ET antagonists for both acute and chronic HF show no benefit.⁸⁸

Both AT-R subtypes, AT1 and AT2, are expressed in the cardiac myocytes and fibroblasts. In mouse models, overexpression of AT1-R induces hypertrophy and HF,^{97,98} whereas knockout of the AT2-R, but not the AT1-R, attenuates the pathologic remodeling.^{99,100} Clinically, AT-R antagonists (ARBs) are used in the treatment of hypertension, and ARBs have consistently proven efficacious in treatment of HF (Review: Ref. 87).

Compartmentalized Gq-receptor Signaling

Several lines of evidence support the hypothesis for compartmentalization of Gq-receptors. Quantitatively, binding assays on fractionated adult cardiac myocytes detect 80% of

endogenous α 1-ARs in the nuclear fraction, whereas 95% of endogenous ET-Rs localize to the sarcolemma,⁴¹ (AT-Rs below the level of detection, unpublished result Wright CD and O'Connell TD, November 2009). However, AT-Rs localize to both the sarcolemma and the nuclei in adult cardiac myocytes based on immunostaining, but quantitative data on their distribution is lacking. Interestingly, ARBs do not cross the sarcolemma¹⁰¹ or block intracrine angiotensin signaling⁴³ suggesting that AT-R pathologic signaling probably initiates at the sarcolemma. Functionally, α 1-ARs and ET-Rs differentially activate PKD and ET-Rs activate PKD at the sarcolemma, whereas α 1-ARs activate PKD at the nuclei in adult cardiac myocytes.¹⁰² Furthermore, AT-Rs and ET-Rs activate calmodulin kinase II through different phosphorylation sites than α 1-ARs.¹⁰³

Alternatively, it is entirely possible that functional differences between Gq-receptors in cardiac myocytes exist due to intrinsic differences in how receptors activate subsequent downstream signaling pathways and interact with G α q. In this case, differential activation of signaling pathways or biased signaling could play a significant role in the physiologic outcome of Gq-receptor signaling. Therefore, future studies will need to define the functional significance of nuclear GPCR signaling in adult cardiac myocytes.

FINAL SUMMARY

There is now significant evidence demonstrating that α 1-ARs localize to and signal at the nuclei in adult cardiac myocytes as follows: (1) identification of endogenous α 1-ARs at the nuclei in adult cardiac myocytes based on radio-ligand binding assays on fractionated myocytes or fluorescent ligands in cultured myocytes provide the basis for this novel model, (2) demonstration of signaling in isolated nuclei, failure of α 1-AR “localization” mutants to signal, failure to detect α 1-AR signaling at the plasma membrane, and rapid and specific catecholamine uptake support the nuclear α 1-AR signaling model, and (3) evidence that nuclear localization is required for α 1-AR-mediated inotropy and survival signaling identifies a physiologic significance to the localization of α 1-ARs at the nuclei in adult cardiac myocytes. Finally, unlike other Gq-receptors, α 1-ARs are cardioprotective and localize primarily to the nuclear membrane, suggesting that compartmentalized signaling at the nuclei in adult cardiac myocytes could be cardioprotective.

ACKNOWLEDGMENTS

The authors thank Brian C. Jensen, MD, Departments of Medicine and Pharmacology and The McAllister Heart Institute, The University of North Carolina for insightful editorial comments during the writing of this manuscript.

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