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Heart Disease and Relaxin: New Actions for an Old Hormone

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Abstract

The hormone relaxin has been long recognized for its involvement in maternal adaptation during pregnancy. However, discoveries during the last two decades on the mechanism of action of relaxin, its family of receptors and newly described roles in attenuating ischemia/reperfusion injury, inflammation and arrhythmias have prompted vast interest in exploring its therapeutic potential in cardiovascular disease. These observations have inspired clinical trials in patients with acute heart failure that were concluded recently. This review will discuss our current understanding of the protective signaling pathways elicited by relaxin in the heart and highlight important new breakthroughs about relaxin signaling that may pave the way to more carefully-designed future trials.

Keywords

Relaxin; RXFP1; Myocardial Infarction; Heart Failure; Fibrosis

Scope and Relevance

The peptide hormone relaxin has traditionally been linked to the maternal adaptation of the cardiovascular system during the first trimester of pregnancy[1]. By activating several molecular signaling events, relaxin has been proposed as a pleiotropic and cardioprotective hormone. In fact, pre-clinical studies were able to demonstrate that relaxin promotes vasodilatation and angiogenesis, ameliorates ischemia/reperfusion (I/R) injury, regulates extracellular matrix turnover and remodeling following **acute myocardial infarction (AMI)** (see glossary), suppresses arrhythmias post MI and reverses fibrosis[2–5]. In the RELAX-AHF phase 3 clinical trial, intravenous administration of serelaxin (recombinant human relaxin-2) in the first 48 hours following admission for **acute heart failure (AHF)** was shown to be safe and was associated with remarkable survival benefit at 180 days[6];

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

however, the recently completed RELAX-AHF-2 trial did not yield significant clinical benefits, despite significant reduction in biomarkers of injury and worsening of HF through day 5. A recent article called into question the design of several HF trials and highlighted that acute HF is not a “48-hour illness”[7]. Due to its safety profile in patients and based on compelling preclinical reports suggesting a protective role of relaxin against AMI[2–5] and other cardiovascular diseases, more rigorous trials involving relaxin with carefully designed strategies are warranted to provide a fair assessment of its clinical efficacy in patients with cardiovascular disease. In this review article, we present updated information on signaling cascades associated with relaxin and its receptor RXFP1. In subsequent sections, we examine cardiac pathophysiology relevant to MI and adverse remodeling while limiting discussion to pathways specifically countered by relaxin signaling. After reviewing the preclinical data demonstrating the cardioprotective effects of relaxin, we present emerging evidence supporting the prospect of biased signaling at RXFP1 and its potential therapeutic implications.

Relaxin-2 and Its Receptor: Current Knowledge and Significance

The endocrine system specializes in evoking various physiological functions throughout the body in response to environmental or innate stimuli, and the advantage in its design allows for a pleiotropic response, wherein a single hormone, including the 6 kDa peptide relaxin, can orchestrate the dynamic interplay of significant downstream events. Relaxin-2, the active form in humans, is secreted by the corpus luteum in the luteal phase of the human ovarian cycle, and by the placenta[8]. However, mRNA levels were also detected in kidney[9] and heart[8]. Apart from its direct influence on pelvic girdle softening, cervical ripening and uterine contractility in the context of female reproduction [8], relaxin specializes in modulating hemodynamic and cardiovascular function during the latter stages of mammalian pregnancy. Cardiac output (CO) and systemic vascular compliance were increased to mid-term pregnancy levels when relaxin was chronically administered to non-pregnant rats[10].

Relaxin-2 exerts its physiologic effects by primarily acting on its cognate receptor – the Relaxin Family Peptide Receptor 1 (RXFP1), a **G-protein Coupled Receptor (GPCR)**[11]. Other receptors include RXFP2 (which shares several structural features with RXFP1, and is activated by the insulin like peptide INSL3), RXFP3 (receptor for neuropeptide relaxin-3) and RXFP4 (activated by the gut hormone INSL5)[11]. While its expression in vascular, neuronal, and reproductive tissue is well established[11], RXFP1 has also recently been documented in atrial and ventricular tissues of the heart[12,13], and more specifically, in the ventricular cardiomyocyte[2]. It has been well documented in the Langendorff isolated-perfused rat heart setting that relaxin also induces positive **inotropy** and **chronotropy**[14].

RXFP1 Signaling

RXFP1 belongs to a class of GPCRs – Leucine-Rich Repeat (LRR) containing GPCRs (LGRs) – that possess a unique LRR domain on the N-terminal extracellular side of the receptor. RXFP1 attains further specialization via the attachment of an LDL-A domain to the LRR[15]. The resulting three-dimensional structure allows for unique receptor conformations upon ligand activation. The B-chain of relaxin interacts with aspartate and

glutamate residues present within the LRR domain, facilitating the interaction between LDL-A and the extracellular loops 1 and 2 (ECL1 and 2) of the transmembrane domain of RXFP1. RXFP1 with truncated LDL-A exhibits no signs of activation despite the presence of relaxin[15]

RXFP1 activates three distinct G α proteins upon initiation of its canonical cascade by nanomolar levels of relaxin. These G α proteins – G α_s , G α_{oB} and G α_{i3} – exhibit spatiotemporally varied activation profiles evident in the biphasic cAMP response generated at the onset of receptor activation. The initial surge of cAMP generation upon activation of G α_s is curbed by G α_{oB} activity; a more gradual cAMP response is elicited by G α_{i3} activation. The latter phase of cAMP generation is in part attributed to G $\beta\gamma$ -induced PI3K activation, as initially implicated in THP-1 cells, where cAMP response was diminished upon pharmacologic inhibition of PI3K[16]. In human atrial tissue, positive inotropy was associated with G γ -PI3K-induced cAMP accumulation[13]. Long-term exposure of serelaxin, recombinant relaxin-2, in human umbilical arterial smooth muscle cells (HUASMCs) and human cardiac fibroblasts (HCFs) also led to increased nNOS-driven NO generation, along with vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs)-2 and 9. Serelaxin induced phosphorylation of ERK1/2 and cGMP signaling in both of these cell lines[17]. Recent evidence also points to PKA-induced activation of p38 MAPK in hepatic stellate cells[18]. Therefore, promiscuous coupling of RXFP1 to its G proteins leads to a varied response contingent upon cell type and further studies are required to elucidate the diversity of pathways evoked.

Pathologic Signaling in Ischemic Heart Disease

The prevalence of ischemic heart disease and the multitude of pathophysiological mechanisms at play necessitate an ever-increasing demand for novel therapies that counter aberrant signaling, minimize loss of functional tissue and improve quality of life for affected patients. MI involves cessation of blood supply to the affected region of the heart, causing necrosis and extravasation of inflammatory cells into infarcted tissue. Ischemia also damages mitochondria and impairs the functionality of the electron transport chain[19]. Although timely reperfusion is essential to salvage myocardium and limit the extent of injury, reintroducing oxygen in the presence of deranged mitochondria leads to the generation of **reactive oxygen species (ROS)** – which can exacerbate the ischemic insult and associated pathophysiology. In the wake of I/R injury to the myocardium, adverse remodeling coupled with systemic neurohormonal dysregulation and inflammation ultimately lead to clinical presentation of HF[20,21]. Within the broader picture of adverse remodeling, the following subtopics are explored in further detail as they particularly pertain to the cardiac actions of relaxin.

Inflammation

Prolonged myocardial ischemia leads to tissue necrosis and release of intracellular contents upon cell rupture. The resulting cell debris consists of damage-associated molecular patterns (DAMPs), which act on pattern recognition receptors (PRRs) expressed on the surface of resident cells[21]. This initiates a sterile inflammatory cascade resulting in the aggregation

of a macromolecular complex – the inflammasome, which consists of the sensor NLRP3 (cryopyrin), the scaffold ASC, and the effector enzyme caspase-1[21]. The consequences of inflammasome activation in the post-infarcted heart vary with the cell type: leukocytes produce a surge in active IL-1 β , whereas cardiomyocytes respond via increased pyroptosis and apoptosis[22,23]. Genomic knockdown of NLRP3[24], caspase-1 or ASC[25] in mice led to significant reduction in infarct size upon I/R injury, validating the causality between inflammasome activation and adverse remodeling.

Events leading up to activation of the inflammasome involve priming, and subsequent triggering[21]. The inflammasome is ‘primed’ by DAMPs and other mediators of innate sterile inflammation released upon cardiac cell death. Priming leads to transcription of inflammasome components and post translational modification of cryopyrin[26]. The ‘triggers’ are more specific, involving ATP released upon cell death; the ATP receptor P₂X₇ is directly implicated. A mouse I/R model employing either pharmacologic or RNA silencing inhibition of the receptor leads to a reduction in infarct size and cardiac enlargement following MI. In addition to ATP, ROS generated from the reperfusion injury also activates the inflammasome in isolated cardiomyocytes subjected to hypoxia-reoxygenation (HR) injury[27]. Both of these pathways (P₂X₇ signaling and ROS generation) converge to result in mitochondrial dysfunction and oxidization of mitochondrial DNA (mtDNA)[28], which also triggers inflammasome activity[28,29].

Cardiac Fibrosis and Adverse Remodeling

The acute inflammatory phase seen in response to MI eventually subsides as mediators of chronic inflammation direct the course of fibrosis and adverse remodeling. Fibrotic tissue is less compliant than ventricular muscle and leads to elevated diastolic filling pressures in the failing ventricle. Upregulation of renin-angiotensin-aldosterone system (**RAAS**) due to disruption within the cardiorenal axis mediates the signaling mechanisms observed in fibrosis after MI[30,31]. Elevated levels of Angiotensin II induce expression of TGF- β within cells, which also occurs in the presence of ROS[32,33].

TGF- β is a key modulator of the fibrotic program in the ischemic heart. Activation of its target receptor leads to SMAD-2 and SMAD-3 phosphorylation, which after combining with SMAD-binding element (SBE) increases transcript levels of pro-fibrotic genes[30,34]. SMAD 3 knockout mice had reduced cardiac extracellular collagen deposition and mitigated diastolic dysfunction in comparison with WT mice post MI, despite both having similar infarct size[35]. MMPs and their inhibitors (TIMPs) play a crucial role in scar remodeling, and SMAD3-null cardiac tissue demonstrated a profile tipped in favor of an anti-fibrotic profile of MMPs[35].

Cardiac Arrhythmias

Macrostructural disarray within the cardiac muscle due to fibrotic infiltration and disruptions of ionic currents and conductance at a cellular level predispose the infarcted heart to increased arrhythmic activity[36]. The incidence of **atrial fibrillation** (AF), one of the most prevalent arrhythmias encountered clinically, is estimated to be between 6–21% after MI[37], and is correlated with a higher mortality and adverse events during and after in-

hospital period. Inflammatory mediators such as C-reactive protein (CRP), IL-6, and TNF α are elevated in AF, lending credence to I/R-associated inflammation as a possible etiology[38]. A potential correlation between ROS generation after MI and AF exists, as patients with new-onset AF after cardiac surgery had a significantly higher oxidized protein and serum peroxide levels[39]. Myocardial remodeling orchestrated by RAAS contributes to atrial fibrosis, a risk factor highly associated with AF[36,38].

Rampant electrical remodeling also contributes to arrhythmogenesis in AF. Maintenance of AF is possible due to a shorter atrial refractory period within the myocyte; sustained atrial tachycardia within canine atrial myocytes reduces inward Ca²⁺ current and consequently **action potential duration** (APD), which leads to a shorter refractory period[40]. Atrial tachypacing also reduces inward Na⁺ current, along with reduced channel mRNA levels in canine cells[41]. Reduction in Na⁺ current can lead to a slower action potential upstroke, reducing **conduction velocity** (CV) – a mechanism observed in Nav1.5 mutation-induced AF[42,43]. Lowered CV is also observed as a result of reduced **connexin** expression[43].

The interplay of inflammation, oxidant injury, fibrosis and aberrant electrical activity present after MI necessitates treatment strategies countering multiple cascades of pathologic signaling via pleiotropic activity.

Therapeutic Potential of Relaxin for Cardiovascular Disease

The prospect of relaxin as a cardioprotective agent has been investigated in several preclinical animal models of cardiovascular disease. We present the following evidence in the context of pathophysiology in the order discussed in the previous section.

Inflammation

Relaxin administration prior to ischemia reduced the extent of I/R-associated damage, neutrophil extravasation and activity, mast cell granule release and Ca²⁺ overload in the rat heart after MI[44]. Relaxin therapy upon reperfusion in a swine model of MI yielded dose-dependent reduction in cardiac troponin release, apoptotic markers (caspase 3, TUNEL), and improved ventricular performance. The protective mechanism was attributed to a decrease in oxidative injury as shown by reduced malondialdehyde (MDA) within treated tissue[45]. Guinea pig hearts subjected to I/R injury also had a significant decrease in MDA production and improved contractility with relaxin administration and the protective effect was blunted upon treatment with L-NMMA, a NOS inhibitor[46]. The benefits of NO signaling in the context of I/R injury have been reported comprehensively in literature[47]. Relaxin has also been shown to reduce human and rabbit platelet aggregation *in vitro* in a NO dependent manner[48]. A recent study investigated the cardioprotective effect of serelaxin in adult mice at the onset of reperfusion. In this study, serelaxin was shown to reduce mortality and infarct size while preserving LV function. However, these protective effects were abrogated in endothelial (e)NOS knockout mice. Serelaxin treatment also attenuated caspase-1 activity following MI indicating attenuation of NLRP3 inflammasome activation; an effect that was absent in eNOS knockout mice[2]. *In vitro* studies in macrophages provide evidence to suggest the inhibitory role of NO in attenuating caspase-1 activity[49]. However, the precise role of eNOS-induced NO production on inflammasome activity is yet to be elucidated.

Cardiac Fibrosis and Adverse Remodeling

The anti-fibrotic effects of relaxin have been well studied in the context of cardiac disease. TGF- β -stimulated cardiac fibroblasts increased MMP-2 expression and decreased collagen expression upon treatment with relaxin[50]. Rats grafted with genetically modified C2C12 myoblasts expressing relaxin showed improved histologic outcomes pertaining to cardiac fibrosis after being subjected to permanent ligation of the left anterior descending coronary artery[51]. Anti-fibrotic effects were also observed in mice with permanent left coronary artery ligation receiving exogenous relaxin, illustrated by reduced TGF- β 1 expression and myocyte apoptosis, along with improved MMP13 production[52]. Mechanistic studies determined that relaxin-induced inhibition of TGF β and phosphorylation of Smad-3 were dependent on the Notch-1 pathway, whereby pharmacologic inhibition of Notch-1, or its ligand Jagged-1, significantly abolished the inhibitory effect exerted by relaxin on TGF- β signaling[53]. Treatment of H9C2 cardiomyoblasts with relaxin demonstrated that upregulation of MMP2 and MMP9 and downregulation of TIMP-1 were dependent on activation of sphingosine kinase 1 (Sphk1)[54]. Evidence implicating Erk1/2 in Sphk1 phosphorylation and its subsequent activation corroborates this mechanism[54,55], although the significance of this pathway in adult cardiomyocytes in the context of pathologic fibrosis is unclear. Nevertheless, the consensus from preclinical studies evaluating the effect of relaxin on fibrosis following cardiac injury indicates significant attenuation of pro-fibrotic signaling and cardiac scar formation.

Cardiac Arrhythmias

Relaxin therapy suppresses ventricular and atrial arrhythmias by countering electrical remodeling, in addition to preserving the cardiac substrate by thwarting fibrotic changes. To this end, incidence of ventricular arrhythmias was significantly reduced in a swine model of MI after treatment with relaxin at the onset of reperfusion[56]. Interestingly, the reported cardioprotective effects of relaxin were not exclusive to ischemic heart disease. Spontaneously hypertensive rats (SHR) treated with serelaxin for 2 weeks reversed atrial hypertrophy, increased CV and connexin-43 phosphorylation, and improved restitution kinetics (reduced dependence of APD on diastolic interval)[57]. Relaxin also upregulated Na⁺ current density in induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs), possibly through Nav1.5 channel overexpression[57]. Similar effects were observed in aged rats, whereby voltage-clamp studies indicated a 46% increase in atrial Na⁺ current after 2 days of relaxin treatment[3]. Another study in rats subjected to MI evaluated the influence of relaxin on inducibility of tachyarrhythmias and electrophysiological remodeling. Relaxin treatment for 2 weeks improved connexin 43 physiology at the infarct border zone. Treatment also reduced dispersion of APD after MI[58]. In a mouse cryoinfarction model, relaxin therapy reduced mean duration of AF episodes, while improving CV in the atrial tissue[5]. In another cryoinfarction model, chronic low-dose administration of serelaxin following MI reduced inducibility of ventricular tachycardia, but no changes in connexin or ion channel expression were found[59]. Taken together, these studies demonstrate the ability of relaxin to attenuate substrate and electrical remodeling. However, the mechanism behind improved gap junction function and increased Na⁺ channel expression is yet to be uncovered.

Clinical trials investigating the efficacy of relaxin as a therapeutic agent were motivated by its vasodilatory actions through NO stimulation and functional antagonism of endothelin-1 activity[60]. Currently available vasodilatory agents such as nitrates and nesiritide have limited applicability in the context of AHF due to reservations pertaining to tolerance and long-term safety[61]. The benefits of relaxin treatment were hypothesized to significantly improve outcomes in a subset of AHF patients with normal to high blood pressure. Results from Pre-RELAX-AHF, a randomized, double-blind, placebo-controlled phase IIb study, showed improvement of dyspnea at 24 hours and reduced mortality and readmission rates owing to heart or renal failure at day 60[61]. In the phase III RELAX-AHF trial, 48-hour intravenous infusion of serelaxin improved the primary endpoint of dyspnea measured via visual analogue scale area under the curve (VAS AUC), but did not increase the Likert scale proportion of patients with dyspnea improvement. The additional endpoint of reduced 180 day mortality was satisfied[6]. Serelaxin was subsequently rejected by FDA due to failure to address all the primary endpoints, and insufficiency of single-trial data to justify improvement in symptomatic endpoints[62], although it received “novel breakthrough therapy for HF” designation from the FDA. Post-hoc analyses of the RELAX-AHF are summarized in Table 1. Clinical objectives were reformulated in RELAX-AHF2 trial to include reduced cardiovascular death and worsening HF as primary endpoints, but 48-hour infusion of serelaxin did not lead to beneficial changes in these outcomes[7]. These results, while significant in a clinical sense, fail to underscore the complexity of pathophysiology in AHF, which is not a 48-hour illness, but rather a chronic syndrome with profound ramifications[7]. In this context, recent studies have shown the potential utility of assaying endogenous relaxin-2 levels as a biomarker for predicting disease severity in chronic HF patients[63,64]. In fact, assessing relaxin-2 in addition to BNP had better diagnostic predictability when compared to BNP alone[64].

Biased Signaling at RXFP1: Potential for Novel Drug Development

The ligand-GPCR complex was previously thought to regulate the magnitude of a static signaling response, whether it involved activation in the case of agonism, or cessation of the same through antagonism. However, advances in GPCR physiology have led to the understanding that a diversity of pharmacological profiles can be evoked at the same receptor by different ligands – a phenomenon termed as biased signaling[65]. Biased signaling could lead to variable recruitment of G proteins or other signal transducers, leading to a cellular response that is qualitatively distinct from that produced by the orthosteric, cognate ligand. Such ligand-receptor behavior has been observed in a variety of GPCRs[66], including RXFP1. Concentration bias also exists at RXFP1, whereby the receptor recruits a signalosome, which characterizes its constitutive activity. The signalosome involves an adenylyl cyclase 2 (AC2) and a β -arrestin-2-associated scaffold[67]. Therefore, this apparatus generates cAMP despite the absence of ligand stimulation. Sub-picomolar levels of relaxin amplify this pathway, and at a nanomolar concentration, the signalosome disassembles to lead way to canonical G-protein signaling[67].

Two ligands, namely B7-33 and ML290 have been reported to elicit biased signaling at RXFP1[68,69]. B7-33 is a modified B-chain of relaxin that lacks the A chain present in the native peptide[70]. B7-33 biases the receptor response to prioritize Erk1/2 phosphorylation

over cAMP synthesis, as evident by increased MMP2 production in HEK293 cells and reduced fibrosis in an ISO-induced mouse HF model[69]. ML290 is a recently characterized small molecule agonist at human RXFP1. Evidence from site-directed mutagenesis experiments showed that ML290 bound allosterically to RXFP1; residues within transmembrane domain 7 (TM7) and ECL3 of the receptor were found to be crucial to produce a dose-dependent cAMP response[71]. The measured cAMP response generated upon ML290 activity was abolished when the residues G659 and T660 within the ECL3 domain of human RXFP1 were mutated. Interestingly, these residues are not conserved in murine RXFP1, or in human RXFP2[71,72]. The possibility of a biased, cAMP-independent response cannot be ruled out. In fact, a cGMP biased response was observed in human cardiac fibroblasts (HCF) treated with ML290, inhibiting TGF- β -induced SMAD-2 or SMAD-3 phosphorylation. ML290 influenced receptor-G protein interactions, strengthening $G\alpha_s$, $G\alpha_oB$ over $G\alpha_{i3}$ recruitment[68]. More studies on cAMP-independent pathways elicited by RXFP1 activation to investigate previously undiscovered protective mechanisms employable through biased signaling are warranted.

Concluding Remarks and Future Perspectives

The preponderance of preclinical evidence and some clinical outcomes outlined in this review are suggestive of a therapeutic role for relaxin in MI and HF. Nevertheless, the requirement for intravenous administration, short terminal half-life (1–4 hours), and cost associated with recombinant protein therapy limit the utility of serelaxin for chronic treatment[73]. Therefore, modulation of the route of administration of relaxin or identification of novel agonists at RXFP1 that can overcome these practical hindrances and offer cardioprotective benefits is a vital area of ongoing research. In fact, the development of gastro-protected porcine oral relaxin that was tested in patients with peripheral arterial disease is a promising approach and provided first clinical evidence of oral relaxin in long-term treatment in patients[74]. While awaiting publication of post-hoc analyses from RELAX-AHF2 in the near future, which will provide valuable details on serelaxin treatment in AHF, it is notable that studies investigating the expression profile of RXFP1 in cardiovascular diseases are lacking. Whether cardiac RXFP1 expression is altered in patients with cardiovascular disease, and depending on the profile change in different etiologies, such knowledge may provide new insight into the potential efficacy of exogenous recombinant relaxin-2 treatment or novel RXFP1 agonists. This will also pave the way for precision cardiovascular medicine by taking advantage of focusing relaxin therapy in patients with preserved RXFP1 expression. Clearly, extensive studies are needed to address this important issue. The new knowledge of biased signaling at RXFP1 also provides a wide range of opportunities to better understand downstream protective signaling and also to develop novel drugs with selective pathway activation, depending on disease etiology. To this end, B7-33 and ML290 have significantly improved our knowledge about RXFP1 signaling and new ML290 analogs are being investigated[74].

Finally, although most preclinical findings suggest a protective role of relaxin against MI and its related inflammation, adverse remodeling, arrhythmias and HF, translating these findings in patients with MI requires careful evaluation of the safety profile of relaxin in the setting of emergent cardiac catheterization. The encouraging outcomes reported in various

animal models of cardiovascular disease, however, provide a strong foundation for this promising field of research.

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Glossary terms

MI

Myocardial Infarction. Ischemia (loss of blood supply) to the myocardium can lead to ATP depletion, cell swelling due to failure of active transport mechanisms, and increased intracellular Ca^{2+} concentration. These changes result in irreversible tissue necrosis

Heart failure

Failure of the heart to pump blood to body organs at a level appropriate for homeostasis. Variety of etiologies exist; ischemic heart failure is characterized by depletion of cardiac muscle from a prior ischemic insult. Heart failure can clinically manifest as either reduced ejection fraction (HFrEF) or impairing ventricular filling despite no overt changes in ejection fraction (HFpEF).

Acute Heart Failure

Acute exacerbation of heart failure due to decompensation. Clinically manifests as volume overload, dyspnea and exercise intolerance. Potentially life-threatening

GPCRs

G protein coupled receptors. These cell surface receptors play a crucial role in mediating cell signaling in various organ systems. GPCRs have an extracellular ligand-interacting domain, a transmembrane region within the lipid bilayer, and an intracellular domain that interacts with G proteins or other mediators of signal transduction

Inotropy

The contractile function of the heart. Mechanisms that increase the strength of cardiac muscle contractions are defined as positive inotropic agents

Chronotropy

Pertains to heart rate. Positive chronotropic agents increase heart rate

Reactive oxygen species

A by-product of several biochemical reactions in the cell. Detrimental increases are observed in the context of inflammation and cell damage. Highly unstable due to the presence of an unpaired electron, and can therefore initiate chain reactions

RAAS

Renin-angiotensin-aldosterone system. The feedback mechanisms exercised by these hormones are critical in maintaining plasma volume and renal function. RAAS disruption in heart failure leads to elevated levels of plasma angiotensin, which mediates pro-fibrotic signaling

Atrial Fibrillation

Impulse generation at several ectopic foci within the atrial tissue, leading to rapid fibrillations. Can lead to hemostasis in the atrial chamber and subsequent embolus formation, increasing risk for cerebrovascular stroke

Action potential duration

The total duration of an electrical impulse in a cell – starting from depolarization and ending at resolution to resting membrane potential. Abnormal prolongation or shortening of this interval can be proarrhythmic

Conduction velocity

Velocity of impulse conduction within cardiac tissue

Connexins

Components of gap junctions present at the functional syncytium between cardiomyocytes. Critical for maintaining conduction velocity

 β -arrestin

Scaffolding protein that has been associated with GPCR internalization and cessation of activity. However, relevant isoforms are now identified to play a role in transducing biased signaling mechanisms

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Trends

- Relaxin is an endogenous hormone associated with mammalian pregnancy. It acts on relaxin family peptide receptor 1 (RXFP1), a G protein-coupled receptor to generate cell type specific responses.
- In the context of myocardial ischemia/reperfusion (IR) injury, preclinical evidence demonstrates the efficacy of relaxin therapy in countering acute inflammation and its consequences.
- Relaxin therapy prevents worsening of cardiac function after I/R injury by limiting fibrosis and suppressing arrhythmogenesis. Investigative efforts are focused on uncovering precise mechanisms underlying the protective effects.
- Other RXFP1 agonists – including peptide mimetics and small molecules – evoke biased signaling at the receptor and may offer promising future therapeutic alternatives to recombinant relaxin.

Outstanding Questions

1. What can be inferred from the outcomes of RELAX-AHF and RELAX-AHF2 when placed in context with preclinical evidence suggesting a protective role for relaxin signaling after acute MI? Clinical investigation of the efficacy of serelaxin in combating adverse remodeling remains unexplored.
2. How does relaxin signaling associate with decrease in inflammasome activity? Apart from NO mediated signaling and mitoprotection, do other protective pathways play a role?
3. What specific mechanisms underlie the observed ion channel and gap junction protein expression changes with relaxin therapy?
4. Can biased signaling at RXFP1 be exploited for therapeutic purposes? Differential recruitment of G proteins and resultant variation in cyclic nucleotide-associated downstream signaling could have meaningful implications in cardiovascular disease.
5. How is the expression of RXFP1 affected in various cardiac disease states? Could significant differences within populations account for varied responses in observed benefits? Gain/loss-of-function studies involving RXFP1 can fill the gap in knowledge and raise prospects for targeted gene therapy.

Figure 1.

Schematic outlining pathologic signaling after myocardial I/R injury over the course of adverse remodeling (red) and protective mechanisms conferred upon activation of RXFP1 by serelaxin (green) in the mammalian heart. Pathways that require further validation for mechanistic insight are also indicated (dashed). NO produced downstream of $\beta\gamma$ subunit activation is central to protection. This involves, but is not limited to, cGMP generation and activation of Protein Kinase G (PKG): targets attributed to mitigating ROS injury and countering fibrosis. NO also counters activation of the inflammasome. RXFP1 signaling can upregulate sodium channel and connexin 43 expression, which lead to increased conduction velocity.

Figure 2.

Pleiotropic cardioprotective actions of relaxin: The physiological roles of relaxin involve mediating vasodilatation by interacting with smooth muscle and endothelial cells within vascular beds, and increasing cardiac inotropy and chronotropy. In the context of MI, relaxin attenuates inflammation by reducing mast cell degranulation, neutrophil extravasation (infiltrating cells) and inflammasome activity (in resident and infiltrating cells of myocardium). Relaxin also reduces platelet aggregation and counters adverse remodeling by inhibiting fibrosis and arrhythmogenesis in the post-infarcted heart.

Table 1

Summary of post-hoc analyses of RELAX-AHF

Study, year	Topic of investigation	Results	Notes	Ref.
Metra et al, Jan 2013	Biomarkers of organ damage in patients with acute heart failure	Improvement in cardiac (troponin T), renal (creatinine and cystatin-C) and liver (ALT, AST) biomarkers upon administration of serelaxin, correlating with reduced 180-day mortality		[75]
Metra et al, Oct 2013	Assessment of dyspnea relief, CV and 180-day mortality in patient sub-groups with AHF upon treatment with serelaxin	Forest plots of subgroup analyses show no subgroup based variations in dyspnea relief upon treatment. CV mortality was reduced in patients 75 years ($P=0.0337$), and with no HF hospitalization in previous year ($P=0.0119$), no baseline beta blocker use ($P=0.0432$) and with 12% blood lymphocytes ($P=0.0137$) and eGFR < 50 ml/min/m ² ($P=0.0286$). Similar subgroup based trends observed in 180 day all-cause mortality reductions	Pre-defined subgroups: demographic (age, race, sex, region), time from presentation to randomization, eGFR, SBP, past medical history (AF, diabetes, ischemic heart disease, cardiac devices and <i>i.v.</i> administration at the time of randomization)	[76]
Filippatos et al, Apr 2014	Response of AHF patients with HFpEF to serelaxin treatment	Serelaxin well tolerated in patients with HFpEF. Dyspnea relief measured via VAS-AUC was similar to observed results in patients with HFpEF; however, Likert scale results for moderate or marked dyspnea improvement at 6,12 and 24 hours show difference in effects, with increased odds ratio for improvement in patients with HFpEF ($P=0.03$). Survival outcomes were similar in both groups	26% of recruited patients in RELAX-AHF had HFpEF	[77]
Cotter et al, Nov 2015	Association of growth differentiation factor – 15 (GDF-15) levels with RELAX AHF end points	Large increase in GDF-15 associated with higher risk of 60 and 180-day CV mortality. Randomization to serelaxin treatment correlated with decrease in GDF-15 levels at days 2 and 5.	GDF-15 is a member of the TGF- β super family, and is associated with poor outcomes in chronic heart failure	[78]
Liu et al, Sept 2016	Effect of treatment with serelaxin on renal function	Renal impairment was defined in patients with an eGFR < 60 ml/min/1.73 m ² . Renally impaired patients on serelaxin had further all-cause mortality reduction (HR 0.53, 95% CI 0.34–0.83), compared to patients without impairment (HR 1.30, 95% CI 0.51–3.29)	Renal dysfunction led to an overall increase in CV and all-cause mortality in RELAX AHF	[79]
Filippatos et al, Feb 2017	Assessment of RELAX-AHF endpoints in patients with and without AF	Serelaxin demonstrated similar efficacy and safety profile in patients with and without atrial fibrillation. Although stroke incidence was higher in AF patients in general, a trend of lower incidence was observed in patients treated with serelaxin (OR 0.31, $P=0.0759$) versus placebo (OR 3.88, $P=0.2255$; interaction $P=0.0518$)		[80]