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S100A1: a novel inotropic regulator of cardiac performance. Transition from molecular physiology to pathophysiological relevance

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Most P, Remppis A, Pleger ST, Katus HA, Koch WJ. S100A1: a novel inotropic regulator of cardiac performance. Transition from molecular physiology to pathophysiological relevance. Am J Physiol Regul Integr Comp Physiol 293: R568–R577, 2007. First published April 25, 2007; doi:10.1152/ajpregu.00075.2007.—Here we review the considerable body of evidence that has accumulated to support the notion of S100A1, a cardiac-specific Ca2+-sensor protein of the EF-hand type, as a physiological regulator of excitation-contraction coupling and inotropic reserve mechanisms in the mammalian heart. In particular, molecular mechanisms will be discussed conveying the Ca2+-dependent inotropic actions of S100A1 protein in cardiomyocytes occurring independently of β-adrenergic signaling. Moreover, we will shed light on the molecular structure-function relationship of S100A1 with its cardiac target proteins at the sarcoplasmic reticulum, the sarcomere, and the mitochondria. Furthermore, pathophysiological consequences of disturbed S100A1 protein expression on altered Ca2+ handling and intertwined systems in failing myocardium will be highlighted. Subsequently, therapeutic options by means of genetic manipulation of cardiac S100A1 expression will be discussed, aiming to complete our current understanding of the role of S100A1 in diseased myocardium.

excitation-contraction coupling; cardiomyocyte; heart failure; gene therapy

IN 2001, WE PUBLISHED A STUDY (21) showing that elevated levels of the Ca2+-binding protein S100A1 in myocardium exert profound inotropic actions through modulation of cardiomyocyte Ca2+ homeostasis and myofilament function. This was by no means the first study to demonstrate the potent effects of the Ca2+ sensor on cardiac performance. Due to its altered expression in diseased myocardium (31), clinical interest immediately sparked in its pathophysiological relevance and therapeutic potential. After identification of S100A1 as the predominant cardiac S100 protein isoform among normal human tissues, Remppis et al. (31) were the first to describe altered expression of S100A1 in failing human myocardium. Since then, large efforts have been undertaken to elucidate the role of S100A1 in cardiovascular biology and its contribution to cardiovascular disease. In this review, we focus on concepts supporting a physiological role for S100A1 in the regulation of cardiac excitation-contraction (EC) coupling and inotropic reserve mechanisms. Moreover, pathophysiological consequences of altered S100A1 expression in cardiovascular disease and therapeutic options by the means of genetic manipulation of S100A1 expression will be highlighted, aiming to complete our current understanding of the role of S100A1 in diseased myocardium.

S100 PROTEINS: AN INTRODUCTION

S100 protein family. S100 proteins (so named for being soluble in 100% ammonium sulfate), described first by Moore (20), constitute the largest subfamily of Ca2+-sensors within the EF-hand Ca2+-binding protein superfamily [see the excellent previous reviews (3, 18, 47)]. S100 proteins are encoded by a multigenic family, and 21 members have been reported in the human genome so far (18). Characteristic of the S100 protein family is that most s100 genes form a cluster on human chromosome 1q21 (18). Similarly, a likewise-organized s100 gene cluster is well conserved in mouse and rat on chromosome 3 and 2, respectively (18). As proposed by Schaefer et al. (35), s100 genes located within the cluster on chromosome 1q21 are designated by consecutive Arabic numbers placed behind the stem symbol s100a, e.g., s100a1, s100a2, s100a3, etc. In contrast, s100 genes from other chromosomal regions outside this cluster carry the stem symbol S100 followed by a single letter, e.g., s100b on human chromosome 21q22.3 (18).

S100 protein structure. Individual S100 proteins have long been viewed as cell and tissue specific, as opposed to the ubiquitous intracellular Ca2+ receptor calmodulin, pointing toward a higher degree of specification (3). Although S100 protein sequences exhibit some variety, the key structural features of all S100 proteins are highly conserved. Generally, S100 proteins (M, between 9,000 and 13,000) are characterized by the presence of two EF-hand Ca2+-binding domains interconnected by an intermediate region, referred to as the hinge...
region (47). The COOH-terminal EF-hand contains the classical, canonical Ca\(^{2+}\)-binding motif common to all EF-hand proteins and has a typical sequence signature of 12 amino acids confined by a linear COOH-terminal extension. The noncanonical NH\(_2\)-terminal “pseudo” EF-hand, containing two additional amino acids, is characteristic of S100 proteins. As a consequence, Ca\(^{2+}\) binding to individual EF-hands occurs with different affinities, lower in the case of the NH\(_2\)-terminal site (\(K_d \approx 200–500\) \(\mu\)M) and \(-10\) times higher at the COOH-terminal site (\(K_d \approx 10–50\) \(\mu\)M) (reviewed in Ref. 47). Importantly, Ca\(^{2+}\) affinity of both Ca\(^{2+}\)-binding sites is tightly regulated by posttranslational mechanisms, i.e., redox-dependent S-glutathionylation of cysteine residues (9, 45), that facilitate Ca\(^{2+}\) binding to S100 proteins at nanomolar free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]), thereby enabling them to sense [Ca\(^{2+}\)] from the nanomolar to the micromolar range.

Within cells, S100 proteins mostly exist as antiparallel homodimers held together by noncovalent bonds. Both outer \(\alpha\)-helices, flanking each Ca\(^{2+}\)-binding domain at the COOH- and NH\(_2\)-terminal sides, are the major contributors to the dimer interface (47) (Fig. 1). On Ca\(^{2+}\) binding, S100 proteins undergo a conformational change, forming a hydrophobic cleft in each monomer, which is mainly defined by residues of the hinge region and the COOH-terminal extension (Fig. 1). The exposed hydrophobic surface represents the interaction site of most S100 proteins for Ca\(^{2+}\)-dependent binding of target proteins (47). In general, the hinge region and COOH-terminal extension exhibit the highest sequence variety and might, therefore, contribute to the specificity of individual S100 proteins (34).

Clinical interest in S100 proteins has been sparked due to their altered expression in various human pathologies, including cancer, neurodegenerative disorders, inflammation, and, in particular for S100A1, in cardiovascular diseases (31).

**S100A1: Expression Pattern, Subcellular Location, and Molecular Targets in the Heart**

Cardiac S100A1 expression pattern. S100A1 protein is preferentially abundant in the heart, although the protein is also found in lower amounts in skeletal muscle, brain, and kidney (10, 12, 13, 16, 46). In the heart, S100A1 is the predominant cardiac S100 protein among other S100 isoforms such as S100A4, S100A6, and S100B and is mainly found in ventricular cardiomyocytes. Dominant cardiac expression is seen early in embryonic development, and S100A1 mRNA and protein levels rise constantly throughout maturation of the heart to reach a plateau in the postnatal state (16). In the adult heart, S100A1 protein is not uniformly expressed but exhibits its highest mRNA and protein levels in the left ventricle, with descending concentrations in the right ventricle and atria (6, 31). Most recently, large-scale analyses of the human transcriptome by Su et al. (39) and Shmueli et al. (37) comprehensively confirmed that the heart is the predominant location of s100a1.

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**Fig. 1.** Three-dimensional (3-D) solution structure of S100A1 determined by nuclear magnetic resonance spectroscopy. A: 3-D S100A1 structure in the Ca\(^{2+}\)-free (apo) state. S100A1 is composed of 2 identical subunits (blue and red), each composed of a repetitive helix-loop-helix Ca\(^{2+}\)-binding motif that is connected by a short linear linker (hinge) region. Dimerization occurs in an antiparallel manner between helices (H) 1 and H1', stabilized by hydrophobic bonds. Hydrophobic residues in the hinge region and H4 in the blue dimer are shown in yellow. This 3-D structure reflects the characteristic molecular backbone of S100 proteins. B: 3-D S100A1 structure in the Ca\(^{2+}\)-bound state. Ca\(^{2+}\) binding both to the NH\(_2\)- and COOH-terminal EF-hand motif mainly results in altered orientation of H3 and H4 as well as the hinge region, whereas the residual structure of the dimer remains nearly unaffected. As indicated by arrows, the NH\(_2\)-terminal part of H3/H3' and the COOH-terminal part of H4/H4' in both dimers swing apart, exposing hydrophobic residues of both helices and the hinge region to the surface, facilitating their Ca\(^{2+}\)-dependent interaction with target molecules. S100A1 COOH terminus (S100A1ct) is indicated by the dashed red box. Reproduced with modifications from Ref. 43 (Wright NT et al., J Mol Biol 353: 410–426, 2005).
Invited Review

S100A1 REGULATION OF CARDIAC PERFORMANCE

CARDIAC S100A1: A Ca\textsuperscript{2+}-DEPENDENT REGULATOR OF CARDIomyocyte EC COUPLING

Gain- and loss-of-function studies comprehensively characterize S100A1 as a molecular inotrope in vivo and ex vivo. S100A1 is a key factor in cardiac EC coupling and is critical for cardiac inotropic reserve. Strong evidence for this notion stems from studies demonstrating that chronic or acute elevation of S100A1 protein levels in cardiomyocytes by the means of gene or protein delivery enhances systolic and diastolic performance in vivo and ex vivo primarily through modulation of cellular Ca\textsuperscript{2+} handling and, potentially, myofilament function (14, 21, 23, 25, 27, 29, 32, 33, 42, 44) (Fig. 2).

Lessons from in vivo and ex vivo cardiac S100A1 gain-of-function studies. Cardiac-specific overexpression of S100A1 in transgenic mice (STG) displayed a phenotype of chronically improved cardiac performance without development of cardiac hypertrophy and failure or detrimental effects on survival (25). Notably, the in vivo gain of basal cardiac function in STG mice was independent of heart rate and was preserved under sympathetic stimulation. Subsequent ex vivo functional analysis exhibited enhanced Ca\textsuperscript{2+} transients and contractile performance in isolated STG ventricular cardiomyocytes, suggesting that the hypercontractile state in vivo is provoked through improved Ca\textsuperscript{2+} fluxes at the level of the cardiomcyocyte. Expression analysis showed that this effect does not depend on altered abundance or phosphorylation status of major sarcoplasmic and sarcolemmal Ca\textsuperscript{2+} regulators in the heart, respectively (21–27). Biochemical studies further demonstrated that S100A1 inotropic effects neither rely on nor involve components of the β-adrenergic receptor (β-AR) signaling system (21–30). Strikingly, as with transgenic manipulation in mice, adenosine-mediated S100A1 gene delivery to hearts and isolated ventricular cardiomyocytes of rats and rabbits in vivo and ex vivo also provided a hypercontractile phenotype based on enhanced Ca\textsuperscript{2+} cycling but independent and additive to β-AR signaling (21, 23, 25, 29, 32, 33).

Thus elevated S100A1 protein levels in cardiomyocytes evoke a cardiac phenotype of sustained inotropy, which is essentially based on enhanced Ca\textsuperscript{2+} cycling. This is important to note because chronic inotropic stimulation of the heart, i.e., through sustained activation or genetic manipulation of cardiac β1-ARs (7) and downstream effectors such as cAMP-dependent protein kinase A (PKA) (1), respectively, eventually lead to cardiac hypertrophy with transition to failure. Moreover, alterations of cytosolic Ca\textsuperscript{2+} fluxes, i.e., through enhanced sarcolemmal Ca\textsuperscript{2+} fluxes via the L-type Ca\textsuperscript{2+} channel, can evoke Ca\textsuperscript{2+}-dependent hypertrophic signaling involving activation of protein kinase C (PKC), calmodulin dependent kinase II (CaMKII), or calcineurin (PP2B) (36). However, S100A1-mediated inotropy is independent of β-AR signaling, and S100A1-provoked cytosolic Ca\textsuperscript{2+} fluxes seem to bypass Ca\textsuperscript{2+}-dependent hypertrophic pathways. This supports the notion that S100A1- and β-AR-mediated inotropy differently involve cardiomyocyte Ca\textsuperscript{2+} cycling and might target a diverse subset of Ca\textsuperscript{2+} effectors.

Molecular targets and effects involved in S100A1 regulation of cardiac Ca\textsuperscript{2+} cycling. Subsequent studies therefore focused on mechanisms underlying S100A1 inotropic actions and provided a comprehensive molecular concept showing that intracellular S100A1 targets SR but not transsarcolemmal Ca\textsuperscript{2+}...
Fig. 2. Mechanisms conveying S100A1 inotropic actions in ventricular cardiomyocytes. A: normal excitation-contraction (EC) coupling in a nonfailing cardiomyocyte requires action-potential-dependent systolic opening of the L-type calcium channel (LLC) (1), enabling a transsarcolemmal Ca\(^{2+}\) entry, triggering sarcoplasmic reticulum (SR) Ca\(^{2+}\) release via the ryanodine receptor 2 (RyR2) (2) that in turn activates myofilament cross-bridge cycling and force development (3). During diastole, SR Ca\(^{2+}\) resequestration occurs through the SR Ca\(^{2+}\)-ATPase 2a (SERCA2a) (4), allowing Ca\(^{2+}\) dissociation from myofilaments and relaxation to occur. Under steady-state conditions, the sodium-calcium exchanger (NCX) exerts balanced extrusion of LLC-mediated Ca\(^{2+}\) influxes and reentry (5). S100A1 interacts both with RyR2 and the SERCA2a-phospholamban (PLB) complex and is present at myofilaments and in mitochondria. B: proposed mechanistic model for S100A1 actions in cardiomyocytes. Increased cardiomyocyte S100A1 protein levels result in enhanced EC coupling gain and systolic SR Ca\(^{2+}\) release through enhanced RyR2 but not LLC activity (a). Myofilament compliance is increased via S100A1-titin interaction (b) accompanied by facilitated diastolic Ca\(^{2+}\) dissociation independent of protein kinase A (PKA) (c). Augmented systolic SR Ca\(^{2+}\) release is balanced by enhanced diastolic SERCA2a activity independently of protein kinase A (PKA) and calmodulin kinase II (CaMKII) (d). Enhanced SERCA2a activity, together with diminished diastolic RyR2 activity (e), result in increased SR Ca\(^{2+}\) resequestration and prevent SR Ca\(^{2+}\) leak, whereas S100A1 does not affect diastolic Ca\(^{2+}\) extrusion through the NCX. Finally, S100A1 interaction with F1-ATPase is associated with enhanced ATP generation (f). Insets: net effect of enhanced cardiomyocyte S100A1 protein levels evoking augmented cytosolic Ca\(^{2+}\) transients and force generation.

The first evidence for this notion came from numerous experiments demonstrating a Ca\(^{2+}\)-dependent interaction of S100A1 both with RyR2 (14, 23–25, 42) and the SERCA2a-PLB complex (14, 15, 23, 24), which is in line with its location at the junctional and longitudinal SR (10, 12, 23, 24). Molecular approaches identified the S100A1 COOH terminus (S100A1ct, amino acids 75-94) as a critical structure for its interaction both for S100A1 actions in cardiomyocytes. On the basis of these results, molecular structure-function relationship of important S100A1 targets in cardiac muscle. On the basis of these results, several studies explored the impact of S100A1 both on transsarcolemmal and SR Ca\(^{2+}\) fluxes in adult ventricular cardiomyocytes and consistently found that S100A1 increases systolic and diastolic performance through enhanced Ca\(^{2+}\)-induced SR Ca\(^{2+}\) release (CICR) (14, 23, 25, 27) and augmented SR Ca\(^{2+}\) resequestration (14, 21, 22, 24, 25, 27, 32). These effects are due to enhanced systolic RyR2 and diastolic SERCA2a activity through S100A1, thereby providing a molecular mechanism for enhanced balanced SR Ca\(^{2+}\) fluxes. Importantly, Kettlewell et al. (14) provide compelling evidence that intracellular S100A1 actions occur independently of transsarcolemmal Ca\(^{2+}\) fluxes. S100A1 neither altered L-type Ca\(^{2+}\) current (\(I_{\text{Ca,L}}\)) nor sodium-calcium exchanger (NCX) reverse or forward mode, respectively, in adult ventricular cardiomyocytes (14). Similar results were found for intracellular S100A1 in neonatal ventricular cardiomyocytes (23). Hence, these findings strongly support the notion that S100A1 specifically targets SR Ca\(^{2+}\) cycling, providing a mechanistic concept for improved cardiac contractile performance seen in vivo and ex vivo (Fig. 2). These results are also in line with the observation that S100A1-regulated Ca\(^{2+}\) cycling does not evoke cardiac hypertrophy, because the S100A1 Ca\(^{2+}\) sensor apparently does not enhance subsarcolemmal Ca\(^{2+}\) fluxes participating in the activation of Ca\(^{2+}\)-dependent hypertrophic cardiac growth.

Of note, studies have also suggested that S100A1 actions are not only confined to modulation of systolic RyR2 activity but also affect diastolic RyR2 activity and SR Ca\(^{2+}\) leak (24, 27). Voelkers et al. (42) most recently strengthened this notion by demonstrating S100A1 interaction with RyR2 even at diastolic [Ca\(^{2+}\)], thereby decreasing Ca\(^{2+}\) spark frequency and diastolic...
SR Ca\(^{2+}\) leak. Thus improved diastolic SR Ca\(^{2+}\) storage through decreased SR Ca\(^{2+}\) leakage in combination with enhanced SR Ca\(^{2+}\) reuptake most likely potentiates S100A1 stimulatory effects on systolic CICR from the SR. Moreover, a regulatory effect of S100A1 on skeletal muscle ryanodine receptor isoform (RyR1) activity has previously been described (8, 26, 40) that results in enhanced SR Ca\(^{2+}\) release and contractile function in skeletal muscle fibers. Because Treves et al. (40) identified three different epitopes in the cytoplasmic portion of the RyR1, enabling S100A1 binding at different [Ca\(^{2+}\)], it is tempting to speculate that S100A1 might also assemble with the RyR2 through more than one binding site, thereby providing a potential molecular clue for its biphasic effect on RyR2 function. Moreover, it is currently unclear whether the interaction between S100A1 and the RyR2 is accomplished through direct binding or requires adaptor proteins. Further studies mapping the binding domains in both proteins and the potential interference with other modulatory proteins of RyR2, such as the 12.6-kDa FK-506 binding protein (FKBP12.6) or sorcin, have to address this important issue. At least under diastolic conditions, a previous study ruled out that S100A1-RyR2 interaction altered RyR2 stoichiometry with FKBP12.6 and sorcin (42), rather suggesting a direct effect of S100A1. Moreover, given the redox sensitivity of RyR2 function (19), it is tempting to speculate that NO-dependent posttranslational modification of S100A1 might mechanistically contribute to enhanced CICR, i.e., through transfer of NO moieties to the RyR2. However, irrespective of the precise molecular mechanism, S100A1 acts as a biphasic modulator of RyR2 activity, participating in enhanced cardiac EC coupling gain.

The detailed molecular mechanisms underlying S100A1 enhancement of SR Ca\(^{2+}\) resequestration and SERCA2a activity, respectively, have not been explored yet, although the S100A1ct (amino acids 75-94) was able to reproduce functional effects of the native S100A1 protein on SERCA2a activity (24). This is important to note, because each S100A1 monomer opens up to accommodate a target protein on Ca\(^{2+}\) binding by exposing hydrophobic residues, including the COOH terminus, to the surface (34, 47). On the basis of its antiparallel composition, it has been suggested that the S100A1 homodimer might function as a molecular clamp, binding target proteins on opposite sides (34, 47). By this mechanism, S100A1 dimers might either functionally cross-link or separate homologous or heterologous multimeric target proteins such as the homotetrameric RyR2 or the heterodimeric SERCA2a-PLB complex, respectively (Fig. 3). Interposition of the S100A1 homodimer between SERCA2a and PLB might attenuate the inhibitory effect of PLB on SERCA2a, resulting in enhanced SR Ca\(^{2+}\) uptake. In this regard, it also important to point out that S100A1 does not affect the serine 16 nor threonine 17 PLB phosphorylation state (5, 21, 25), suggesting that neither PKA nor CaMKII are involved in S100A1 actions at the SR. Future studies will have to address the S100A1 structure-function relationship in recognition and modulation of cardiac target protein activity in more detail.

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**Fig. 3.** Ca\(^{2+}\)-dependent interactions of S100A1 with cardiac target proteins. Proposed simplified model for Ca\(^{2+}\)-dependent interaction of S100A1 with cardiac target proteins, i.e., RyR2 and SERCA2a-PLB, is shown. A: at low Ca\(^{2+}\) concentrations, S100A1 resides in the apo state, disabling Ca\(^{2+}\)-dependent interaction with, for example, RyR2 and SERCA2a-PLB. Removal of cytosolic Ca\(^{2+}\), i.e., through NCX Ca\(^{2+}\) efflux or SR Ca\(^{2+}\) uptake, results in dissociation of Ca\(^{2+}\) and target proteins from S100A1, returning in its apo state. B: on increased cytosolic Ca\(^{2+}\) concentrations, i.e., through L-type Ca\(^{2+}\) influx or SR Ca\(^{2+}\) release, S100A1 binds Ca\(^{2+}\) and undergoes a conformational change that modifies the hydrophobic surface properties. This allows the protein to interact with, for example, RyR2 and SERCA2a-PLB to modulate target protein activity. Because of its antiparallel formation, S100A1 might functionally crosslink (i.e., RyR2) or separate (i.e., SERCA2a-PLB) homo- or heteromultimeric target proteins, thereby altering their functional state.
Molecular targets and effects involved in S100A1 modulation of cardiac myofilaments and mitochondrial function. Inotropic actions of S100A1 are apparently not confined to the SR, because additional molecular effects for S100A1 have been proposed, including regulation of myofilament Ca²⁺ sensitivity (21) and compliance (44). In accord with its sarcomeric location, Ca²⁺-dependent interaction between S100A1 and the PEVK subdomain of cardiac titin has been shown to result in improved sarcomeric compliance (44). At a molecular level, Granzier and co-workers (44) propose S100A1 to inhibit cardiac titin-actin interaction, providing the sarcomere with a mechanism to free the thin filament from titin before active contraction and thereby reduce titin-based passive tension. In addition, S100A1 has been shown to reduce Ca²⁺ sensitivity of myofilaments without affecting maximal force development (21) and the phosphorylation state of troponin I (5, 25). Thus, like regulation of SR function, S100A1 modulation of myofilament function appears to be independent of PKA. S100A1-mediated reduction in sarcomeric Ca²⁺ sensitivity might in turn facilitate diastolic Ca²⁺ dissociation from myofilaments and thereby support cardiac relaxation. Thus the overall effect of S100A1-mediated reduction of systolic titin spring constant and modulation of myofilament Ca²⁺ responsiveness might add to the augmentation of myocardial inotropy and lusitropy so that the net effect of S100A1 results in enhanced contractile force development and accelerated relaxation.

Because S100A1 is also found in cardiac mitochondria (2, 10), its actions might also extend into cardiac energy homeostasis. Börries et al. (2) provided compelling evidence for S100A1 to be located within cardiac mitochondria by applying immunofluorescence and immunoelectron microscopy. There, S100A1 directly interacts with the α- and β-chains of the mitochondrial F₁-ATPase in a Ca²⁺- and pH-dependent manner, resulting in enhanced activity of the ATP-generating enzyme at physiological pH (2). Accordingly, enhanced and decreased S100A1 protein concentrations resulted in a reciprocal energetic phenotype in ventricular cardiomyocytes displaying augmented and diminished ATP levels, respectively, both under basal conditions and in response to β-AR agonists (2). Thus it is tempting to speculate that S100A1 couples cardiac Ca²⁺ cycling with Ca²⁺-dependent mitochondrial energy production, because it regulates SR Ca²⁺-ATPase on the one hand and the mitochondrial F₁-ATPase on the other.

Overall, a striking body of evidence strengthens the notion that S100A1 functions as a Ca²⁺-dependent molecular inotrope, conveying its inotropic actions through balanced improvement of SR Ca²⁺ fluxes and modulation of sarcomeric function. In addition, our data support the notion that S100A1 actions extend into regulation of cardiac energy production and, potentially, its adaptation to cardiac work.

Lessons from in vivo and in vitro cardiac S100A1 loss-of-function studies. Strong additional support for this mechanistic picture comes from the cardiac phenotype of S100A1-deficient mice. Although heterozygous and homozygous S100A1 knockout (SKO) mice showed unaltered in vivo baseline cardiac function and heart rate, they displayed deficiencies in vivo contractile function in response to β-AR stimulation and enhanced transsarcomemal Ca²⁺ influx (5). This is important to note because heterozygous SKO hearts, with only 50% of S100A1 protein levels found in wild-type hearts, exhibit an identical defective phenotype to homozygous SKO hearts with complete lack of S100A1 expression. Thus >50% of normal left-ventricular S100A1 protein levels are required for cardiac adaptation to acute hemodynamic stress. Of note, loss of β-AR-dependent inotropy in SKO mice occurs despite regular β-AR signaling ranging from unaltered β-AR density to proper PKA-dependent target phosphorylation, thereby indicating that β-AR-mediated positive inotropy essentially relies on normal S100A1 protein levels in the heart. This is important because our current molecular understanding of β-AR stimulation effects increased function of target proteins following enhanced phosphorylation through PKA. The anticipated net effect is to increase the amplitude of the Ca²⁺ transient that in turn increases the strength of contraction. However, the reciprocal phenotypes of STG and SKO hearts reviewed here profoundly challenge this concept and expand our molecular knowledge of cardiac inotropy. Obviously, effective β-AR signaling in the heart requires an additional S100A1-dependent molecular switch beyond PKA-dependent phosphorylation of target proteins (Fig. 4).

Functional exploration of isolated SKO cardiomyocytes revealed blunted Ca²⁺ transients in response to sympathetic stimulation and increased extracellular [Ca²⁺] (27). Similar results were obtained in ventricular cardiomyocytes, with a S100A1 protein knockdown to 20–30% of control protein levels (2). Thus defective mobilization of Ca²⁺ from intracellular stores might provide a reasonable mechanism for the impaired inotropic reserve in S100A1-deficient and -depleted cardiomyocytes. In support of this, SKO ventricular cardiomyo-

![Image](https://ajpregu.physiology.org/doi/fig/10.1152/ajpregu.00124.2006)
ocytes show enhanced $I_{c,a,L}$ (Most P, unpublished data), suggesting desensitized CICR under basal conditions that is compensated by increased $I_{c,a,L}$. However, further studies will have to precisely address the question of whether SKO ventricular cardiomyocytes compensate with enhanced transsarcomemal Ca$^{2+}$ for desensitized RyR2 activity to maintain cellular Ca$^{2+}$ cycling.

Overall, several important conclusions and hypotheses can be drawn from these studies: 1) S100A1 is a molecular inotrope in cardiomyocytes exerting a cAMP-independent enhancement of cardiac Ca$^{2+}$ cycling; 2) S100A1 acts beyond and independently of PKA, but effective β-AR-mediated inotropy critically relies on S100A1; 3) S100A1 specifically targets SR Ca$^{2+}$ fluxes, improving both systolic SR Ca$^{2+}$ release capability (enhances systolic RyR2 activity) and diastolic SR Ca$^{2+}$ storage capability (decreases diastolic RyR2 open probability and enhances SERCA2a activity); 4) synchronous enhancement of SERCA2a activity and decreased RyR2 activity in diastole provides a unique synergistic molecular mechanism that amplifies its inotropic actions but might prevent Ca$^{2+}$-triggered arrhythmias; 5) because $I_{c,a,L}$ and NCX are unaffected, S100A1 inotropic actions are mainly conveyed through enhanced CICR and EC coupling gain, respectively; 6) S100A1 modulation of cardiac titin function might adapt cardiac passive tension and sarcomere Ca$^{2+}$ sensitivity to improved Ca$^{2+}$ cycling, thereby facilitating systolic and diastolic cardiac performance; and 7) S100A1 might couple cardiac work with Ca$^{2+}$-dependent mitochondrial energy production.

However, despite these encouraging results, exploration of the role of S100A1 in cardiovascular physiology has just begun, and further studies are required to investigate its function in cardiac EC coupling and energy homeostasis. Moreover, it is important that we advance our molecular understanding of the structure-function relationship of S100A1 with cardiac target proteins that are critically involved in the regulation of Ca$^{2+}$ homeostasis and adjacent processes such as gene expression, cytoskeletal dynamics, and apoptosis.

DYSREGULATION OF S100A1 IN DISEASED myocardium: A KEY FACTOR IN HEART-FAILURE DEVELOPMENT

On the basis of its functions in nondiseased myocardium, the clinical significance of S100A1 in the cardiovascular system has become apparent, because failing human myocardium displays a marked loss of S100A1 expression at both the transcriptional and translational levels (31). A previous study (27) has addressed potential pathways involved in S100A1 dysregulation and has provided evidence for Gs-coupled receptor agonists (i.e., endothelin-1) and PKC to diminish cardiac S100A1 mRNA and protein abundance. Importantly, a recent proof-of-concept study (27) taking advantage of STG and SKO mouse models provided strong evidence that down-regulation of S100A1 protein critically contributes to the progressive contractile dysfunction of the diseased heart and cardiac-related death. The same study also supplies critical evidence that preservation of cardiac S100A1 protein levels can prevent the development of postischemic heart failure and cardiac remodeling, resulting in superior survival. The prevention of progressive loss of cardiac S100A1 expression after ischemic myocardial damage protected cardiomyocytes from the transition to contractile failure, whereas cardiac S100A1 deficiency tremendously accelerated progression to cardiac failure accompanied by excessive mortality (27).

Mechanistically, S100A1 preserved myocardial SR Ca$^{2+}$ cycling and therefore enabled the remote myocardium to maintain adequate cardiac output (27), presumably preventing enhanced wall stress and maladaptive sustained activation of neurohumoral systems such as the sympathetic and the renin-angiotensin-aldosterone system. The latter is reflected by abrogated expression of fibrotic genes, marked protection from apoptosis and hypertrophy, as well as prevention of β-AR desensitization through S100A1 (27). In contrast, accelerated loss of contractile function in S100A1-deficient hearts exacerbated the common maladaptive sequence of cardiac hypertrophy, accompanied by enhanced expression of fibrogenic functions and apoptosis, accelerated development of cardiac hypertrophy leading to premature failure (27), and potentially lethal arrhythmias.

Transaortic constriction (TAC) of homozygous SKO revealed immediate transition to contractile failure, in contrast to TAC wild-type control mice (WT), which showed functional compensation (5). Of note, both groups displayed similar hypertrophic cardiac growth, indicating that S100A1 is indispensable for functional compensation for chronic hemodynamic stress (5). However, heterozygous SKO subjected to TAC were able to normalize cardiac S100A1 protein levels, resulting in compensated function similar to TAC WT mice (5). Hence, these findings strengthen the notion of S100A1 as an indispensable factor in cardiac adaptation to cardiac damage and hemodynamic stress.

In addition, S100A1, like other S100 proteins, seems to exert extracellular actions that potentially contribute to its intracellular beneficial effects. Shortly after ischemic myocardial damage in humans, S100A1 appears in the serum, rising to 1–2 μM by 6–8 h after the clinical onset (41). Because S100A1 does not possess a classical secretion sequence but mimics serum kinetics of creatine kinase isoenzyme MB (41), its extracellular release most likely reflects ischemic disintegrity and necrosis of cardiac cells. This is important because extracellular S100A1 (1 μM) seems to exert direct antiapoptotic actions on ventricular cardiomyocytes through activation of the extracellular-regulated kinase 1/2 (p44/42) (22). However, further studies have to explore the in vivo significance and role of extracellular S100A1 protein in the context of myocardial damage in more detail.

Therefore, from a clinical point of view, preservation of cardiac contractility and output in the damaged heart through S100A1 inotropic actions appears as a key event protecting or even interrupting the onset of a maladaptive sequence, leading to pathological hypertrophy and cardiac failure. Our current understanding of S100A1-mediated antihypertrophic effects in particular is fragmentary, and it is imperative to conduct further studies on that subject.

S100A1 GENE THERAPY FOR heart failure: EXPERIMENTAL STATE-OF-THE-ART AND CLINICAL PERSPECTIVES

S100A1 might be a future prototype of a novel class of Ca$^{2+}$-modulating inotropes targeting SR function (both release and uptake). This notion is based on profound proof-of-concept for its therapeutic effectiveness in clinically relevant experi-
mental animal models of chronic and acute heart failure (24, 30, 28). Because of the pathophysiological relevance of altered cardiac S100A1 expression, adenoviral and adeno-associated viral (AAV) intracoronary S100A1 gene delivery to failing myocardium restored diminished S100A1 protein levels, resulting in short- and long-term heart-failure rescue (24, 28, 30) (Fig. 5). Mechanistically, restoration of diminished cardiac S100A1 protein levels through viral S100A1 gene transfer resulted in normalization of dysfunctional Ca\(^{2+}\) cycling (24, 28, 30) mainly via restoration of diminished SR Ca\(^{2+}\) content and SR Ca\(^{2+}\) release as well as through reversing the augmented SR Ca\(^{2+}\) leak in failing myocardium (24, 27). These mechanistic findings are in line with previously characterized effects of the Ca\(^{2+}\) sensor both at the level of the RyR2 and the SERCA2a-PLB complex in healthy myocardium (14, 21, 25, 27, 42). In turn, cardiac S100A1 gene therapy not only rescued contractile function but also restored several other defective systems, such as energy and sodium homeostasis, that are closely intertwined with cardiac Ca\(^{2+}\) cycling (24). Finally, most likely as a result of restored cardiac output, S100A1 gene therapy partially resensitized β-AR responsiveness and effectively reversed cardiomyocyte remodeling at the molecular and organ levels, potentially reflecting abrogated wall stress and an interrupted sympathetic drive (24, 28, 30).

From a clinical point of view, it is important to point out that AAV6-S100A1 gene therapy exerted superior therapeutic effects to the clinically established β-AR antagonist metoprolol (28). Metoprolol orally ingested at a therapeutically effective dosage prevented the progressive decrease in cardiac performance and reversed remodeling of infarcted hearts but failed to restore cardiac contractility. In contrast, AAV6-S100A1 cardiac gene therapy significantly restored cardiac performance and reversed cardiac hypertrophy without adverse effects. Most interestingly, the combination of a positive and a negative inotropic intervention in the form of application both of S100A1 and metoprolol neither negatively interfered with nor neutralized each other, supporting the notion that established clinical drugs can be beneficially combined with emerging novel molecular therapies.

**CONCLUSION**

Previous studies have comprehensively characterized S100A1 as a Ca\(^{2+}\)-dependent molecular inotrope with a key role in cardiac EC coupling. At a molecular level, S100A1 apparently regulates both RyR2 and SERCA2a-PLB function, resulting in a balanced enhancement of SR Ca\(^{2+}\) release and uptake and thereby facilitating a heightened contractile state. Cardiomyocyte S100A1 protein concentrations therefore seem to critically determine the responsiveness of cardiac Ca\(^{2+}\) cycling to trigger contractile force, in particular to sympathetic stimulation, that ultimately relies on acute Ca\(^{2+}\) mobilization from the SR. Thus, unlike the β-AR cascade, S100A1 is involved in chronic rather than acute modulation of cardiac contractile performance, and sustained S100A1-mediated inotropy sharply opposes the detrimental cardiac phenotype of chronic sympathetic stimulation of the heart. From a pathophysiological point of view, this is essential to note, because current studies strengthen the notion that cardiac S100A1 protein levels occurring in nondiseased myocardium are at a critical threshold and are an indispensable key factor in the cardiac stress response both to ischemic damage and chronic hemodynamic overload.

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**Fig. 5.** Mechanisms conveying therapeutic actions of S100A1 in failing ventricular cardiomyocytes. A: failing ventricular cardiomyocytes displaying decreased S100A1 abundance show defective EC coupling characterized by impaired EC coupling gain, enhanced SR Ca\(^{2+}\) leak, and diminished SR Ca\(^{2+}\) resequestration. Further hallmarks are impaired β-AR responsiveness, increased intracellular sodium concentrations, and altered energy homeostasis. B: S100A1 gene therapy restores S100A1 protein levels and reverses defective Ca\(^{2+}\) cycling (a, b, c) that in turn rescues contractile performance, improving β-AR responsiveness (d), sodium homeostasis (e), and energy supply (f).
Dysregulation of S100A1 expression in diseased myocardium resulting in a progressive decrease of S100A1 protein accelerates decompensation and transition to failure under both aforementioned conditions. Preservation and restoration of S100A1 protein levels to (supra)normal levels in diseased myocardium therefore appear as a promising therapeutic strategy that has already been successfully addressed by means of viral cardiac S100A1 gene transfer. The profound beneficial effects of viral-based S100A1 gene transfer strengthen the notion that cardiac S100A1 gene therapy might become a clinical reality, complementing and adding to established drug regimens against heart failure.

Therefore, the attempt to elucidate the role of S100A1 in the context of normal cardiac function ultimately advanced to the exploration of its pathophysiological relevance in diseased myocardium. Subsequently, translational research aims to discover the therapeutic potential of S100A1 in the context of heart failure. Advancing both basic and translational research on S100A1 could potentially pioneer a clinical revival of inotropic therapeutic interventions targeting defective Ca\(^{2+}\) cycling, thereby providing a novel weapon to combat heart failure, a devastating disease reaching epidemic proportions worldwide.

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Invited Review

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