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A New Mechanism Links Preamyloid Oligomer Formation in the Myocyte Stress Response Associated with Atrial Fibrillation

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Atrial fibrillation remains the most common clinical cardiac arrhythmia, affecting 1-2% of the population [1-6]. It is characterized by uncoordinated depolarization of the atria in an intermittent (paroxysmal or persistent AF) or constant (permanent AF) fashion [7]. AF leads to impaired atrial contraction and is associated with increased morbidity and mortality [8]. Underlying this arrhythmia are specific electrical phenomena controlled by a diverse set of remodeling mechanisms. These electrical phenomena are reentrant waves, rapidly firing ectopic foci, and rotors which in combination can lead to initiation and maintenance of a fibrillating wave pattern [9]. Much of current research has focused on identifying remodeling mechanisms that develop an AF-prone substrate. These mechanisms can be generally grouped into three types: electrical, structural, and autonomic which have been reviewed in greater detail elsewhere [10-12]. Electrical remodeling includes alterations in K^+ currents, L-type Ca^{2+} currents, and gap junction function. Structural remodeling includes atrial fibrosis, size, and ultrastructure. Autonomic remodeling includes hyperinnervation of the atria and surrounding region as well as increased sympathovagal activity. Though research to date has revealed a detailed and complex view of remodeling in AF, the specific myocyte stressors that activate these remodeling mechanisms are less well understood. The factors contributing to initiation of AF include inflammation, cell death, oxidative stress, hypertrophy and fibrosis [10-12]. Human clinical studies as well as mouse models have provided strong evidence of AF secondary to cardiac disease, such as congestive heart failure (CHF) where many of these remodeling mechanisms are activated by the failing heart [13-15]. These mechanisms can also be activated by AF itself, i.e. “AF begets AF,” which leads to progressive worsening of the disease as the atrial substrate becomes more and more AF-prone [16]. In cases of AF occurring independent of other diseases, referred to as lone AF, an understanding of these triggering factors can be especially important. In the recent publication, “Reactive γ -Ketoaldehydes Promote Protein Misfolding and Preamyloid Oligomer Formation in Rapidly-Activated Atrial Cells,” Sidorova et al. identify a new

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molecular component that may link oxidative stress to the development of an AF-prone substrate [17]. Their study exploits a rapidly-paced atrial cell line model to mimic early AF stress responses in order to highlight a major role for oxidative stress pathways in atrial myocytes involving γ -ketoaldehydes (γ -KA) in the formation of preamyloid oligomers (PAO), which are soluble precursors to amyloid deposits.

PAO complexes refer to a diverse set of misfolded proteins grouped together by a common structural epitope linked to the conformation of the peptide backbone of PAOs [18, 19]. PAOs play an important role in disease pathogenesis across various organ types, with their most well known role in neurodegenerative disorders such as Alzheimer's disease [18, 20]. However, recent studies have highlighted a role for PAOs and amyloid deposits in the heart. Cardiac amyloidosis has previously been observed in systemic amyloidosis diseases and ischemic heart disease [21, 22]. The role of protein misfolding and amyloid oligomer formation in the setting of cardiac disease has also been more directly assessed by Sanbe et al. where a mutant/misfolded small heatshock protein alpha-B-crystallin (CryAB(R120G)), previously associated with desmin-related cardiomyopathy, was overexpressed in the mouse heart [23]. Transgenic mice overexpressing CryAB(R120G) exhibited a cardiomyopathy associated with desmin aggregates and increased PAO levels. A study by Pattinson et al. also showed that overexpression of an 83 amino acid polyglutamine preamyloid peptide, modeled after the Huntington's disease protein, leads to dilated cardiomyopathy and premature death [24], suggesting a direct causative link between PAOs and heart disease. Although little is known about the role of PAOs in development of AF, PAO levels can be detected in human atrial samples [25] and a small clinical study has shown a correlation between atrial amyloid deposits and AF [26], suggesting a potential role for PAOs in the development of AF.

The study by Sidorova et al. sheds light on a new molecular mechanism contributing to atrial myocyte injury and cell death that may play a role in AF [17]. Sidorova and colleagues exploit a rapidly-paced atrial cell line (HL-1) to investigate the connection between PAOs and oxidative stress in atrial myocytes. The authors show that rapid pacing is a trigger for oxidative stress in this system resulting in increased PAO levels. They further show the accumulation of a particular oxidative stress product, γ -ketoaldehydes, previously implicated in formation of PAOs in non-cardiac disease models, through their crosslinking activity [27, 28]. The authors specifically highlight that γ -ketoaldehydes may crosslink atrial natriuretic peptide (ANP) to subsequently form PAOs. A mechanism involving γ -ketoaldehydes was further validated in causing PAO formation in this model system by elegant experiments that involved using a specific scavenger (salicylamine) of γ -ketoaldehydes, which rescued/prevented PAO formation.

Beyond showing the importance of particular oxidative stress products in development of PAOs in atrial myocytes, the findings of this article suggest that PAO mechanisms may crossover between the brain and heart. Previous groups have shown the importance of γ -ketoaldehydes in Alzheimer's disease. Davies et al. observed increased levels of γ -ketoaldehyde adducts in the hippocampus of brains from patients with Alzheimer's disease [29]. The same group also showed that salicylamine treatment improved spatial working memory in a mouse model of dementia (hApoE4 mouse) [29]. Boutaud et al. have also

shown that incubation of γ -ketoaldehyde with amyloid- β (1-42), a neurotoxic peptide in Alzheimer's disease, increases amyloid- β (1-42) crosslinking and neurotoxicity [30]. Sidorova et al. have now demonstrated the effectiveness of scavenging γ -ketoaldehydes with salicylamine in a cardiomyocyte setting where the peptides forming PAOs are presumably different than a neurodegenerative setting [17]. Altogether these studies emphasize similarities in oxidative stress mechanisms between the brain and heart, which may be of value in future research in better understanding PAO biology as well as designing therapeutic approaches for PAO related diseases.

There is emerging clinical data to suggest that amyloid deposits are associated with AF [26, 31], however, it is unknown whether PAOs themselves or their downstream product, amyloid deposits, are the direct toxic player in AF. Proteotoxicity of PAOs has been demonstrated in neurodegenerative settings [18, 20, 32], therefore it will be important to determine whether PAOs versus amyloid deposits are causative for disease in the cardiac setting. PAOs have only recently been examined in human atria in a recent study by the same authors [25], though it remains unknown how their expression relates to myocyte injury or AF. Based on the suggested role of PAOs in myocyte injury by Sidorova et al., it will be important to examine the effects of this response in cardiac disease settings both *in vivo* in mice and humans. Currently, Congo red, a chemical dye, is used to assess amyloid deposits in the heart; however, it may be important to also include analysis of PAO levels, which can be accomplished using a specific antibody that detects the misfolded conformation [25]. Such experiments may begin to address the question of how PAO levels correlate with disease in comparison to amyloid deposits. These studies will also provide important insight into the mechanisms underlying PAO and amyloid deposit toxicity in the atria. Furthermore, Sidorova et al., show *in vitro* that PAOs can accumulate prior to formation of amyloid deposits [17], therefore it may be helpful to also measure their levels in patient hearts to determine whether PAOs accumulate in the absence of amyloid deposits and whether their levels can be used for predictive or diagnostic purposes in AF.

Another important unknown, concerns the particular peptides forming the PAOs and their role in disease mechanisms in the atria. Though the authors identify ANP as a potential PAO forming peptide which has previously been associated with amyloid bodies in the heart [33], western blot analysis highlights numerous protein bands that corresponds to an antibody that stains for either PAOs in general or proteins with γ -ketoaldehyde adducts. Thus, it remains to be proven which peptide or peptides are forming PAOs and, moreover, whether these PAOs are directly involved in the disease mechanisms underlying atrial disease. Previous studies have shown γ -ketoaldehydes can alter Na^+ channel function in HL-1 cells, though it is unknown if this is a direct or indirect effect [34]. It may be interesting to determine whether γ -ketoaldehyde adducts form on other proteins that are not forming PAOs. For example, a characteristic feature of AF is atrial fibrosis [10-12], therefore it would be of interest to determine whether γ -ketoaldehyde adducts or PAOs themselves can contribute to the fibrotic response in AF.

Within this model system, there also remains an important unanswered question on whether reducing PAO levels can reverse molecular defects associated with AF. It has previously been shown in a rapidly paced atrial cell system that molecular changes associated with

human AF can be observed, including reduced levels of L-type Ca^{2+} channels and increased calpain levels [35]. Sidorova et al. were able to reduce PAO levels as well as transcription of genes related to PAO formation using salicylamine, a γ -ketoaldehyde scavenger [17]. It will be interesting to determine whether salicylamine treatment is sufficient to prevent other more specific AF-associated molecular defects that can occur in this model system.

The findings of Sidorova et al. also have potential clinical implications. The authors present a model system in which increased rate of firing of atrial cells leads to oxidative stress leading to accumulation of PAOs and atrial myocyte injury [17]. A question this article raises is why rate control therapies for AF do not prevent worsening of the disease, since the excessive atrial rate is reversed [36]. If PAOs are the product of oxidative stress arising from increased atrial rates, there is a possibility that rate control drugs could effectively block worsening of AF. This line of reasoning, however, assumes that increased atrial rates are the only stimulus promoting AF *in vivo* which is unlikely to be the case, as there are numerous mechanisms that can contribute to AF aside from atrial rate [10-12]. An alternative approach lies in targeting an underlying or upstream mechanism such as the oxidative stress itself, though the data on targeting oxidative stress pathways for therapeutic effects in the setting of AF has been controversial. Free radical scavenger (tempol) treatment of angiotensin-II treated endothelial nitric oxide synthase deficient mice resulted in reduced atrial fibrillation and fibrosis [37]. In humans; however, approaches targeting oxidative stress (antioxidant therapy) and upstream pathways (renin-angiotensin-aldosterone system inhibitors) implicated to have actions on oxidative stress, resulted in patients showing little to no benefit for primary or secondary prevention of AF [38, 39]. These results may be tempered by the lack of information on whether oxidative stress (e.g., reactive oxygen species) levels were effectively blocked in the target tissues. For example, orally administered vitamin C cannot reach plasma concentrations high enough to scavenge superoxide ions, making it unlikely to confer positive effects in this particular system [40]. Along the same lines, vitamin E and fish oil treatments were also found to be ineffective at reducing antioxidant levels [41-43]. Renewed focus on improved delivery methods for antioxidants or targeting antioxidants of specific oxidative species, such as using salicylamine to scavenge γ -ketoaldehydes, offers a promising new approach that may prove to be more effective.

The myocyte stress response in AF remains a poorly understood process in spite of its importance in disease development and progression. Sidorova et al. present a potential link between a well-documented cardiac stressor, oxidative stress, and myocyte injury via production of reactive γ -ketoaldehydes and subsequent formation of PAOs [17], which may highlight a new structural player in AF (**Figure 1**). Based on the importance of oxidative stress in various cardiac diseases, such as AF, these finding suggest the importance of more closely examining γ -ketoaldehydes and PAOs in cardiac disease settings. A better understanding of PAO biology and the specific molecular players responsible for oxidative stress-related atrial myocyte injury may better focus future research and therapeutic approaches for AF.

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Figure 1.

Schemata of the mechanisms underlying atrial remodeling and fibrillation, which highlight a potential link between oxidative stress and atrial myocyte injury via production of reactive γ -ketoaldehydes and subsequent formation of preamyloid oligomers (PAOs) based on findings from Sidorova et al. Components of the PAO pathway and scavengers (salicylamine) are highlighted in the atria by solid lines. Dotted lines and arrows denote the potential link to atrial structural remodeling.