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Oxidized CaMKII: a “heart stopper” for the sinus node?

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Each normal heart beat is triggered by an electrical impulse emitted from a group of specialized cardiomyocytes that together form the sinoatrial node (SAN). In this issue of the *JCI*, Swaminathan and colleagues demonstrate a new molecular mechanism that can disrupt the normal beating of the heart: angiotensin II — typically found in increased levels in heart failure and hypertension — oxidizes and activates Ca²⁺/calmodulin-dependent kinase II via NADPH oxidase activation, causing SAN cell death. The loss of SAN cells produces an electrical imbalance termed the “source-sink mismatch,” which may contribute to the SAN dysfunction that affects millions of people later in life and complicates a number of heart diseases.

The electrical impulses that trigger the heart to beat originate from a group of specialized cardiomyocytes that together form the sinoatrial node (SAN). The SAN is a complex anatomical structure located in the wall of the right atrium, near the entrance to the superior vena cava. The SAN triggers billions of heart beats during an individual's lifetime. Neurohormonal regulation of the SAN allows us to adapt our cardiac output to precisely match life's rapidly changing demands: cardiac out-

put is reduced during times of rest and increased during physical and emotional exercise. Not surprisingly, SAN dysfunction (SND) affects millions of individuals later in life; it also complicates a number of heart diseases. While a large body of work has elucidated the molecular signaling processes that regulate physiological pacemaking, much less is known about the molecular signaling that causes SND (1). SND is characterized by physiologically inappropriate heart rates, most often sinus bradycardia (a regular but abnormally slow heart rate), and the only currently available treatment option is implantation of an electrical pacemaker. The typical patient is elderly and presents with additional cardiac

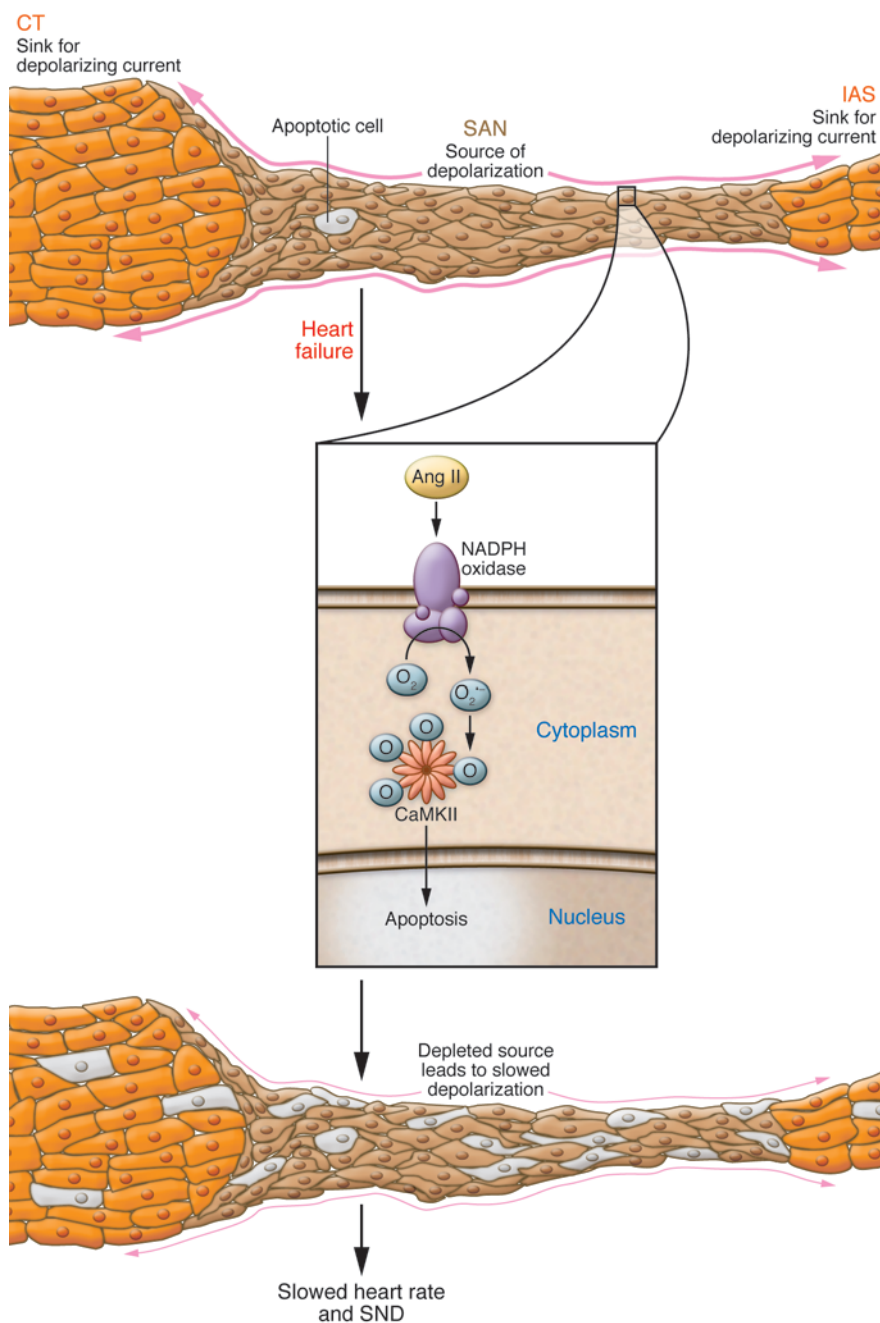
pathology of an ischemic, inflammatory, or degenerative nature. SND also frequently occurs when an individual develops heart failure. Histological studies demonstrate a loss of SAN cells and increased fibrosis in SAN tissue obtained postmortem (2, 3), suggesting that cell death and tissue remodeling importantly contribute to SND. In this issue of the *JCI*, Swaminathan and coworkers elegantly combine studies in mice and human tissue to demonstrate a molecular chain reaction that can cause SND (4). The study demonstrates a heretofore unrecognized molecular mechanism responsible for SND and provides a clear target for developing new treatments aimed at preventing SND in the future.

Ang II-induced Ca²⁺/calmodulin-dependent kinase II oxidation causes SAN cell death and SND

Anderson and colleagues have shown previously that Ang II induces myocardial dysfunction and heart failure at least in part via myocyte apoptosis (5). They recognized at that time that inhibition of Ca²⁺/calmodulin-dependent kinase II (CaMKII) provided protection against myocyte cell

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**Figure 1**

Mechanism of Ang II-induced SND. Normally, the small volume of excited tissue in the SAN (source) depolarizes the neighboring quiescent atrial tissue (sink). In conditions with increased Ang II, NADPH oxidase is activated, leading to oxidation of two methionine residues of CaMKII, rendering the enzyme autonomously active. Elevated activity of CaMKII leads to SAN cell death, reducing the threshold volume of automatic cells of the SAN and increasing non-excitable tissue in the form of fibrosis. This increased electrotonic loading produces a source-sink mismatch (explained in detail in the text), slows the beating rate, and causes SND. CT, crista terminalis; IAS, inferior atrial septum.

mally. Under normal circumstances, SAN cells steadily depolarize until an action potential is triggered. Cardiomyocytes contract synchronously because SAN cells are coupled to neighboring cells by intercellular gap junction channels that connect the cytoplasm of the cells. The heart beat frequency depends not only on intrinsic regulation of the depolarizing currents, but also on the total number of coupled SAN cells that form a critical mass needed to allow for SAN depolarization despite the electrotonic clamping by adjacent atrial tissue (which acts as the “sink” for the depolarizing current). Loss of SAN cells therefore reduces the collective “source” of the current, and it takes longer before depolarization reaches the threshold required to trigger an action potential. Interruption of SAN cell-to-cell coupling as a result of the presence of fibroblasts and/or potential SAN cell coupling to non-excitable fibroblasts has a similar effect. As a result of both, SAN depolarization is slowed, and triggering of an action potential may be delayed, thereby slowing the heart rate. Even more severe loss of SAN cells ultimately renders the SAN incapable of exciting atrial tissue, and normal beating of the heart ceases.

oxCaMKII: molecular trigger or innocent bystander of SND in heart failure?

It is well recognized that CaMKII is a versatile signaling molecule with many important substrates in excitable tissue such as the heart (6). Its unique properties make it attractive to postulate that CaMKII is involved in many disorders (7). For example, it is activated by calcium/calmodulin, so it might be involved in diseases with altered calcium homeostasis

death, as did prevention of ROS production by NADPH oxidase. Logically, they then reasoned that specialized small structures such as the SAN should be more susceptible to loss of function by cell death and investigated the role of CaMKII in SND. In this issue of the *JCI* (4), Swaminathan, Anderson, and colleagues report that SND in humans with heart failure is indeed associated with higher levels of oxidized CaMKII (oxCaMKII) and confirm this finding in a dog model of pacing-induced heart failure. To then study

the role of oxidative stress and CaMKII in the development of SND, they performed Ang II infusion for 3 weeks to induce SND in mice. The data derived from the Ang II-perfused mice together with additional mathematical modeling support a plausible molecular mechanism for SAN cell death, subsequent fibrosis, and SND due to an electrical imbalance termed the “source-sink mismatch” (Figure 1).

To understand the source-sink mismatch, it is important to consider what happens when the SAN functions nor-



(such as heart failure); its activation can be prolonged by autophosphorylation, so it might be involved in diseases with altered phosphatase activity (again such as heart failure; ref. 8); and its activation is also prolonged by oxidation of methionine residues, so it might be involved in diseases with increased oxidative stress (such as myocardial infarction). The current report by Swaminathan et al. (4) now ties oxidation, and thus prolonged activity of CaMKII, to the development of SND. Strictly speaking, the results presented allow for only two conclusions: ROS is involved in Ang II-induced SND; and CaMKII inhibition in the SAN prevents, or at least delays, Ang II-induced SND. However, whether oxCaMKII really is the “toxic” species that leads to SND still needs to be elucidated. ROS may activate CaMKII simply by raising the intracellular concentration of Ca^{2+} (e.g., via oxidation and dysregulation of ryanodine receptor 2 [RyR2]; ref. 9). In general, it seems still unclear how significant activation of CaMKII via the oxidation pathway is under physiological conditions. Western blots with epitope-specific antibodies only detect relative levels, and it is impossible to conclude whether 100% signal increase is a rise from 0.1% to 0.2% (likely irrelevant) or 5% to 10% (possibly relevant) of total CaMKII. While technically more difficult, it would be preferable to quantify the amount of oxCaMKII as percentage of total in absolute, not relative terms.

Regardless of whether oxCaMKII is the molecular trigger for SND, the work of Swaminathan et al. clearly demonstrates that CaMKII inhibition is effective in preventing the development of SND due to high Ang II levels (4). The authors not only demonstrate this using a genetic mouse model with global CaMKII inhibitor expression, but they also use a gene therapy approach to target CaMKII inhibition to the SAN via local application of recombinant DNA (a process termed “gene painting”) and find that it prevents SND. CaMKII is clearly an important mediator that is crucial during the induction of apoptosis by many interventions (10–15). Thus, activation of CaMKII may be a common signaling step in response to a variety of triggers, and CaMKII inhibition may prove universally effective in preventing SAN apoptosis and the development of SND.

Treatment of SND: time for change?

Current treatment for SND consists of implanting an electrical pacemaker, an effective and time-tested therapy for millions of patients with heart rates that are too slow. Does the work by Swaminathan et al. (4) suggest it is time for change in clinical practice? Probably not immediately, for the following reasons. First, targeting the Ang II/oxCaMKII pathway therapeutically would only be expected to slow down the progression of SAN cell death, and not treat established SND. Second, heart failure patients at high risk for developing SND are already treated with ACE inhibitors and/or angiotensin receptor blockers (ARBs), given the seminal studies in the 1990s demonstrating improved survival with those agents (16–18). It would be interesting to go back and determine whether these agents also prevented SND. Of note, Swaminathan et al. report that oxCaMKII is increased in atrial tissue of heart failure patients with SND, even though four of five patients were already treated with ACE inhibitors (4). This result suggests that CaMKII can be oxidized and/or activated independently of the Ang II pathway in heart failure.

Regardless of the mode of CaMKII activation, the work of Swaminathan et al. convincingly demonstrates the efficacy of oxCaMKII inhibition for preventing SND in mice (4). The findings establish a new molecular mechanism of SND, an area that is ripe for clinical studies once a small molecule inhibitor of CaMKII has been developed. While promising, it remains to be tested whether CaMKII inhibition will be effective in preventing SND in humans, where the etiology is multifactorial. For the time being, one might consider starting ACE inhibitors or ARBs earlier in patients at risk for heart failure in order to prevent the development of SND and thereby reduce the need for pacemakers.

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