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EDITORIAL

Cardiac Na/Ca Exchange Suppression: A Late-Breaking Knockout Story Showing That There Is No Free Lunch

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At each heartbeat, Ca^{2+} enters the cardiac myocyte via L-type Ca current (I_{Ca}), triggering additional Ca^{2+} release from the sarcoplasmic reticulum (SR) via ryanodine receptors. In the steady state, the SR Ca pump must resequester the exact amount of Ca^{2+} that was released and the Na/Ca exchange (NCX) is almost entirely responsible for extruding all of the Ca^{2+} that entered, mainly by I_{Ca}.[1,2] Although NCX is critical for this Ca^{2+} flux balance and preventing acute cellular Ca^{2+} overload, digitalis-induced inotropy takes advantage of this relationship to raise myocyte Ca^{2+} by inhibiting the Na^+/K^+-ATPase to increase intracellular $[Na^+]$, which limits Ca^{2+} extrusion via NCX. The downside of this Ca^{2+} loading is that it can cause arrhythmogenic diastolic Ca^{2+} leak and NCX itself carries the transient inward current that causes delayed afterdepolarizations (DADs), which can trigger ectopic action potentials (APs) and tachyarrhythmias, especially in heart failure.[3]

See Article by Lotteau et al.

The critical role of NCX for cardiac myocyte Ca^{2+} extrusion made it a complete surprise that the initial cardiac-specific NCX1 knockout mouse was viable into adulthood.[4] Although these NCX1-knockout (KO) mice did not fare well long-term, follow-up studies revealed remarkable developmental adaptations to limit Ca^{2+} influx via Ca^{2+} current, without apparent upregulation of the plasma membrane Ca^{2+}-ATPase (the only other known Ca^{2+} extrusion mechanism)¨. Three key factors limited Ca^{2+} influx in these NCX-KO myocytes: (1) reduced AP duration (already short in mouse) mediated by (2) an increased transient outward K^+ current (I_{to}), thereby abbreviating I_{Ca} duration, and (3) decreased I_{Ca} density that appeared to be attributable to local elevation of $[Ca^{2+}]_i$ in the junctional cleft and Ca^{2+}-dependent inactivation of Ca^{2+} channels.[5]

INDUCIBLE NCX1 KNOCKDOWN, HOMEOSTATIC COMPENSATIONS, AND PROTECTIVE EFFECTS

In this issue of the *Journal of the American Heart Association* (*JAHA*), Lotteau et al[6] circumvent the apparent developmental adaptations by acute knockdown of NCX with a tamoxifen-sensitive Mer-Cre-Mer promoter, allowing inducible knockdown by breeding with NCX1 exon 11 floxed mice. Intraperitoneal administration of hydrotamoxifen (40 mg/kg) resulted in NCX

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protein expression knockdown by 95% in 1 week and 98% in 4 weeks, allowing assessment of early and later adaptations in adult hearts. At 1 week, with 95% of NCX gone, there was no change in heart weight, ejection fraction, fibrosis, AP duration, or I_{to}, but already a reduction in I_{Ca} and increases in myocyte diastolic and systolic $[Ca^{2+}]_i$, Ca^{2+} waves, and Ca^{+}-calmodulin dependent protein kinase II (CaMKII) activation, as well as an increase in plasma membrane Ca^{2+}-ATPase expression. However, the increased Ca^{2+} waves did not increase DADs or spontaneous APs, consistent with the role of NCX in mediating DADs and triggered APs and thus their suppression in the NCX KO.

During the next 3 weeks, these NCX-KO mice develop cardiac and myocyte hypertrophy, interstitial fibrosis, increased $I_{to,}$ and a virtual abolition of DADs in parallel with the disappearance of the observable caffeine-induced myocyte NCX current. In addition, both 1 and 4 weeks after NCX knockout, the hearts were partially protected from the damage caused by reperfusion after a 20-minute period of global ischemia, with infarct size reduced by ≈50% in the 4-week group. That also makes mechanistic sense, because the increase in $[Na^{+}]_i$ during ischemia and rapid recovery of intracellular pH during reperfusion are known to cause dramatic acute myocyte Ca^{2+} overload mediated by $[Na^{+}]_i$-dependent Ca^{2+} influx via NCX.

This all sounds really great! But even without any ischemic challenge, the NCX-KO mice start dying rapidly 5 weeks after tamoxifen, and 75% die in the next 5 weeks. So what is going on? Reducing NCX prevents some major pathophysiological problems, arrhythmogenic DADs, and ischemia-reperfusion injury, so why do the mice end up dying?

THE DARK SIDE OF NCX KNOCKDOWN

The results in this inducible NCX model are interesting in revealing how effectively the heart compensates for the loss of a major physiological player, but how in the end there is still a major price to pay. Reduced NCX function prevents cellular Ca^{2+} extrusion, tending to cause cellular Ca^{2+} overload. The latter can easily cause cell death, so the body rapidly brings to bear major and effective compensations. In the study by Lotteau et al, the compensations included reduced I_{Ca} attributable to Ca^{2+}-dependent Ca^{2+} channel inactivation and reduced AP duration, likely attributable to K^{+}-current upregulation (both of which decrease Ca^{2+} entry) on one hand; and increased removal of cytosolic Ca^{2+} via enhanced SR uptake through upregulation of the SR Ca^{2+}-ATPase and enhanced transport to the extracellular space across the sarcolemma via the plasma membrane Ca^{2+}-ATPase pump.[6] However, these compensations cannot fully prevent the consequences of cellular Ca^{2+} loading, which they only partly offset. One week after tamoxifen administration, both diastolic and systolic cytosolic $[Ca^{2+}]$ are elevated. Three weeks later, at 4 weeks after tamoxifen, cytosolic $[Ca^{2+}]$ is no longer elevated, but SR Ca^{2+} content is now increased: presumably, more effective SR Ca^{2+} uptake removes excess Ca^{2+} from the cytosol, but at the cost of increased SR Ca^{2+} loading.

Nuclear Ca^{2+} content is a key regulator of cardiac gene transcription and driver of adverse remodeling.[7,8] A key regulator of nuclear Ca^{2+} content is the nuclear envelope, a double-bilayer structure that is in direct continuity with the SR.[9] It is therefore highly likely that the compensatory SR Ca^{2+}-ATPase upregulation and SR Ca^{2+} loading that cannot be extruded without NCX function result in nuclear Ca^{2+} loading and the activation of remodeling programs. This idea is consistent with the RNA-sequencing analysis in the study by Lotteau et al, which showed that only 182 transcripts had changed at 1 week, but 2699 had changed by 4 weeks.[6] It would be of great interest to measure nuclear $[Ca^{2+}]$ as a function of time in inducible NCX KO mice and to relate the changes to alterations in gene expression, cardiac remodeling indexes, and more precise observations on the causes of death in these animals.

CLINICAL RELEVANCE

These observations might have direct clinical relevance. Small molecules that block NCX1 have been under development for many years,[10] based on evidence that they suppress DAD-related arrhythmias and ischemia-reperfusion injury. However, by inhibiting NCX1, they might engage compensatory mechanisms that ultimately promote adverse remodeling and enhance longer-term mortality, as occurs in NCX1 KO mice.

It is possible that there may be levels of NCX1 inhibition that do not produce such consequences. Relatively normal myocyte Ca^{2+} handling might be maintained by the compensations noted in the study by Lotteau et al, with a 30% to 50% reduction in NCX current amplitude, whereas this degree of DAD suppression may virtually abolish DAD-triggered APs. That is because the relationship between $[Ca^{2+}]_i$ and DAD amplitude is highly nonlinear, and a certain threshold amplitude is required for AP induction.[3] Although the poor long-term prognosis of the complete NCX-KO is notable, it would be interesting to know if a heterozygous NCX1-KO mouse would be protected from long-term pathological changes. In addition, the reliability with which greater levels of inhibition could be avoided in patients is a critical

question, given the substantial variability in drug pharmacokinetics and pharmacodynamics that is typical of clinical populations.

Another long-standing question that began with the surprising initial NCX-KO mouse result is, would larger mammals equally tolerate NCX1-KO? That is, the mouse (versus rabbit or human) has an extremely short AP, with less Ca^{2+} influx via I_{Ca}, and therefore much less Ca^{2+} that must be extruded by NCX.[1] Would the same adaptations suffice in humans, in whom transsarcolemmal Ca^{2+} fluxes are more important? Moreover, the observed upregulation of NCX1 in rabbit and human heart failure may be adaptive (when SR Ca^{2+} content and release may be impaired).[3,11] That is, in human and rabbit HF with reduced SR Ca^{2+} release and elevated $[Na^+]_i$, NCX actually mediates some Ca^{2+} entry during the AP to support contraction.[12–14] In addition, removal of all the Ca^{2+} that entered via I_{Ca} plus NCX may require greater NCX function to remove the entering Ca^{2+}, because in heart failure the high $[Na^+]_i$ slows Ca^{2+} extrusion by NCX and the longer AP duration shortens the diastolic time for Ca^{2+} extrusion.[12–14] Would reducing NCX function in heart failure further impair diastolic cardiac function?

CONCLUSIONS

The study by Lotteau et al is an important contribution to the literature, which shows that the powerful adaptations previously noted in models involving NCX KO from birth are also noted when NCX expression is strongly suppressed at adulthood in an inducible KO model, albeit with some differences in the details of compensatory responses. Furthermore, this work shows that although the associated adaptations prevent major Ca^{2+} overload, the animals nevertheless eventually get sick and die. These observations urge caution in the development of therapeutic approaches that target pathological consequences related to NCX activity: with cellular Ca^{2+} handling, as in many areas of life, there is no such thing as a free lunch and potentially disastrous adverse consequences of what seems like a good idea have to be seriously considered.

ARTICLE INFORMATION

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Disclosures

None.

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