Editorial

Cardiac sodium–calcium exchanger: a double-edged sword

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Received 31 May 2001; accepted 31 May 2001

See article by Schäfer et al. [31] (pages 241–250) in this issue.

Development of heart failure is associated with an impairment of intracellular calcium (Ca²⁺) handling. During the past decade, pharmacological management of heart failure has mainly focused on therapies that are aimed at improving haemodynamics and modulating neurohormonal pathways. However, despite optimal inhibition of these pathways with drugs such as ACE inhibitors, beta-blockers, digoxin and, most recently, spironolactone, the mortality rate remains unacceptably high [1].

Recently, the failing cardiomyocyte within the heart itself has become the target for potential therapies — particularly via the regulation of Ca²⁺ transport. The slower rate of decay of Ca²⁺ transients within the failing heart, due to the reduced Ca²⁺ uptake into the sarcoplasmic reticulum (SR), ultimately results in a Ca²⁺ overload that leads to mechanical and electrical dysfunction of the cardiomyocytes.

Pathological conditions such as coronary artery disease and an inefficient supply of blood to the heart muscle can cause cardiac ischemia/hypoxia or reoxygenation injury, which results in a Ca²⁺ overload and acute lethal cardiomyocyte cell injury. Intracellular changes in Na⁺ levels, pH and rigor shortening of the cardiomyocyte following brief exposure to ischemia/hypoxia [2–4], has been shown to be mediated by an increase in the Na⁺/H⁺ exchanger function [4]. This leads to a rapid increase in intracellular Na⁺ that upsets the sarcolemmal Na⁺ gradient, which in turn results in Ca²⁺ overload [5] and swelling of the SR. In order to compensate, it has been proposed that Na⁺/Ca²⁺ exchange is upregulated to assist the SR in maintaining Ca²⁺ homoeostasis [6]. However, following long periods of ischemia/hypoxia, reoxygenation of the cardiomyocyte (in which the cell tries to recover and reestablish the Na⁺ and Ca²⁺ gradients) is not able to promote recovery. In fact, reoxygenation exacerbates the existing cardiomyocyte cell injury. This phenomenon is termed the ‘oxygen paradox’ and is characterized by a sudden development of hypercontracture [2,7] and enzyme release due to tissue necrosis [8]. If uncontrolled, reoxygenation-induced injury leads to further cardiomyocyte death and ultimately cardiac failure.

In order to protect the cardiomyocytes from reoxygenation-induced injury, several strategies have been used to control Ca²⁺ levels by using a variety of inhibitors of specific cellular cardiac functions. For instance, research has demonstrated cardio-protective effects of inhibitors of Na⁺/H⁺ exchange and intracellular pH [4], SR function, contractile elements [7], Na⁺ levels [5], and Na⁺/Ca²⁺ exchange during ischemia mediated Ca²⁺ overload [9].

The Na⁺/Ca²⁺ exchanger is one of the essential regulators of Ca²⁺ homeostasis within cardiomyocytes and is thus an important determinant of cardiac contractile function. Na⁺/Ca²⁺ exchange catalyses electrogenic exchange of Ca²⁺ and Na⁺ across the plasma membrane in either the Ca²⁺-influx or Ca²⁺-efflux mode, depending on the prevailing electrochemical driving forces (i.e. the Na⁺ and Ca²⁺ concentration gradients and the membrane potential) [10]. While the role of Na⁺/Ca²⁺ exchange as a primary Ca²⁺ extrusion mechanism in the heart is widely accepted (reviewed in Ref. [10]), the Ca²⁺-influx or ‘reverse mode’ remains controversial. Experimental evidence has shown that Na⁺/Ca²⁺ exchange also serves as a source for Ca²⁺-influx into the cell [11–13], and that this Ca²⁺-influx mode can be enhanced by either decreasing the Na⁺ or increasing the Ca²⁺ gradients across the transsarcolemmal membrane [11]. Additionally, upregulation of Na⁺/Ca²⁺ exchange activity [14–17] and Na⁺/Ca²⁺ exchanger expression has been observed [18,19] in the failing myocardium [20,21], leading to an increase in Ca²⁺-influx or decrease in Ca²⁺-efflux due to a rise in intracellular Na⁺ resulting in Ca²⁺ overload of the SR [22]. The increase in intracellular Ca²⁺ after ischemia/reperfusion is thought to
result from the Ca\(^{2+}\)-influx mode of the Na\(^+\)/Ca\(^{2+}\) exchange mechanism. In fact, Ca\(^{2+}\)-influx mode of Na\(^+\)/Ca\(^{2+}\) exchange has been reported to play a cardioprotective role in failing human heart. The idea stems from the fact the failing human cardiomyocytes are known to have impaired SR function due to a reduced levels/activity of SR ATPase proteins and an increase in expression of the Na\(^+\)/Ca\(^{2+}\) exchanger, which is thought to be a compensatory mechanism for dysfunctional Ca\(^{2+}\) handling [23,24]. In fact the upregulation of the Na\(^+\)/Ca\(^{2+}\) exchanger in failing human hearts has been credited with helping to preserve normal diastolic function [23]. A functional role of Ca\(^{2+}\)-influx mode of Na\(^+\)/Ca\(^{2+}\) exchange has also been implicated in SR loading of Ca\(^{2+}\) [25–27]. It has also been implicated in modulating the effectiveness of L-type Ca\(^{2+}\) channels [28–30]. Additional reports have suggested that Ca\(^{2+}\)-influx mode of Na\(^+\)/Ca\(^{2+}\) exchange may be involved in direct release of Ca\(^{2+}\) [23,24,31] during EC-coupling, but this idea is highly controversial [32]. However, the precise roles of adult Na\(^+\)/Ca\(^{2+}\) exchange within both the Ca\(^{2+}\)-influx and efflux modes, is still unclear because of the lack of specific inhibitors.

Given the wide ranging multiple functions, controversies and conflicting data regarding Na\(^+\)/Ca\(^{2+}\) exchanger — several groups have employed a gene targeting approach to delete the gene, enabling us to begin to precisely determine the role of Na\(^+\)/Ca\(^{2+}\) exchange in structural and functional development of the mammalian heart. These studies have clearly demonstrated that the ubiquitously expressed adult cardiac Na\(^+\)/Ca\(^{2+}\) exchanger (Ncx1) gene plays an important ‘heart-specific’ role during embryonic development, as the targeted removal of Ncx1 results in abnormalities in the generation of the heartbeat and embryonic lethality within the Ncx1-null mutants [33–35]. Additionally, Ncx1-nulls displayed relatively normal transient Ca\(^{2+}\) signals when electrically stimulated, suggesting that the Ca\(^{2+}\) delivery mechanism was fundamentally intact and that Ncx1-null cardiomyocytes are capable of regulating intracellular Ca\(^{2+}\) concentrations despite the absence of Ncx1. However, in contrast to the essential role that Na\(^+\)/Ca\(^{2+}\) exchange plays within the embryo, several studies have shown that Ncx1 expression reaches a maximum near birth and then decreases postnatally to a significantly lower level in the adult stages [36,37]. Thus, the contribution of Na\(^+\)/Ca\(^{2+}\) exchange to the control of intracellular Ca\(^{2+}\) levels is greater in the immature heart than the mature heart [22].

Given the present absence of the necessary genetic tools (i.e. conditional tissue-specific inactivation of Ncx1 within the adult heart), pharmacological blockade of Na\(^+\)/Ca\(^{2+}\) exchange provides a useful alternative. In this issue of Cardiovascular Research, Schafer et al. [31] have very carefully and methodically shown that the inhibition of the Ca\(^{2+}\)-influx mode of Na\(^+\)/Ca\(^{2+}\) exchange only during reperfusion can protect the myocardial cells against reperfusion injury. Using the selective Na\(^+\)/Ca\(^{2+}\) exchange inhibitor 2-[2-[4-(4-nitrobenzoyl)phenyl]ethyl] isothio-urea methanesulfonate (KB-R7943), Schafer et al. have used isolated adult rat cardiomyocytes and whole hearts to determine whether reoxygenation-induced Ca\(^{2+}\) oscillations are a result of Na\(^+\)/Ca\(^{2+}\) changes inability to do its primary function (i.e. remove Ca\(^{2+}\) from the cell) and to determine the mechanisms involved in development of the Ca\(^{2+}\) oscillations. The experimental design consisted of comparing three different sets of experiments: first, a control set where ischemia and reoxygenation was allowed to proceed unhindered; second, where they used the KB-R7943 inhibitor of reverse mode of the Na\(^+\)/Ca\(^{2+}\) exchange; and finally, a third group in which the reverse mode of the Na\(^+\)/Ca\(^{2+}\) exchange was blocked by using nominally Ca\(^{2+}\)-free medium.

In order to address whether the reoxygenation-mediated development of Ca\(^{2+}\) oscillations and hypercontracture was initiated by transsarcomemmal influx of Ca\(^{2+}\) via Na\(^+\)/Ca\(^{2+}\) exchange, the authors show that, both in the presence of KB-R7943 or the absence of Ca\(^{2+}\), the frequency of Ca\(^{2+}\) oscillations and hypercontracture was reduced when compared to non-treated cardiomyocytes. Additionally, although the frequency of oscillations was less, the rate of Ca\(^{2+}\) removal was unaffected. In order to confirm that the cytoprotective effects of KB-R7943 and Ca\(^{2+}\) removal were mediated by the Ca\(^{2+}\)-influx mode of Na\(^+\)/Ca\(^{2+}\) exchange, the authors pretreated the cardiomyocytes with specific inhibitors of SR function (thapsigargin and ryanodine), Na\(^+\)/K\(^+\) ATPase (ouabain), and Na\(^+\)/H\(^+\) exchanger (HOE 642). These results clearly show that Ca\(^{2+}\)-influx mode of Na\(^+\)/Ca\(^{2+}\) exchange is involved in ischemia-mediated Ca\(^{2+}\) oscillations.

Additional in vivo experiments were performed in isolated rat heart on the Langendorff system, and perfused with the KB-R7943 inhibitor. Three different parameters (left ventricle end diastolic pressure, left ventricle developed pressure and lactate dehydrogenase secretion) indicated the KB-R7943 inhibitor protected ischemic hearts during reoxygenation, as witnessed by the lowering of left ventricle end-diastolic pressure and improving left ventricle developed pressure, and marked reduction of lactate dehydrogenase release when administered during reoxygenation. The importance of this work lies in the fact that KB-R7943 was not only able to protect cardiomyocytes but it was also able to protect whole hearts from ischemia and reperfusion mediated injury.

The selective inhibition of the Ca\(^{2+}\)-influx mode of Na\(^+\)/Ca\(^{2+}\) exchanger by KB-R7943 is intriguing and suggests that there are significant differences in the properties of Na\(^+\)/Ca\(^{2+}\) exchange in the forward and reverse modes [22]. However, it should be noted that this selective inhibition may disappear under certain conditions [38]. KB-R7943 is thought to function externally and to compete with extracellular Ca\(^{2+}\) [39,40], possibly through the highly conserved α-2 repeat of Ncx1 [41]. Additionally, KB-R7943 has no effects on normal Ca\(^{2+}\) transients and
contractions suggesting that Ca$^{2+}$-influx via Na$^+$/Ca$^{2+}$ exchange is not important for physiological excitation-contraction coupling, at least in the rat cardiomyocytes [22].

Additionally, KB-R7943 has also been reported to significantly protect against anoxia/reoxygenation or ischemia/reperfusion damage in the brain by significantly improving the recovery of population spike amplitudes in rat hippocampal slices [42], and in the kidney [43] by suppressing the endothelin-1 (ET-1) content in the kidney. These results suggest that Ca$^{2+}$ overload via the Ca$^{2+}$/Na$^+$ exchange mode of Na$^+/Ca^{2+}$ exchange, followed by ET-1 overproduction within the kidney at least, seems to play an important role in the pathogenesis of the ischemia/reperfusion-induced damage.

The authors have suggested that KB-R7943 may be useful as a therapeutic drug for treatment during ischemia and reperfusion-mediated recovery. However, it has been shown that KB-R7943 inhibits both modes of Na$^+/Ca^{2+}$ exchange under special circumstances [38]. Furthermore, KB-R7943 has been shown to be a more potent inhibitor of Ncx$\scriptstyle 1$ when compared to Ncx$\scriptstyle 2$ [44]. Given these findings and the fact that only Ncx$\scriptstyle 1$ is expressed within the heart (Ncx$\scriptstyle 2$ and Ncx$\scriptstyle 3$ are expressed within limited tissues, particularly the brain [32]), it can be argued that the cardioprotective role for KB-R7943 needs to be investigated in greater detail using animal models — in order to determine its usefulness. However, the current study provides one mechanism (Ca$^{2+}$/Na$^+$ exchange via Na$^+/Ca^{2+}$ exchange) that may play a role in aggravating the deleterious consequences of cardiac failure.

Although there are currently no Na$^+/Ca^{2+}$ exchange blockers in clinical use and we do not yet know the precise mechanism of KB-R7943 action, studies like these reported in this issue by Schafer et al. [31] and further transgenic and pharmacological studies should begin to define the precise functions of Na$^+/Ca^{2+}$ exchange during both normal and abnormal heart function. Thus indicating ways to enable us to modulate Na$^+/Ca^{2+}$ exchange function, and maybe one day to regulate Ca$^{2+}$ transport and minimize the effects of heart failure.

References

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