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Review article

NHE-1: Still a viable therapeutic target

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ABSTRACT

The concept of Na–H exchange (NHE) involvement in cardiac pathology has been espoused for decades and supported by a plethora of experimental studies demonstrating salutary effects of NHE inhibition in protecting the myocardium against ischemic and reperfusion injury as well as attenuating myocardial remodelling and heart failure. NHE is actually a family of sodium and proton transporting proteins of which 10 isoforms have been identified. Myocardial NHE is represented primarily by the ubiquitous NHE-1 subtype which is expressed in most tissues. The robust positive results seen with NHE-1 inhibitors in experimental studies have led to relatively rapid development of these pharmacological agents for clinical assessment especially as potential cardioprotective therapies. Yet clinical studies have revealed, at best, inconsistent results as evidenced by poor efficacy and serious side effects, the latter revealed with the use of the NHE-1 inhibitor cariporide in high-risk patients undergoing coronary artery bypass grafting and evidenced by an increased incidence of cerebrovascular events of thromboembolic origin. The lack of success in clinical trials coupled with potential for toxicity has had a negative impact on development of cardiac therapeutic agents which have been developed based on the concept of NHE-1 inhibition. Whether this response is justified is open for discussion although a close scrutiny of clinical trial outcomes suggests that it may not be and that NHE-1 inhibition, if applied appropriately continues to represent an effective, if not the most effective approach for myocardial salvage following ischemic insult. Moreover, in addition to its cardioprotective effects, emerging evidence further suggests that NHE-1 inhibition is an effective strategy to minimize myocardial remodelling as well as a potentially effective strategy to improve efficacy of resuscitation following cardiac arrest. Thus, NHE-1 inhibition continues to represent a potentially highly effective therapeutic approach for the treatment of heart disease. This article is part of a Special Issue entitled "Na^+ Regulation in Cardiac Myocytes".

Contents

1. Introduction

NHE is an important regulator of intracellular pH in most cells including cardiomyocytes. To date, 10 NHE isoforms have been identified (NHE-1 to NHE-10) [1–3]. NHE-1 is the ubiquitous isoform whereas more newly-identified isoforms (NHE-6 to NHE-9) function to maintain acidic pH values in Golgi and post-Golgi compartments due to their localization within these organelles [4]. NHE-10 is restricted to osteoclasts (3). However, in terms of the heart, NHE-1 is the primary and most relevant NHE isoform serving as a target for therapeutic intervention. NHE-1 contains 815 amino acids and can be separated into two

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distinct functional domains: a 500-amino acid transmembrane domain, made up of 12 transmembrane spanning segments (responsible for proton extrusion) and a 315-amino acid hydrophilic cytoplasmic carboxy-terminal domain which contains regulatory sites [5–8]. The major determinant of NHE-1 activity under normal physiological conditions is intracellular pH [9,10]. Detailed discussion of the regulation of NHE-1 can be found elsewhere in this issue of the Journal. This review summarizes the basic concepts underlying the role of NHE-1 in myocardial ischemic injury and discusses the nature of the challenging results seen in clinical trials of NHE-1 inhibitors. It suggests that despite the disappointing results seen in these trials, the bases for these effects can be rationalized and that the potential for NHE-1 inhibitors in cardiac therapeutics should still be pursued.

2. NHE-1 in myocardial ischemic and reperfusion injury

The proposal that NHE contributes to myocardial injury was first espoused by Lazdunski and colleagues primarily as a reperfusion-mediated phenomenon [11]. According to this scheme, rapid reperfusion activates NHE resulting in large elevations in intracellular Na^+ concentrations. Inhibition of the $Na^+ - K^+$ ATPase as a result of the previous period of ischemia supresses Na^+ extrusion resulting in inhibition of Ca^{2+} removal *via* the $Na^+ - Ca^{2+}$ exchanger thereby resulting in elevations in intracellular Ca^{2+} concentrations. Although this hypothesis relates primarily to reperfusion-associated events, it is critical to emphasize that that activation of the antiporter during ischemia *per se* is of major importance in mediating cardiac injury. Indeed, the conditions necessary for NHE-mediated intracellular Na^+ accumulation are present in the ischemic myocardium, including intracellular acidosis, $Na^+ - K^+$ ATPase inhibition, the increased production of intracellular ischemic metabolites as well as hormonal, paracrine, and autocrine factors that activate NHE without the necessity of reperfusion. A close association between Na^+ and Ca^{2+} accumulation in the ischemic non-reperfused myocardium and the ability of NHE inhibitors to attenuate these changes has been reported resulting in reduced ischemia-induced injury and enhanced recovery of function following reperfusion [12–15]. Critically, the elevation in intracellular Na^+ concentrations *during* ischemia likely also sets the stage for subsequent reperfusion injury upon reflow [15,16]. Moreover, as will be discussed below, the salutary effects of NHE inhibitors are markedly more pronounced when the drug is present during the ischemic period compared with its addition before reperfusion. Thus, the basis for NHE involvement in myocardial injury reflects a close interaction between ion-regulatory processes found in the cardiac cell, especially NHE, $Na^+ - Ca^{2+}$ exchange, and the $Na^+ - K^+$ ATPase; indeed, inhibition of the latter during ischemia is an important prerequisite for NHE involvement in ischemic and reperfusion injury and forms the basis for a Na^+-dependent elevation in intracellular Ca^{2+} levels resulting in cell injury. The protection of the ischemic and reperfused heart by NHE-1 inhibition is also associated with reduced apoptosis possibly as a consequence of mitochondrial protection *via* inhibition of mitochondrial permeability transition pore opening due to reduced intracellular Ca^{2+} levels [17,18].

3. Cardioprotective effects of NHE inhibitors

Very strong support for NHE involvement in cardiac injury has originated from many studies that have used drugs which inhibit the antiporter. The first study demonstrating protection was reported from the author's laboratory which showed that amiloride, the prototypical non-specific NHE inhibitor, enhanced ventricular recovery, and diminished enzyme efflux from reperfused ischemic isolated rat hearts [19]. Since that initial observation, support for the concept of NHE-1 inhibition as an effective cardioprotective strategy has been greatly assisted by the development of highly specific and selective inhibitors targeting the NHE-1 isoform [20–24]. Virtually all animal studies using these agents have consistently demonstrated excellent cardioprotection irrespective of experimental model [5,6,13,15,19–21,25–27].

A question which is important to consider, particularly in terms of understanding the trials and tribulations associated with clinical assessment of NHE-1 inhibitors as cardioprotective agents concerns the locus of NHE-1 involvement in the ischemic and reperfused heart. Our initial study with amiloride showed that this agent failed to improve recovery of isolated ischemic rat hearts when it was administered only at reflow [19]. This observation has been supported by a number of other studies using highly specific NHE-1 inhibitors on a variety of experimental models, both *in vitro* and *in vivo* which showed either no protection or minimal protection when the drug was administered solely at the time of reperfusion or additional protection when the drug was administered before ischemia [19,25–27]. The superior protection observed with NHE-1 inhibitors present during ischemia most likely reflects the ability of these agents to inhibit injury in the ischemic nonreperfused myocardium by preventing NHE-1 dependent elevations in intracellular Na^+ and Ca^{2+} concentrations. The locus of action of NHE-1 inhibitors, that is ischemic vs reperfusion phases, remains an important consideration in assessing the results seen in clinical trials.

4. Clinical assessment of NHE-1 inhibitors for cardioprotection

The robust nature of consistent cardioprotective properties seen in experimental studies has led to rapid development of highly-specific NHE-1 inhibitors by the pharmaceutical industry and the assessment of two of these agents in clinical trials. Four such clinical studies have been carried out with rather complex but generally less than satisfying results. These trials are summarized in Table 1

Salutary effects of cariporide were observed in a small (N = 100) multicenter, randomized, placebo-controlled clinical trial which showed that administration of the NHE-1 inhibitor as a 40 mg intravenous bolus to patients with acute anterior myocardial infarction and treated with percutaneous transluminal coronary angioplasty produced cardioprotection [28]. At 21 days follow-up, patients receiving cariporide demonstrated significantly enhanced ejection fractions, reduced end-systolic volumes as well as improvement in regional wall motion abnormalities. These effects were associated with diminished release of myocardial enzymes including creatine kinase (CK), CK-MB and lactate dehydrogenase indicative of infarct size reduction [28].

4.1. The ESCAMI study

Although the above-mentioned study suggests that NHE-1 inhibition at the time of reperfusion following myocardial infarction could reduce infarct size, a subsequent large study using a different NHE-1 inhibitor raised serious doubts concerning the efficacy of this approach. The ESCAMI (Evaluation of the Safety and Cardioprotective effects of eniporide in Acute Myocardial Infarction) study was a Phase 2 (total 1389 patients) prospective, randomized, double-blind, placebo-controlled study to determine the potential efficacy of the NHE-1 inhibitor eniporide in patients undergoing either thrombolytic therapy or angioplasty after myocardial infarction on infarct size as determined by the release of alpha-hydroxybutyrate dehydrogenase [29]. This study was carried out in two stages. In stage 1 (430 patients), 50, 100, 150 or 200 mg eniporide was given as a 10 min infusion before reperfusion basically to identify the optimal dose(s). Indeed, the results from the stage 1 component revealed that the administration of 100 mg and 150 mg eniporide resulted in infarct size reductions of 10% and 23%, respectively. However, administration of either of these two doses failed to reduce infarct size in stage 2 of the study consisting of 959 patients and no benefit was seen on any clinical outcome parameter. Interestingly, a significant reduction in heart failure symptoms was observed with 150 mg eniporide in a subgroup of patients receiving late reperfusion, more than 4 h after onset of symptoms. Overall, the conclusion of the study was that