Na⁺/H⁺ exchange inhibition in hypertrophied myocardium subjected to cardioplegic arrest: an effective cardioprotective approach

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Abstract

Objective: This study was designed to assess whether the protective effects of Na⁺/H⁺ exchange (NHE) inhibition, which have been largely demonstrated in normal hearts, are also manifest in a more surgically relevant model of hypertrophied myocardium subjected to cardioplegic arrest. Methods: Left ventricular hypertrophy was created in 3-week-old rats by coarctation of the ascending thoracic aorta with a hemoclip. Eight weeks later, hearts were excised, isovolumetrically perfused and subjected to 1 h of potassium cardioplegic arrest followed by 2 h of reperfusion. Hearts were allocated to one of the following four groups: sham-operated and aortic banding hearts without any treatment or treated with the NHE inhibitor cariporide (1 μmol/L) given as an additive to cardioplegia and over the first 15 min of reperfusion. Results: The major effect of cariporide was to reduce ischemic peak contacture and to improve post-ischemic diastolic function in both sham-operated and hypertrophied hearts. Total creatine kinase release over the first 45 min of reperfusion was significantly reduced in hypertrophied hearts treated with cariporide. The endothelium-dependent coronary vasodilation to 5-hydroxytryptamine was observed in all sham-operated hearts before cardioplegia; Ischemia/reperfusion; Left ventricular function; Myocardial injury; Myocardial hypertrophy; Na+/H+ exchange inhibitor

Keywords: Cardioprotective effects of the NHE inhibitor cariporide are also manifest in hypertrophied myocardium, which supports the potential usefulness of NHE inhibition in the setting of cardiac surgery.

1. Introduction

Ventricular hypertrophy is an adaptive increase in ventricular mass in response to chronic pressure or volume overload. Hypertrophied hearts are more susceptible to ischemic injury than non-hypertrophied hearts [1]. As the number of cardiac surgical patients with pre-existing myocardial hypertrophy increases, the strategies that are focused on protection of hypertrophied myocardium against ischemia/reperfusion injury may have therapeutic benefit [2].

Assuming that Ca²⁺ overload is a landmark of severe myocardial tissue damage following cardioplegic arrest, most of these strategies have focused on limiting this intracellular calcium accumulation. In this setting, one approach which is gaining increased popularity is Na⁺/H⁺ exchange (NHE) inhibition. NHE allows Na⁺ entry in exchange for extrusion of intracellular excess protons. In the setting of ischemia/reperfusion, intracellular accumulation of protons leads to NHE activation and Na⁺ overload. As the adenosine triphosphate-dependent Na⁺/K⁺ pump becomes inoperable during ischemia, Na⁺ overload leads to reduced Ca²⁺ efflux and/or increased influx through a reverse-mode Na⁺/Ca²⁺ exchange, resulting in intracellular Ca²⁺ overload [3,4]. A large number of experimental studies have demonstrated the ability of NHE inhibitors to blunt the ischemia/reperfusion injury; however, recent clinical studies with the NHE inhibitors provided mixed and somewhat contradictory data [5]. Thus, the GUARDIAN trial failed to show an overall clinical benefit of NHE inhibition by cariporide [6] and no cardioprotective benefit was observed in the ESCAMi trial, in which the NHE inhibitor eniporide was administered as an adjunct to reperfusion therapy in patients with evolving myocardial infarction [7]. From a surgical
standpoint, the approach of NHE inhibition is particularly appealing because in the GUARDIAN trial the most pronounced cardioprotective effect of cariporide was observed in the subset of patients who underwent coronary artery bypass graft surgery [6]. This is consistent with the observation that a prerequisite for NHE inhibitors to be fully cardioprotective is their sustained presence in myocardial tissue from the onset of ischemia, a timing of administration which easily fits the practical conduct of cardiac operations [5,8]. Although it is sound to hypothesize that the cardioprotective effects of NHE inhibition demonstrated in normal hearts subjected to cardioplegic arrest should also be relevant to hypertrophied hearts subjected to the same type of injury, this assumption has yet to be supported by objective data. The present study was therefore designed to meet this objective.

2. Materials and methods

2.1. Experimental preparation

Pressure overload of the left ventricle (LV) was produced in 3-week-old female Wistar rats (body weight 48–57 g) by coarctation of the ascending thoracic aorta with a Weck hemoclip [9]. Animals were anesthetized with 0.18 mL of 2.1. Experimental time course

The hearts were initially allowed to equilibrate for 15 min after being instrumented and stable recordings were established. The LV balloon was inflated to the volume that gave a left ventricular end-diastolic pressure (LVEDP) of about 8 mmHg. At the end of the 1 h control pre-ischemic period, all hearts were arrested and subjected to 1 h of potassium cardioplegic arrest at 33 °C followed by 2 h of normothermic (37 °C) reperfusion. Initial asystole was achieved by adding concentrated KCl directly to the Krebs-Henseleit perfusate reaching the final concentration of 15 mmol/L, which was infused through a side arm on the aortic cannula at a pressure of 40 mmHg over a 5 min period. Hearts were maintained in a globally ischemic state for the remainder of the arrest period. The LV balloon was kept inflated throughout this period at the same volume that had been set throughout the pre ischemic period and after 60 min of reperfusion, the endothelium-dependent coronary flow response was tested by a 5 min perfusion with 5-hydroxytryptamine (5-HT, 10^-7 mol/L). Coronary flow was measured during the last 4 min of 5-HT administration. This was followed by a 12-min washout perfusion with drug-free Krebs-Henseleit solution to reestablish baseline coronary flow. The hearts were subsequently perfused with the endothelium-independent vasodilator papaverine (5 × 10^-6 mol/L) for 5 min and the coronary flow was again measured over the last 4 min of this perfusion.

At the end of reperfusion, the ventricles were weighed. Wet weights were measured after ventricles were incised and the excess fluid was blotted. Dry weights were measured after drying for 24 h at 80 °C. Water content was computed from the formula: 100 × (wet weight − dry weight)/wet weight.

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2.3. Experimental groups

Hearts were allocated to one of the following 4 groups: sham-operated (group 1, n = 9) and aortic banding hearts (group 2, n = 13) without any treatment and sham-operated (group 3, n = 9) and aortic banding hearts (group 4, n = 13) treated with the NHE inhibitor cariporide (1 μmol/L) given as an additive to cardioplegia and over the first 15 min of reperfusion. In addition, LV from the sham-operated and hypertrophied hearts (n = 4 for each group) were snap-frozen before cardioplegic arrest and stored at −80 °C until processing for NHE-1 protein expression by Western blot analysis.
2.4. Immunoblot analysis of NHE-1 protein expression

Left ventricular samples stored at −80 °C were allowed to thaw at room temperature. Samples weighing 146–148 mg were homogenized in 5 ml of homogenization buffer (containing 120 mmol/L NaCl, 10 mmol/L Tris pH 7.4, 0.1 mmol/L PMSF, 0.1 mmol/L benzamidine, 27.5 μmol/L ALLN and a protease inhibitor cocktail comprising 0.02 μmol/L aprotinin, 1.4 μmol/L E-64, 1 μmol/L leupeptin, 1 μmol/L phosphoramidon, 100 μmol/L TLCK, 200 μmol/L TPCK and 10 μmol/L APMSF), using a glass hand grinder. The homogenate was then centrifuged at 3000 rpm for 10 min and the pellet resuspended in resuspension buffer (containing 120 mmol/L NaCl, 10 mmol/L Tris pH 7.4, 0.1 mmol/L PMSF, 0.1 mmol/L benzamidine, 27.5 μmol/L ALLN and 1% SDS). The suspension was recentrifuged at 10,000 rpm for 15 min and the pellet once again resuspended in resuspension buffer. The pellet obtained after further centrifugation at 30,000 rpm for 1 h, which contains the membrane fraction, was resuspended in 150 μL resuspension buffer for a final time. Sample buffer (250 μL, containing 30% glycerol, 3% β-mercaptoethanol, 6% SDS, 130 mmol/L Tris pH 6.8 and 1.5 mmol/L bromophenol blue) was then added to each tube before analysis by SDS-PAGE. Protein samples (60 μL) were separated by SDS-PAGE on 9% acrylamide gels. Expression of the NHE-1 protein was determined by immunoblot analysis, using a monoclonal mouse NHE-1 antibody (Chemicon International, Ltd.). In a parallel experiment, levels of calsequestrin expression, to account for differential protein loading, were compared by paired two-tailed t-tests. Pre-ischemic and post-ischemic coronary flow responses to 5-HT and papaverine within the same group were compared by paired t-tests. A P-value < 0.05 was considered significant. Data are reported as mean ± standard deviation (SD).

3. Results

3.1. Development of left ventricular hypertrophy

Significant hypertrophy developed in the aortic banded hearts compared with sham-operated hearts. There was a significant increase in LV/body weight ratio by 79.6 ± 34.9% and 98.6 ± 38.5% in aortic banded hearts in groups 2 and 4, respectively, (P < 0.001 with sham-operated controls of groups 1 and 3). The difference in LV/body weight ratio between the two groups (untreated and drug-treated) of aortic banded hearts was statistically not significant (P = 0.37).

3.2. Left ventricular diastolic and systolic function

Baseline diastolic data were not significantly different among the groups (Fig. 1). After 60 min of reperfusion, the value of LVEDP was significantly (P < 0.001) lower in hypertrophied hearts treated with cariporide, as compared with untreated aortic banded hearts (Fig. 1). Not unexpectedly, hypertrophied hearts demonstrated significantly (P < 0.001) higher values of LVEDP during reperfusion compared to sham-operated hearts. In this non-hypertrophied group, cariporide also significantly (P = 0.02) reduced end-reperfusion LVEDP values compared with their untreated counterparts (Fig. 1).

Although post-ischemic maximum LV dP/dt decreased significantly (P < 0.001) compared to pre-ischemic baseline values in all hearts, the treatment with cariporide did not improve significantly LV contractility in both hypertrophied and sham-operated hearts (Fig. 2).

3.3. Ischemic peak contracture

Treatment with cariporide significantly reduced ischemic peak contracture in both hypertrophied and sham-operated hearts (Fig. 2).

![Graph showing LVEDP values before and after reperfusion.](https://example.com/graph.png)

Fig. 1. Left ventricular diastolic function at baseline (before cardiac arrest) and after 60 min of reperfusion. All values are mean ± standard deviation. *P = 0.02 vs. sham, **P < 0.001 vs. aortic banding, ***P < 0.001 vs. sham. LVEDP, left ventricular end-diastolic pressure.
hearts treated with cariporide, as compared with their respective untreated controls. Thus, the maximal contracture during cardioplegic arrest reached 40.6 ± 12.7 and 35.1 ± 9.2 mmHg in aortic banded hearts and sham-operated controls, respectively. Cariporide-treated hearts developed ischemic peak contracture of 23.6 ± 8.1 and 17.1 ± 6.2 mmHg in hypertrophied and sham-operated non-hypertrophied hearts, respectively (P < 0.001 vs. untreated hearts).

3.4. Creatine kinase leakage and water content

Total creatine kinase release during the initial 45 min of reperfusion was significantly (P = 0.032) lower in cariporide-treated hypertrophied hearts than in their untreated counterparts (Fig. 3). Cariporide-treated sham-operated hearts demonstrated a statistically insignificant (P = 0.14) decrease of creatine kinase leakage into the coronary effluent compared with the untreated shams (Fig. 3).

The percentage of LV tissue water was insignificantly (P = 0.22) smaller in cariporide-treated hypertrophied hearts (81.6 ± 0.7%) than in their untreated counterparts (82.1 ± 1.2%). Likewise, there was not significant difference (P = 0.34) in LV tissue water content between cariporide-treated sham-operated hearts (82.3 ± 1.2%) and untreated shams (82.9 ± 1.3%).

3.5. NHE-1 protein expression

There was a slight but statistically insignificant (P = 0.080) increase in NHE-1 protein expression, which was normalized relative to calsequestrin expression, in the hypertrophied LV samples (34.9 ± 4.6 arbitrary units) compared to those from the shams (29.2 ± 3.0 arbitrary units).

3.6. Coronary vascular responsiveness

The administration of 5-HT significantly (P < 0.001) increased coronary flow above baseline values in sham-operated controls during the pre-ischemic period (Fig. 4). Hypertrophied hearts demonstrated markedly impaired endothelium-dependent coronary vasodilation to 5-HT which was converted to vasoconstriction during both
the pre- and post-ischemic periods (Fig. 4). The administration of papaverine significantly (P<0.001) increased coronary flow above baseline values in sham-operated controls during both the pre- and post-ischemic periods; however, the endothelium-independent relaxations to papaverine were significantly (P<0.001) reduced in hypertrophied hearts (Fig. 5). Both endothelium-dependent and -independent coronary vasodilation were not affected by cariporide in either hypertrophied hearts or sham-operated controls (Figs. 4 and 5).

4. Discussion

The main results of this study are that: (1) the NHE inhibitor cariporide given as an additive to cardioplegia and during the initial phase of reperfusion greatly reduced ischemic peak contracture, total creatine kinase leakage and improved post-ischemic LV diastolic function in a rat model of pressure-overload hypertrophy and (2) hypertrophied hearts demonstrated a marked impairment of endothelium-dependent and -independent coronary vasodilation during both the pre- and post-ischemic periods that were not effected by the treatment with cariporide.

The cardiac NHE represents one of the major mechanisms to maintain physiological intracellular pH. Under conditions of myocardial ischemia and reperfusion, this physiological mechanism seems to exert detrimental effects on the myocardium, probably by intracellular Na\(^+\) overload, which finally results in elevated intracellular calcium via the Na\(^+\)/Ca\(^{2+}\) exchange [3,4]. Cariporide is a highly selective NHE-1 inhibitor with 60-fold selectivity over NHE-2 and 3000-fold selectivity over NHE-3 [10]. With the concentration of cariporide used in this study (1 \(\mu\)mol/L), the sarcolemmal NHE, which is encoded by the NHE-1 gene, is expected to be markedly inhibited [11]. The activity of the NHE is expected to become still more operative at the time of reperfusion when its potential blockade by extracellular acidosis is suddenly relieved. In addition to limiting sodium-driven calcium overload, NHE inhibition delays the intracellular realkalization during reperfusion and reduces post-ischemic diastolic stiffness without affecting systolic function in the porcine myocardium in situ [12]. The specific relationship between NHE-1 inhibition and post-ischemic diastolic function might be related to reduced hypercontracture that was demonstrated in isolated cardiomyocytes [13] and in an experimental model of in vivo regional myocardial ischemia [14]. These findings are further supported by the present study showing reduced ischemic peak contracture and improved post-ischemic LV diastolic function in both sham-operated and hypertrophied hearts treated with cariporide. This improvement of post-ischemic LV diastolic function seen in our study was not caused by a reduced myocardial edema, since LV tissue water content was not significantly different between hearts treated with cariporide and untreated controls. Thus, although tissue Ca\(^{2+}\) levels were not measured directly, it is sound to hypothesize that the improved preservation of diastolic function seen in the present study was likely related to a drug-induced limitation of Ca\(^{2+}\) overload.

Another finding of this study is that hypertrophied hearts showed a greater impairment of post-ischemic LV diastolic function than sham-operated controls. This increased susceptibility of hypertrophied hearts was to some extent blunted by cariporide, which provided a protection against ischemia/reperfusion injury of greater magnitude in this group than in sham-operated controls, as demonstrated by a significantly reduced total creatine kinase leakage during early reperfusion in hypertrophied hearts. Myocardial hypertrophy is linked with the enhancement of the NHE activity in spontaneously hypertensive rats [15]. Enhanced NHE activity might result from increased expression of the transporter protein and/or increased turnover rate of each unit. Although our data did not show a statistically significant increase in NHE-1 protein expression, they do not rule out the possibility that the hypertrophied hearts may have enhanced NHE activity in the absence of upregulated NHE-1 protein expression, since NHE activity is regulated by many post-translational mechanisms [16], which may be altered in hypertrophy [17]. In fact, chronic inhibition of NHE-1 by cariporide prevents the development of myocardial hypertrophy and heart failure in \(\beta_1\)-adrenergic receptor transgenic mice [18] and induces regression of cardiomyocyte hypertrophy in spontaneously hypertensive rats [19].

The present experiments finally demonstrated that coronary vasodilator responses were markedly impaired in hypertrophied hearts. Thus, responses to endothelium-dependent (5-HT) and -independent (papaverine) exogenous stimuli were depressed during both the pre- and post-ischemic periods. The loss of vasodilatory mechanisms in pressure-overload LV hypertrophy may lead to coronary flow dysregulation and ischemic manifestations that are frequently observed in patients with LV hypertrophy due to aortic stenosis, even though the coronary arteries appear angiographically normal [20]. Endothelium-dependent vasodilation is also impaired in coronary resistance vessels of patients with hypertension and ventricular hypertrophy [21]. Contributing factors may include extrinsic vascular compression and intrinsic coronary wall changes, e.g. increased arteriolar wall thickness [22]. The development of endothelial dysfunction in aortic banding-induced hypertrophy in rats is linked to an enhanced nitric oxide synthase III expression and superoxide anion production [23]. NHE-1 inhibition by cariporide before ischemia reduces endothelial dysfunction of isolated coronary resistance arteries from rat hearts subjected to coronary occlusions (2\(\times\)10 min separated by 5 min reperfusion) but does not alter myocardial contractile function in the area at risk [24]. An adjunctive pre-treatment with cariporide also delays myocardial and endothelial injury in pig hearts subjected to 30 min of nonisothermic ischemia and reperfusion [25]. This contrasts with our findings that NHE-1 inhibition was not effective in improving the protection of either endothelium-dependent or -independent coronary vasodilation. The duration of ischemia in our study (60 min at 33 °C) may have been too long for allowing a protection of coronary vasodilation by NHE-1 inhibition. In addition, pre-treatment with cariporide 15 min before ischemia, as used in other studies [24,25], may be more effective for protection than administration of the drug only started at the onset of cardioplegia, as in
the present study. These results should be tempered by the fact that they were obtained in an isolated heart model using buffer perfusion, which is known to be associated with a high baseline coronary flow. This could increase baseline nitric oxide production in a way that may modify responses to exogenous endothelial stimulation.

In conclusion, the present study demonstrates that the cardioprotective effects of the NHE-1 inhibitor cariporide, which have been extensively demonstrated in normal animal models of global ischemia, also extend to hypertrophied myocardium, which supports the potential usefulness of NHE inhibition in the setting of cardiac surgery.

References