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Journal of Molecular and Cellular Cardiology 38 (2005) 135–143
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original Article

# NHE-1 inhibition improves impaired mitochondrial permeability transition and respiratory function during postinfarction remodelling in the rat

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Received 30 July 2004; received in revised form 23 September 2004; accepted 12 October 2004

Available online 08 December 2004

**Abstract**

The mechanism by which $Na^+$–$H^+$ exchange (NHE) inhibition results in attenuation and reversal of postinfarction remodelling and heart failure remains controversial. In this study, we investigated the possible contribution of mitochondrial involvement by determining the effect of the NHE-1-specific inhibitor EMD-87580 (EMD) on mitochondrial permeability transition (MPT) and respiratory function during the postinfarction remodelling process. Male Sprague–Dawley rats were subjected to either 12 or 18 weeks of coronary artery ligation (CAL) or sham procedure. EMD was provided in the diet immediately after ligation. MPT pore opening was determined by perfusing hearts with 2-deoxy-[$^3$H]-glucose ([$^3$H]-DOG) and measurement of mitochondrial [$^3$H]-DOG entrapment. The respiratory function of isolated mitochondria was measured using Clark-type oxygen electrode. Remodelling was associated with significant hypertrophy and there was an increase in MPT pore opening in hearts both 12 and 18 weeks following CAL. Mitochondrial respiratory function, especially state 2 and state 3 rates were significantly decreased in hearts subjected to CAL. EMD suppressed MPT pore opening by 40% ($P < 0.01$) and 35% ($P < 0.01$) 12 and 18 weeks after ligation, respectively. Mitochondria isolated from EMD treated hearts exhibited increased respiratory chain activity for oxidation of substrates at complex I and II. These beneficial effects of EMD were associated with decreased mitochondrial vulnerability to exogenous $Ca^{2+}$. We conclude that NHE-1 inhibition has a protective effect on mitochondrial function, attenuating MPT pore opening and improving the respiratory function, which may contribute to the salutary effect of NHE-1 inhibitors in heart failure.


*Keywords:* Coronary artery ligation; EMD-87580; Mitochondrial function; Na–H exchange; Calcium

## 1. Introduction

The myocardial $Na^+$–$H^+$ exchange (NHE) represents a major $H^+$ extrusion system that plays an important role in the intracellular pH regulation in normal physiological processes and pathology, especially during ischaemia and early reperfusion. NHE activation induced by cellular acidosis during ischaemia contributes to cell injury most likely, because NHE is coupled closely to $Ca^{2+}$ overload through the reverse mode activation of $Na^+$–$Ca^{2+}$ exchange [1]. It has been shown that various neurohormonal stimuli, including endothelin-I [2,3], $\alpha_1$-adrenergic agonists [4,5] and angiotensin II [6,7] that play an important role in the development of hypertrophy, can increase cardiac NHE-1 activity. Emerging evidence suggests that NHE-1 inhibition may offer beneficial effects in the postinfarcted myocardium in terms of cardiac hypertrophy, remodelling and heart failure. Recent studies have demonstrated that NHE-1 inhibition with cariporide attenuates myocardial hypertrophy in vitro in response to neurohormonal adrenoreceptor [8] and mechanical stretch-induced [9] stimuli. Administration of cariporide [10–12] and the potent NHE-1 inhibitor EMD-87580 (EMD) [13], resulted in inhibition and reversal of postinfarction remodelling and heart failure independently of infarct size or afterload reduction.

*Abbreviations:* CAL, coronary artery ligation; DOG, 2-deoxyglucose; DOG-6P, 2-deoxyglucose-6-phosphate; EGTA, ethylene glycol-bis-(2-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EMD, EMD-87580; MPT, mitochondrial permeability transition; NHE, $Na^+$–$H^+$ exchange; RCI, respiratory control index; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylendiamine.

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doi:10.1016/j.yjmcc.2004.10.007

Despite evidence for an antiremodelling effect of NHE inhibition, the potential mechanisms explaining these actions are far from being fully elucidated. Mitochondria play critical roles in both the life and death of cells. The mitochondrial inner membrane is usually impermeable to all but a few selected metabolites and ions. However, when mitochondria are exposed to high [$Ca^{2+}$], especially if accompanied by adenine nucleotide depletion and oxidative stress, this permeability barrier is lost. Known as the mitochondrial permeability transition (MPT), this phenomenon is caused by the opening of non-selective pores involving dynamic multiprotein complexes in the inner membrane of mitochondria. Opening of the pore has dramatic consequences on mitochondrial physiology, including $\Delta\psi_m$ collapse, uncoupling of respiratory chain, and efflux of small molecules from the mitochondria [14–17]. Mitochondrial calcium overload is a major trigger of MPT pore opening. Since MPT pore opening leads to mitochondrial ATP hydrolysis rather than synthesis, energy metabolism would be further impaired, resulting in further $Ca^{2+}$ overload and further MPT pore opening. Thus MPT pore opening would be the critical point at which the injury becomes irreversible. There is now strong evidence that heart failure is a consequence or at least related to impaired energy metabolism and mitochondrial dysfunction [18–20]. Accordingly, we hypothesised that attenuation of the postinfarction remodelling and heart failure with NHE inhibition is a result of decreasing MPT opening and improving mitochondrial oxidative function.

## 2. Methods

### 2.1. Animal groups and surgical procedures

Male Sprague–Dawley rats weighing 285–320 g were purchased from Charles River (St. Constant, Quebec, Canada). All animals were maintained in the Health Sciences Animal Care Facility of the University of Western Ontario in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, Ont., Canada). Animals were randomly assigned to the following four treatment groups: (1) sham surgery with control diet (sham group); (2) sham surgery with EMD diet (sham + EMD group); (3) coronary artery ligation (CAL) with control diet (CAL group); or (4) CAL with EMD diet (CAL + EMD group). The surgical procedure was performed as previously described [12,13]. Briefly, rats were anesthetised with intraperitoneal pentobarbital sodium, intubated, and artificially ventilated by using a rodent respirator (model 683, Harvard Apparatus). A left thoracotomy was performed and the heart was gently exposed. To induce myocardial infarction, the left main coronary artery was ligated ~3 mm from its origin by using a firmly tied silk suture (5–0). For the sham procedure the ligature was placed in an identical fashion but not tied. The chest was then closed in three layers (ribs, muscle, and skin), and the animal was allowed to recover. The animals were then followed for either 12 or 18 weeks after surgery although initial studies were also done on a 6 weeks postinfarction group (see Section 3).

### 2.2. Diets

Rat chow containing the NHE-1 inhibitor EMD-87580 (*N*-[2-methyl-4,5-bis(methylsulphonyl)-benzoyl]-guanidine, hydrochloride) at a concentration of 200 ppm or EMD-free identical chow were generously provided Merck KGaA (Darmstadt, Germany). Details of EMD characteristics in terms of potency and selectivity vs. other NHE isforms have been recently published [13].

### 2.3. Mitochondrial isolation and measurement of the mitochondrial permeability transition (MPT) using 2-[$^3$H]-DOG-6P entrapment

This method and the calculation of the units of 2-[$^3$H]-DOG uptake have previously been described [21,22]. Briefly, 2-deoxyglucose (DOG) enters the myocyte on the glucose transporter and is metabolised to 2-deoxyglucose-6-phosphate (DOG-6P), which remains entrapped in the cytosol. It does not enter the mitochondria unless the MPT pore opens. The optimal conditions under which hearts can be perfused with 2-[$^3$H]-DOG to load them with 2-[$^3$H]-DOG-6P without any effect on function has previously been described [23]. Removal of unmetabolised 2-[$^3$H]-DOG was achieved by subsequent washout perfusion in the absence of DOG. The extent to which 2-[$^3$H]-DOG-6P enters mitochondria of loaded hearts was then used as an indicator of the number that has undergone MPT. Measurement of the mitochondrial 2-[$^3$H]-DOG-6P requires rapid isolation of the mitochondria in sucrose buffer containing ethylene glycol-bis-(2-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), which immediately closes the MPT pores and thus traps the 2-[$^3$H]-DOG-6P within the matrix.

To measure MPT, rats were euthanised by decapitation at the end of the postsurgery follow up period and the hearts were immediately removed and placed in cold Krebs–Henseleit buffer (see composition below) to inhibit any further contractions, and then mounted by the aorta on a stainless steel cannula and arranged for retrograde perfusion with the use of the Langendorff method, as described previously [24]. The hearts were electrically stimulated at 5 Hz and perfused at a constant flow rate of 10 ml/min with the use of a peristaltic pump. The perfusion medium was Krebs–Henseleit buffer composed of the following (in mM): 118 NaCl, 4.8 KCl, 1.2 $KH_2PO_4$, 1.2 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$ and 11 glucose. The buffer was initially equilibrated and then continuously gassed with a 95% $O_2$–5% $CO_2$ mixture. The pH of the buffer was 7.4, and temperature was maintained at 37 °C by enclosing the entire system in a series of water-jacketed coils.

After a 20 min equilibration perfusion in non-recirculating mode for hearts were perfused in recirculating manner with 50 ml of Krebs–Henseleit buffer containing 0.5 mM 2-[$^3$H]-