Chronic NHE-1 blockade induces an antiapoptotic effect in the hypertrophied heart

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Garciaarena CD, Caldzí CI, Portiansky EL, de Cingolani GE, Ennis IL. Chronic NHE-1 blockade induces an antiapoptotic effect in the hypertrophied heart. J Appl Physiol 106: 1325–1331, 2009. First published January 29, 2009; doi:10.1152/japplphysiol.91300.2008.—Na+/H+ exchanger (NHE-1) inhibition was demonstrated to induce the regression of cardiac hypertrophy (CH) in several experimental models and to inhibit mitochondrial death pathway in “in-vitro” experiments. Since recent reports show that NHE-1 inhibition delays the transition from CH to failure, and apoptosis plays a key role in this process, we investigated the effect of chronic treatment with the NHE-1 blocker cariporide on CH and apoptosis in the SHR. One month of cariporide treatment (30 mg·kg⁻¹·day⁻¹) induced the regression of CH (cardiomyocyte cross-sectional area: 468 ± 285 vs. 285 ± 9 μm² in untreated and cariporide-treated spontaneously hypertensive rats; P < 0.05). Apoptosis was assessed by TUNEL staining, the expression of Bcl-2, Bax, and activation of caspase-3 and PARP-1 by immunoblot. Cariporide treatment decreased the TUNEL-positive cells, the Bax-to-Bcl-2 ratio (3.16 ± 0.32 vs. 1.70 ± 0.17, untreated and cariporide-treated, respectively; P < 0.05); caspase-3 and PARP-1 activation (465 ± 62 vs. 260 ± 22 and 2,239 ± 62 vs. 1,683 ± 85 AU, untreated and cariporide-treated, respectively; P < 0.05). Angiotensin II, a growth factor and apoptotic stimulus, was used to induce O₂⁻ production that activated the ERK1/2-p90RSK pathway, increasing NHE-1 phosphorylation. These effects were prevented by losartán, N-(2-mercaptopyrroropinyl)-glycine, and cariporide.

In conclusion, we present data demonstrating that chronic NHE-1 inhibition with cariporide decreases both hypertrophy and apoptosis susceptibility in the spontaneously hypertensive rat heart. The antiapoptotic effect would be the consequence of two different actions of cariporide: the prevention of cytosolic Na⁺ and Ca²⁺ overload due to the inhibition of the sarcosomal NHE-1 and a direct mitochondrial effect preventing mitochondrial permeability transition pore opening.

Cariporide; apoptosis; spontaneously hypertensive rats; hypertrophy

IN THE HYPERTROPHIED MYOCARDIUM of the spontaneously hypertensive rats (SHR), an enhanced activity of the Na⁺/H⁺ exchanger (NHE-1) has been detected (30, 33) due to a kinase-dependent posttranslational modification of its cytosolic tail (14, 34). The regression of myocardial hypertrophy attained by several pharmacological interventions was accompanied by a tendency toward normalization of the NHE-1 activity, suggesting a causal link between both phenomena (3, 14). Moreover, SHR chronic treatment with NHE-1 inhibitors causes load-independent regression of cardiomyocyte hypertrophy and fibrosis (9, 11). Interestingly, there is recent evidence suggesting that NHE-1 pharmacological inhibition can also be beneficial in the prevention of heart failure (4, 7).

Hypertensive cardiac hypertrophy is caused by an autocrine/paracrine chain of events triggered by myocardial stretch that begins with the activation of the AT₁ receptors followed by the release/formation of endothelin (ET) and stimulation of the NHE-1 (10). The increased production of reactive oxygen species (ROS) that results from angiotensin II (Ang II)/ET stimulation of the NADPH oxidase may be responsible for ERK1/2-p90RSK activation and NHE-1 stimulation (8, 17). NHE-1 hyperactivity leads to an increase in intracellular Na⁺ concentration that favors the reverse mode of the Na⁺/Ca²⁺ exchanger (NCX), promoting cytoplasmic Ca²⁺ overload (10). Excessive accumulation of cytosolic Ca²⁺ is taken up by mitochondria leading to mitochondrial Ca²⁺ overload, which, in turn, may stimulate the opening of the mitochondria permeability transition pore (MPT) and the release of proapoptotic factors (21, 29).

Apoptosis is increased in hypertensive cardiac hypertrophy (13, 16, 22, 25), and it has been demonstrated to play a role in the transition from hypertrophy to heart failure that can be attenuated by interfering with the rennin-angiotensin system (25). This active and strictly regulated process of cell death can be initiated by the death receptor/extrinsic pathway or the mitochondrial/intrinsic pathway and typically occurs via activation of caspases that cleave DNA and other cell components. Several proteins are known to regulate the mitochondrial pathway, and between them the Bcl-2 family plays a key role (1). The ratio between two of the members of this family, the proapoptotic Bax and the prosurvival Bcl-2 may be considered as a determinant for survival or death of ventricular myocytes after an apoptotic stimulus (26). At the onset of apoptosis, caspase-3 proteolytically cleaves poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear DNA-binding zinc finger protein that influences DNA repair, DNA replication, modulation of chromatin structure and apoptosis (24). Both caspase-3 and PARP-1 constitute a convergence point of the intrinsic and extrinsic pathways of caspase activation (7) and represent a valuable tool to assess the extent of apoptotic cell death.

From a clinical point of view, it is of great relevance to determine whether chronic treatment with NHE-1 inhibitors, initiated after the establishment of cardiac hypertrophy, is able to reverse or stabilize hypertrophy and apoptosis. Although in...
MATERIALS AND METHODS

vitro experiments suggested an antiapoptotic effect of NHE-1 blockade (12, 36, 37), no data in the hypertensive cardiac hypertrophy is available so far.

We hypothesize that chronic inhibition of NHE-1 can inhibit apoptosis in concert with regression of cardiac hypertrophy. To this purpose, we studied adult SHR rats that received a 1-mo treatment with cariporide initiated once cardiac hypertrophy was already established.

We present herein data demonstrating that chronic NHE-1 inhibition with cariporide reverses both hypertrophy and apoptosis susceptibility in the SHR heart.

Fig. 1. Effect of the Na+/H+ exchanger (NHE-1) blocker cariporide on left ventricular mass and cardiac hypertrophy. A: the average values of left ventricular (LV) weight (LVW) in untreated spontaneously hypertensive rats (SHR) (n = 14) and cariporide-treated SHR for 1 mo (SHR + cariporide; n = 7). B: the average results for cardiac hypertrophy assessed by LVW-to-tibia length (TL) ratio in the same experimental groups. Cariporide treatment induced a significant regression of left ventricular hypertrophy. *Significant difference vs. untreated SHR (P < 0.05).

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MATERIALS AND METHODS

All procedures followed during this investigation conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the experimental protocol was approved by the Animal Welfare Committee of La Plata School of Medicine. Male SHR (4-mo-old) were assigned to either no drug treatment or to cariporide treatment (30 mg·kg⁻¹·day⁻¹) and euthanized after 1 mo of treatment under ether anesthesia. Cariporide was directly dissolved in the drinking water. The drug solution was prepared daily for each rat according to the records of water consumption and body weight (BW), the latter being measured every 2 days to ensure a dose of ~30 mg·kg⁻¹·day⁻¹ of the NHE-1 inhibitor, as previously described (15).

Systolic blood pressure (SBP) was measured weekly by the tail-cuff method. The left ventricle (LV) with the septum was weighed (LVW) and normalized by tibia length (TL) to determine cardiac hypertrophy.

Morphological studies. Ventricular tissue was fixed in buffered 10% formaldehyde and paraffin-embedded. LV coronal sections (5 μm thick) at the equator were stained for determining cardiomyocyte cross-sectional area (CSA) and quantifying collagen volume fraction (CVF) as previously described (9). To assess CSA, only round to ovoid cells with visible round nucleus were considered, and 50 cells were counted in at least 10 images obtained from each LV. CVF was calculated as the sum of all connective tissue areas divided by the total section surface in no less than 10 images. Perivascular collagen was excluded from this measurement.

Nuclei morphology. Cardiac myocytes were identified following morphological parameters (nuclei with elliptical shape and striated cytoplasm). Cardiomyocyte and endothelial cell nuclei were morphometrically measured in hematoxylin and eosin-stained SHR cardiac tissue sections. Measurements (area determination) were performed on five rats counting 500 nuclei/sample on images obtained with a ×40 objective using image analysis software (ImagePro Plus version 6.3, Media Cybernetics).

TUNEL staining. To detect apoptotic cardiomyocytes, a TUNEL assay was performed using the In Situ Cell Death Detection kit, TMR red (Roche). Slices (20 μm) from frozen hearts (6 sections/heart) were mounted on positively charged slides. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). To count stained nuclei, 10 images of every slide were captured using a digital camera (Olympus DP71) mounted on a microscope (Olympus BX61). Capture was performed using image analysis software (ImagePro Plus version 6.3, Media Cybernetics) using a ×40 objective. Monochrome images were then pseudo-colored. Fields containing an average of 125 myocyte nuclei were studied. The percentage of TUNEL-positive nuclei was calculated for each animal (n = 5/group) using the following formula: TUNEL-positive nuclei/DAPI labeled nuclei×100. Only apoptotic nuclei corresponding to cardiomyocytes were considered for the counting based on their morphometric area and distinguished from those of endothelial cells. The assay was standardized with the use of adjacent tissue sections treated with DNase I (Genbiotech) to induce DNA fragmentation as a positive control of apoptosis.

Western blot analysis. LV of untreated and 1 mo cariporide-treated SHR were homogenized in a lysis buffer [300 mM sacarose; 1 mM DTT; 4 mM EGTA, protease inhibitors cocktail (Complete Mini Roche); 20 mM Tris·HCl, pH 7.4]. Protein concentration was determined by Bradford method. For Bcl-2, Bax, caspase-3, and PARP-1 detection, samples were size-fractionated on 4–12% Bis-Tis gels (Invitrogen) and electrotransferred following the manufacturer’s instructions. After blocking, membranes were incubated with the specific antibodies: anti-Bcl-2 and anti-Bax antibodies (Pharmigen BD) and anti-caspase-3 and anti-PARP-1 (Santa Cruz Biotechnology). To determine ERK1/2, p90RSK, and NHE-1 phosphorylation, cardiac tissue was incubated with 1 nM Ang II for 30 min and homogenized in lysis buffer. For NHE-1 phosphorylation, determination samples were immunoprecipitated using a NHE-1 polyclonal antibody (Chemicon). Samples were subjected to PAGE, electrotransferred, and incubated with anti-phospho-ERK1/2 or anti-phospho-p90RSK antibodies (Santa Cruz Biotechnology). To assess NHE-1 phosphorylation, an anti-14-3-3 binding motif antibody was used. Previous reports have shown that the regulatory Ser703 in NHE-1 lies within a sequence that creates a binding motif for 14-3-3 proteins on phosphorylation; thus the phospho-Ser 14-3-3 binding motif antibody represents a useful tool for estimating NHE-1 Ser703 phosphorylation (35). Bands were visualized using the ECL-plus chemiluminescence detection system (Amersham). Autoradiograms were analyzed by densitometric analysis (Scion Image). Actin, total p90RSK, or ERK-2 was assayed as loading control as appropriate.

Fig. 2. Effect of NHE-1 inhibition on LV cardiomyocytes cross-sectional area (CSA) and collagen volume fraction (CVF). A: chronic treatment with cariporide induced a significant decrease in CSA of LV cardiomyocytes. Mean values of CSA determined in untreated SHR (n = 14); and cariporide-treated SHR for 1 mo (SHR + cariporide; n = 7). B: cariporide did not induce any significant regression in myocardial fibrosis after 1 mo of treatment. *Significant difference vs. untreated SHR (P < 0.05).
Superoxide production. Cardiac tissue slices from the left ventricle (1 × 5 mm) were obtained and kept at 4°C until assayed as previously described (8). Cardiac slices were kept in the assay buffer during 30 min in the presence of 1 nM Ang II in a metabolic incubator before anion superoxide (O$_2^-$) production was measured by the lucigenin-enhanced chemiluminescence method as previously described (8, 37). The inhibitors assayed were pre-incubated during 5 min and kept until the end of the experiment. The increase in O$_2^-$ production was expressed as the difference from the control after 15 min in the presence of lucigenin.

Statistics. Data are presented as means ± SE. Student’s t-test was used to assess differences between groups. The significance level was set at $P < 0.05$.

RESULTS

Chronic treatment with the selective NHE-1 inhibitor cariporide (30 mg·kg$^{-1}$·day$^{-1}$) induced the regression of cardiac hypertrophy in SHR. Figure 1A shows the LVW of untreated and cariporide-treated SHR. After 1 mo of treatment, a significant decrease in LVW (~24% below untreated SHR) was detected. This value was not different from that previously reported by our laboratory in age-matched normotensive Wistar rats (11). The LVW-to-TL ratio also evidences the regression of hypertrophy induced by cariporide treatment (Fig. 1B). Therefore, we confirm with these results our laboratory’s previous findings demonstrating that chronic NHE-1 blockade induces the regression of LV hypertrophy after 1 mo of treatment (9, 15). The regression of cardiac hypertrophy was achieved without a significant decrease in arterial pressure (172.5 ± 2.5 vs. 168.6 ± 2.1 mmHg untreated and treated SHR, respectively; $P = $ not significant), in agreement with previous reports (9).

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Fig. 3. Effect of the NHE-1 inhibitor cariporide on myocardial apoptosis. A: representative micrographs of DAPI (top) and TUNEL (bottom) staining of frozen heart sections. Cardiomyocyte apoptosis incidence (nuclei stained in red) was significantly reduced by cariporide treatment. B: the average results from TUNEL staining. C: representative micrograph of hematoxylin and eosin-stained SHR myocardium used to determine cardiac myocyte (arrows) and endothelial cell nuclei area (arrowheads). Magnification, ×40; bar = 30 μm. D: the average results of these determinations. CM, cardiomyocyte; EC, endothelial cell.
The mean values of the CSA of LV myocytes of untreated and cariporide-treated SHR are represented in Fig. 2A. Whereas the normalization of myocyte size [reaching a value similar to that of age-matched normotensive rats previously reported by our laboratory (11)] was evident after 1 mo of treatment, no significant regression of myocardial fibrosis, quantified as LVCVF, was achieved (Fig. 2B), probably due to the long half-life of collagen in the myocardium [~80–120 days (38)].

Since acute in vitro experiments have demonstrated that the blockade of the NHE-1 decreased the incidence of mitochondrial-mediated cell death (12, 36, 37), we investigated the effect of chronic treatment with cariporide on the increased apoptosis of the hypertrophied SHR heart. To this purpose, we measured apoptosis-positive cells as identified by TUNEL staining in cardiac slices from untreated and cariporide-treated SHR. Cariporide-treated hearts had significantly fewer TUNEL-positive cells compared with untreated SHR (Fig. 3). Moreover, we determined the cardiac expression of two apoptotic-related proteins: the proapoptotic Bax and the prosurvival Bcl-2 in both experimental groups. Immunoblotting analysis showed a lower expression of Bax in the LV of the cariporide-treated SHR compared with the untreated ones (P < 0.05; t-test), implying that chronic treatment with the NHE-1 inhibitor induced a decrease in the myocardial expression of this proapoptotic protein. The opposite was appreciated in the case of Bcl-2: higher signal intensities were detected in the bands corresponding to the LV of cariporide-treated animals compared with those of untreated SHR (P < 0.05; t-test). Therefore, the Bax-to-Bcl-2 ratio, an index of cell susceptibility to apoptosis, was significantly decreased by chronic inhibition of NHE-1 (Fig. 4). To confirm the inhibitory effect of cariporide on the apoptotic pathway in the hypertrophied myocardium of the SHR, we determined the activation of procaspase-3 as well as the cleavage extent of PARP by caspase-3 by protein immunoblotting in untreated- and 1 mo-treated SHR. As shown in Fig. 5, chronic treatment with cariporide decreased the extent of procaspase-3 cleaved into fragments of 17 kDa (Fig. 5A) as well as the amount of fragments of 85 kDa form the precursor PARP-1 (Fig. 5B), indicating a decreased activation of both effectors of the apoptotic pathway.

It has been proposed that the reduction in cytosolic Ca2+ levels induced by NHE-1 inhibition prevents mitochondria Ca2+ overload reducing the incidence of apoptosis (36, 37). However, we have recently demonstrated in feline myocardium a direct mitochondrial effect of NHE-1 inhibitors preventing Ang II-induced MPT formation and ROS release that may play a key role in their antiapoptotic action. This additional preventive mechanism would coexist with the cytosolic Ca2+-dependent mechanism previously described. Experiments were performed in rat cardiac slices in which mitochondrial O2− production was induced by Ang II, and the effect of cariporide was evaluated. Figure 6 shows that 1 nM Ang II stimulated myocardial O2− production and that this effect was prevented by the AT1 blocker losartan, the ROS scavenger MPG, and even more importantly by cariporide. Interestingly, the Ang II-induced release of ROS was accompanied by an activation of the ERK1/2-p90RSK pathway that correlates with an increased phosphorylation of the NHE-1 (Fig. 7).

**DISCUSSION**

The data presented herein provide evidence that chronic treatment of SHR with a specific NHE-1 blocker caused the reduction of cardiac hypertrophy and apoptosis susceptibility.

The potential mechanisms explaining the anti hypertrophogenic effect of NHE-1 inhibition have not been completely elucidated yet. An increase in intracellular Na+ concentration ([Na+]i) has been previously recognized in the hypertrophied myocardium (5, 6, 20); and in experimental studies sodium diet restriction has been reported to prevent cardiac hypertrophy (32). An increase in [Na+]i can induce an increase in intracellular Ca2+ concentration ([Ca2+]i) by favoring the reverse mode of operation of the NCX and therefore increasing Ca2+ entry (5, 10). Augmented [Ca2+]i is a well known hypertrophic signal as well as a major trigger of the mitochondrial death pathway. Sarcocellular NHE-1 inhibition in the hypertrophied myocardium would normalize [Na+]i, and [Ca2+]i, and by this way hypertrophy development and apoptosis could be prevented. Sustained elevation in [Ca2+]i results in activation of several signaling molecules, among which the calcium-/calmodulin-dependent protein phosphatase calcineurin is one of the most important. Calcineurin through its downstream principal effector, the nuclear factor of activated T cells (NFAT),

![Fig. 4. Effect of NHE-1 inhibition on the abundance of the proapoptotic Bax and the prosurvival Bcl-2 proteins in the LV myocardium of SHR.](http://jap.physiology.org/Downloadedfromhttp://jap.physiology.org/)

**A**: representative Western blot autoradiograms of the Bax and Bcl-2 proteins. Chronic treatment with cariporide induced an increase in the expression of the prosurvival Bcl-2 in the SHR myocardium. Actin was used as loading control. **B**: the average density (arbitrary units) of Bax and Bcl-2 proteins are represented with white and grey bars, respectively, for untreated rats (SHR) and cariporide-treated SHR. Black bars represent the Bax-to-Bcl-2 ratio. Since it can be appreciated, cariporide treatment reduced the SHR Bcl-2-to-Bax ratio almost to 50%, probably implying better conditions of these hearts to face apoptotic stimulus. *Significant difference vs. untreated SHR (P < 0.05; n = 3 each group).
mediates one of the main hypertrophic pathways in cardiac myocytes. We have recently demonstrated that the inhibition and regression of cardiac hypertrophy by NHE-1 blockade was accompanied by normalization of calcineurin/NFAT pathway activity (15). Although controversial, calcineurin does not seem to have a proapoptotic effect in the myocardium (38).

In agreement with previous results (9), 1 mo of cariporide treatment normalized SHR cardiomyocyte size without inducing significant regression of interstitial fibrosis. This fact should be probably considered as a weakness of the treatment with respect to arrhythmia susceptibility since it has been recently proposed that interstitial fibrosis, particularly differences in its ventricle distribution, underlie dominant arrhythmia vulnerability (28). On the other hand, disturbed calcium handling is recognized as one of the major triggers for arrhythmias, and NHE-1 inhibition by preventing [Na⁺] rise prevents [Ca²⁺] overload, therefore exerting a protective effect on myocardial electrical activity. However, it was out of the scope of the present work to analyze the effect of chronic NHE-1 inhibition on the incidence of cardiac arrhythmias.

Apoptosis is a physiological process in which cell death is the final consequence of a tightly regulated sequence of events. At the cellular level, apoptosis may be initiated either by ligand-binding activation of membrane death receptors, as Fasl/Fas, and/or by activation of mitochondrial-related pro-apoptotic mechanisms in response to unfavorable changes in the intracellular milieu (18). This latter pathway involves the activation of the MPT, an inner mitochondrial large-conductance channel activated by an increase in [Ca²⁺], and ROS, and the release of pro-apoptotic factors such as cytochrome c, thereby initiating a receptor-independent apoptotic death cascade (27). Mitochondria are the primary site of action for key apoptotic regulatory factors, including the Bcl-2 family of proteins. It is believed that the pro-apoptotic Bcl-2 family members, as Bax, trigger cell death by disrupting mitochondrial function resulting in MPT opening; whereas the anti-apoptotic members, as Bcl-2 itself, prevent mitochondria dysfunction (31). Following an apoptotic stimulus, these factors translocate from the cytosol to the mitochondria, where they presumably provoke mitochondrial membrane defects and MPT formation as a result of membrane insertion (19). The data that we present herein show that 1 mo of cariporide treatment decreased the expression of Bax and increased the expression of Bcl-2 proteins, thereby decreasing the Bax-to-Bcl-2 ratio and therefore apoptosis susceptibility in the SHR myocardium. These results were confirmed by the reduction in apoptotic nuclei identified by TUNEL staining and the decrease in caspase-3 activation and PARP-1 cleavage after cariporide treatment. Even though we could not rule out that other cell types different from cardiomyocytes were involved in the change of the apoptotic-related protein expression after NHE-1 inhibition, it has been shown in adult SHR that apoptosis (detected by in situ end-labeling) is confined predominantly to the cardiomyocytes of the LV, being rare in the

Fig. 6. Ang II-induced myocardial reactive oxygen species (ROS) production. Cardiac tissue slices incubated during 30 min in the presence of 1 nM Ang II (n = 8) induced a significant increase in O₂⁻ production that was prevented by losartan (Los; 1 μM; n = 5), MPG (2 mM; n = 7), and cariporide (carip; 10 μM; n = 5). None of the inhibitors used had an effect on the control chemiluminescence signal (inset). *Significant difference vs. all other groups (ANOVA; P < 0.05).
interstitial or endothelial cells and smooth muscle cells of the intramyocardial arteries and arterioles (13). A similar effect in the profile of these apoptotic-related proteins has been reported in the SHR myocardium after AT1 chronic blockade and in isolated neonatal rat cardiomyocytes exposed to hypoxia/re-oxygenation treated with cariporide (16, 36).

Since apoptosis is augmented in hypertensive cardiac hypertrophy and a decrease in its occurrence has been proposed to delay the transition from cardiac hypertrophy to failure, NHE-1 blockade could be beneficial from this perspective (13, 16, 25). Interestingly, it has been reported in a pacing-induced heart failure model in rabbits that chronic treatment with the NHE-1 inhibitor BIIB722 preserved left ventricular function by a mechanism that involved a reduction in myocyte apoptosis (2). These authors proposed that the beneficial effect of the NHE-1 inhibitor was related to a decrease in activity of the p38MAPK due to a reduced accumulation of [Na⁺]i and therefore [Ca²⁺]i (2). We did not explore p38MAPK activation in the present work.

Inhibition of mitochondrial death pathway by NHE-1 blockade was reported in isolated rat hearts subjected to ischemia/reperfusion (12) and in isolated neonatal rat cardiomyocytes exposed to oxidative stress or hypoxia/re-oxygenation (36, 37). A protective effect on mitochondrial function, attenuating MPT formation and improving the respiratory function of a different NHE-1 inhibitor, EMD-87580, was reported in the postinfarction remodelling in the rat heart (23). Interestingly, we have recently proposed a novel mechanism by which the guanidine derivative NHE-1 inhibitors may exert a protective effect against apoptosis: a direct mitochondrial action retarding MPT formation and ROS release (17).

The most relevant question that remains to be answered is the relative contribution of the two proposed mechanisms described for the NHE-1 inhibitors in the prevention of apoptosis: the prevention of cytosolic Na⁺ and Ca²⁺ overload due to the inhibition of the sarcolemmal NHE-1 and the direct mitochondrial effect preventing MPT opening and the release of ROS and Ca²⁺. Fig. 7. Ang II-induced phosphorylation of ERK1/2, p90RSK, and NHE-1. Ang II (1 nM) induced an increase in ERK1/2 (A), p90RSK (B), and NHE-1 phosphorylation in rat myocardium that was prevented by losartan (Los; 1 μM), MPG (2 mM), and cariporide (carip; 10 μM). Total ERK2, p90RSK, and NHE-1 were used as loading control as appropriate. *Significant difference vs. all other groups (P < 0.05; ANOVA; n = 4 for ERK1/2 and p90RSK and n = 5 for NHE-1 phosphorylation).

Fig. 8. Possible sites of action of cariporide in the prevention of apoptosis. We propose two mechanisms for the NHE-1 inhibitors in the prevention of apoptosis: the prevention of cytosolic Na⁺ and Ca²⁺ overload due to the inhibition of the sarcolemmal NHE-1 and the direct mitochondrial effect preventing MPT opening and the release of ROS and Ca²⁺.
cytochrome c. By preventing intracellular Ca\(^{2+}\) overload and consequently mitochondrial Ca\(^{2+}\) overload, MPT formation will be retarded. However, superimposed to this action is the recently proposed direct mitochondrial effect that also inhibits MPT opening. Figure 8 schematizes the two proposed sites of action of calcineurin in the prevention of apoptosis.

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NHE-1 BLOCKADE, APOPTOSIS, AND CARDIAC HYPERTROPHY