Am J Physiol Heart Circ Physiol
280: H830–H834, 2001.

HOE-642 (cariporide) alters pH_i and diastolic function after ischemia during reperfusion in pig hearts in situ

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Received 8 August 2000; accepted in final form 5 September 2000

Portman, Michael A., Anthony L. Panos, Yun Xiao, David L. Anderson, and Xue-Han Ning. HOE-642 (cariporide) alters pH_i and diastolic function after ischemia during reperfusion in pig hearts in situ. *Am J Physiol Heart Circ Physiol* 280: H830–H834, 2001.—The specific Na^+/H^+ exchange inhibitor HOE-642 prevents ischemic and reperfusion injury in the myocardium. Although this inhibitor alters H^+ ion flux during reperfusion in vitro, this action has not been confirmed during complex conditions in situ. Myocardial intracellular pH (pH_i) and high-energy phosphates were monitored using ^{31}P magnetic resonance spectroscopy in open-chest pigs supported by cardiopulmonary bypass during 10 min of ischemia and reperfusion. Intravenous HOE-642 (2 mg/kg; $n = 8$) administered before ischemia prevented the increases in diastolic stiffness noted in control pigs ($n = 8$), although it did not alter the postischemic peak-elastance or pressure-rate product measured using a distensible balloon within the left ventricle. HOE-642 induced no change in pH_i during ischemia but caused significant delays in intracellular realkalinization during reperfusion. HOE-642 did not alter phosphocreatine depletion and repletion but did improve ATP preservation. Na^+/H^+ exchange inhibition through HOE-642 delays intracellular alkalinization in the myocardium in situ during reperfusion in association with improved diastolic function and high-energy phosphate preservation.

magnetic resonance spectroscopy; metabolism; phosphates; intracellular pH

THE SPECIFIC BENZOYL-GUANIDINE Na^+/H^+ exchange (NHE1) inhibitor HOE-642 (cariporide) has been shown to reduce myocardial injury after ischemia and reperfusion in clinical studies (3, 5). The rationale for using such inhibitors to protect the myocardium derives principally from basic research studies performed in isolated hearts (1, 7, 20). The mechanism of protective action in those research models can be directly linked to effects on Na^+, Ca^{2+}, and H^+ fluxes by these NHE inhibitors during ischemia and/or reperfusion (18, 19). Nevertheless, the physiological differences, which exist between the buffer-perfused heart and blood-perfused myocardium in situ, can produce doubts concerning mechanisms of action in a clinically relevant model. A study (4) in research animals in situ demonstrates that HOE-642 reduces myocardial infarct size, contractile dysfunction, and ventricular fibrillation associated with reperfusion. However, alterations in H^+ ion flux or intracellular pH (pH_i) induced by HOE-642 during myocardial ischemia and reperfusion have never been confirmed in the heart in situ. The principal purpose of the present study was to test the hypothesis that NHE inhibition alters intracellular hydrogen ion homeostasis in the myocardium in situ during ischemia and reperfusion. Secondarily, we sought to determine whether such alterations were associated with improvements in systolic or diastolic cardiac function. A porcine model employing magnetic resonance spectroscopy was used to confirm this hypothesis.

METHODS

Cardiopulmonary bypass and surgical procedures. Animals used in this study were handled in accordance with Institutional and National Institutes of Health Animal Care and Use Guidelines. Pigs (age 24–31 days; 10–15 kg) received 10 mg/kg intramuscular ketamine, 0.05 mg/kg atropine, and 0.2–0.4 mg/kg xylazine followed by intravenous α-chloralose (40 mg/kg). They were intubated and ventilated with room air and oxygen before thoracotomy. The aorta and the right atrial appendage were cannulated, and a cardiopulmonary bypass was instituted using a membrane oxygenator (Minimax, Medtronic)-equipped circuit primed with Dextran 40 (500 ml). The circuit/heat exchanger and a heating pad maintained the temperature at 37°C throughout the experiment. A snare was placed around the ascending aorta distal to the aortic valve. A catheter tipped with a highly compliant and inflatable latex balloon was inserted into the ventricular chamber via a small left ventricular apex incision. Conducting patches attached to copper wires were affixed to the flanks of the pigs to perform defibrillation within the magnet bore.

NMR measurements. A 2-cm flexible radiofrequency coil, tuned to 81 MHz and matched to 50 Ω, was sutured to the left ventricular lateral wall. After transfer of the pig into the magnet, NMR data were collected with a General Electric spectrometer operating at 4.7 Tesla. ^{31}P spectra were ob-

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tained using a cardiac gating sequence. Fully relaxed spectra were first obtained using a 16-s interpulse delay. Pulse width in a one-pulse sequence was then optimized according to the phosphocreatine (PCr) and intracellular inorganic phosphate (P_i) signal using a 0.5- to 0.6-s interpulse delay. Spectra were then collected in 18-s blocks. This interpulse delay served to increase overall signal intensity but especially enhanced intracellular P_i peak intensity. Relaxed spectra provided the reference for relative peak area calculations using the least-squares analysis program (15). pH_i was determined from the chemical shift intracellular P_i-PCr difference by using calibration curves for pH_i versus chemical shift.

Cardiac function. After initiation of cardiopulmonary bypass, pressure-volume curves were constructed by inflating the latex balloon with saline and recording the pressure within the balloon. Pressure tracings were then recorded as the balloon was sequentially inflated in 1-ml increments. The atria were paced at rates above the intrinsic sinus rate throughout the protocol and ranged between 150 and 180 beats/min. Afterload was kept constant by maintaining mean aortic pressure between 50 and 60 mmHg through the bypass pump. Peak elastance and diastolic stiffness were determined from the respective slopes of the peak systolic and end-diastolic pressure curves. The pressure-rate product was calculated as the maximal developed pressure times heart rate.

Protocols. Baseline pressure-volume curves were constructed with the pig placed in the spectrometer bore. Subsequently, the balloon was deflated to 5 ml, and cardiac pacing continued while baseline and fully relaxed ^{31}P magnetic resonance spectra were acquired for 10 min. Pigs were randomized into control ($n = 8$) and HOE-642-treated groups ($n = 8$). Intravenous HOE-642 (2 mg/kg) was administered in the drug group 10 min before complete aortic constriction with the snare. Magnetic resonance data were collected throughout a 4-min baseline period, a subsequent 10-min period of ischemia, and through 10 min of reperfusion (after snare removal). Defibrillation was performed if necessary 5 min after reperfusion with a single shock (10 J). Ventricular fibrillation generally occurred immediately at onset of reperfusion. Three pigs within each group required defibrillation. Reperfusion pressure-volume curves were constructed 20 min after snare removal. Cardiac pacing was maintained at the preischemic rate for construction of these curves after reperfusion.

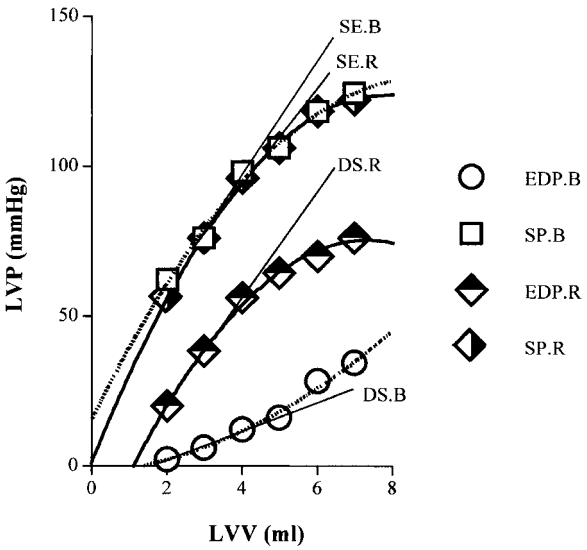

Fig. 1. Left ventricular pressure (LVP) response to left ventricular volume (LVV) loading in a control pig heart. A compliant balloon within the left ventricle was serially inflated in increasing 1-ml increments. Curves are demonstrated for end-diastolic pressure at baseline (EDP.B) and after reperfusion (EDP.R) and systolic pressure at baseline (SP.B) and after reperfusion (SP.R). Peak systolic elastance is determined from the slope of lines for baseline (SE.B) or reperfusion (SE.R). Similarly diastolic stiffness is defined from slopes of DS.B (baseline) and DS.R (reperfusion).

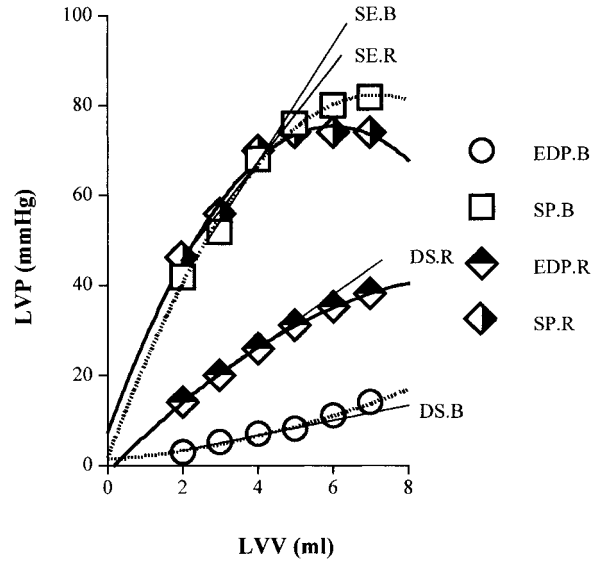

Fig. 2. Curves similar to those presented in Fig. 1 for a pig that received HOE-642. Note that no change in systolic pressure curve occurs between baseline and reperfusion. A more dramatic increase in diastolic stiffness after reperfusion is demonstrated in Fig. 1 than in the current example.

Statistical analyses. Data within groups were evaluated using ANOVA for repeated measures. Data between groups were analyzed using ANOVA and Scheffe's *F*-test. PCr and ATP data are reported as relative to baseline peak areas. Exponential line fittings were performed as previously described to determine half-time (τ) for PCr depletion and repletion, respectively, during ischemia and reperfusion (14). Values for τ were compared between groups using unpaired *t*-tests. All descriptive data are reported as means ± SE. Statistical significance was considered to have occurred when $P < 0.05$.

RESULTS

Cardiac function. Representative curves for intraventricular volume versus developed pressure in a control and HOE-642-treated animal are illustrated in Fig. 1 and 2, respectively. The curves in these particular examples demonstrate minimal change in peak systolic elastance after reperfusion. Changes in diastolic stiffness after reperfusion are much greater in Fig. 1 than Fig. 2. Cardiac performance parameters for each group are summarized in Fig. 3. There were no significant differences in systolic or diastolic parameters at baseline between groups. Ischemia and reperfusion did not cause a significant change in peak elastance for either group, although the pressure-rate product (a systolic performance parameter in this model) was significantly diminished for both. Because

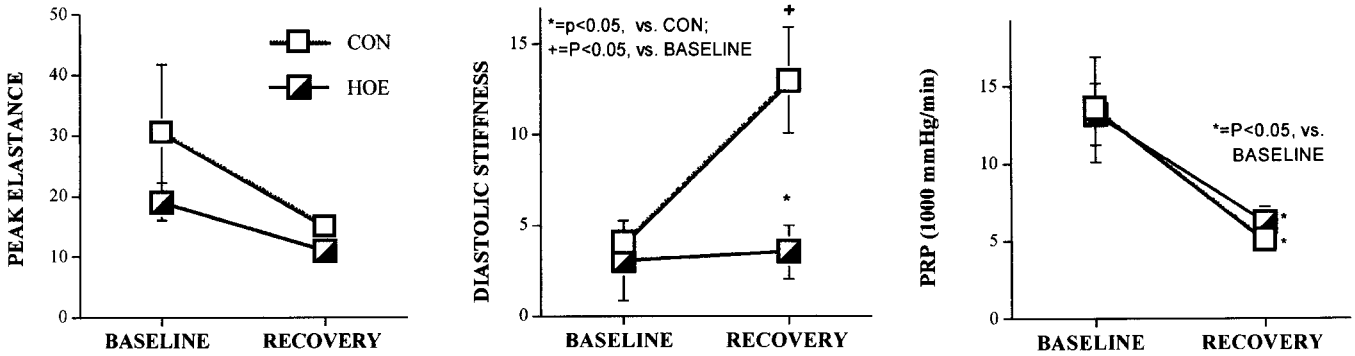

Fig. 3. Systolic and diastolic performance indexes at baseline and recovery. Con, control group; HOE, HOE-642 group; PRP, pressure rate product.

the cardiac pacing rate during reperfusion precisely matched the baseline rate, the changes in the pressure-rate product were principally due to decreases in developed pressure. HOE-642 did not alter these systolic performance parameters after reperfusion. Diastolic stiffness increased dramatically in the control group after reperfusion. This decrease in diastolic performance did not occur in the HOE-642-treated group.

Intracellular pH. Spectra, which demonstrate changes in the intracellular phosphate peak intensity and chemical shift, are illustrated in Fig. 4. Values for pH_i are shown in Fig. 5. Each point represents a summation of four blocks of data acquisition, thus yielding a temporal resolution of 72 s. A steady decline in pH_i occurred after the onset of ischemia in both groups. The pH nadir always occurred in the acquisition block immediately preceding reperfusion. For statistical purposes, the following time points were considered: baseline; postdrug; intial ischemia and consecutive 72-s blocks of final ischemia (nadir); and *reperfusion 1*, *2*, *3*, and *4* (R1, R2, R3, and R4). Administration of HOE-642 did not alter pH_i before or during ischemia. Delayed reversal of acidification in the HOE-642-treated group was noted in the first reperfusion period (R1) ($P < 0.05$) and persisted through R3. By R4, the groups demonstrated similar pH_i values, indicating that realkalinization was delayed but not abrogated. No significant differences in systemic arterial blood pH occurred between groups either before or after reperfusion.

High-energy phosphates. A strong PCr signal coupled with data analysis through the fit to standard program allowed high temporal resolution of the PCr and ATP peaks. Small changes in ATP content were noted for this brief period of ischemia (Table 1). Thus PCr depletion provides the major source of energy during this period. The τ for each of the processes was calculated

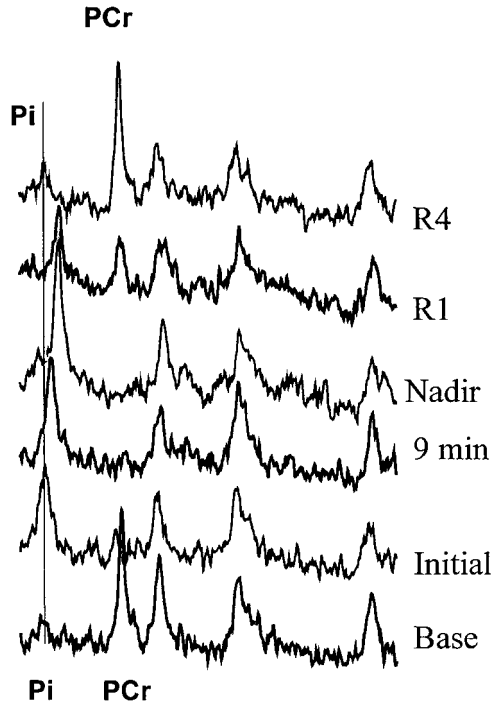

Fig. 4. ^{31}P magnetic resonance spectra representing 72-s acquisitions with minimal line broadening (3 Hz). Stages in protocol are the following: base, baseline; initial; 1st 72-s ischemia; 9 min, during the 9th minute of ischemia; nadir, final period of ischemia; R1, 1st 72-s reperfusion; and R4, 4th 72-s period during reperfusion. PCr, phosphocreatine peak; P_i, inorganic phosphate peak. The vertical line enhances recognition of the P_i peak shift toward PCr during ischemia and away during reperfusion.

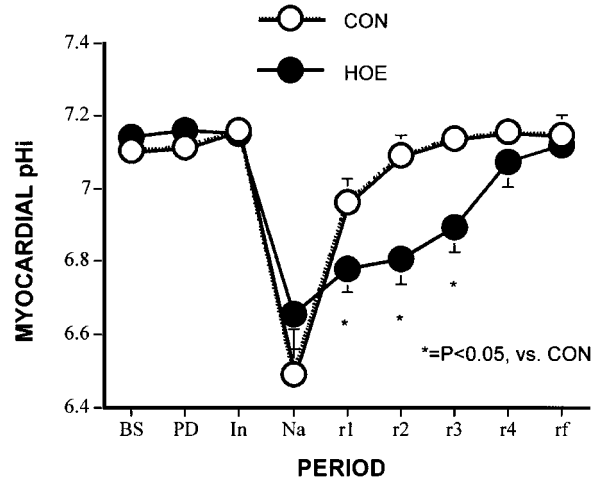

Fig. 5. Intracellular pH (pH_i) for each protocol period. BS, baseline; PD, postdrug (HOE-642); In, initial 72 s of ischemia; Na, pH_i nadir; r1, r2, r3, and r4; sequential 72 s of reperfusion; rf, final 72 s of reperfusion (total 10 min).

Table 1. *High-energy phosphate parameters*

	PCr Depletion τ, s	PCr Repletion τ, s	ATP, % baseline
Control	113 ± 19	67 ± 11	82 ± 1.2
HOE-642	81 ± 12	81 ± 8	90 ± 3.6
P value	0.26	0.33	0.048

Data are means ± SE. *P* values are control vs. HOE-642. ATP percentage was measured as the percent retained after reperfusion. PCr, phosphocreatine; τ, exponentially derived half-time value.

through exponential fitting and provides a basis for comparison between groups. There were no differences in PCr depletion or repletion rates. ATP retention was significantly greater after reperfusion in the HOE-642-treated group.

DISCUSSION

NHE inhibitor (HOE-642) mechanisms during myocardial reperfusion in situ have not been previously studied. Prior investigations of this drug class have included mechanistic examinations in isolated hearts (4) as well as descriptions of drug end effect in animals (13) and human patients (3, 5). Detailed studies employing magnetic resonance spectroscopy in buffer-perfused hearts have established that the benzoyl-guandine derivatives such as HOE-642 delay intracellular alkalinization during reperfusion (6). The current study confirms that this NHE inhibition occurs under the more complex conditions that operate in the intact animal.

The complexities apparent in situ include those related to α-adrenergic receptor activation of NHE. A previous study (16) in our laboratory demonstrated that both α-adrenergic antagonism and NHE inhibition through HOE-642 induce myocardial intracellular acidosis during graded hypoxia in the heart in vivo. Rehring and colleagues (17) further elaborated this relationship in the perfused rat heart by demonstrating that the pH_i decline formerly attributed to NHE inactivation during ischemia can be abrogated by phenylephrine, a recognized α-adrenergic agonist. NHE inhibition by HOE-642 under this α-adrenergic stimulation exacerbates intracellular acidosis, thus exhibiting that NHE operates during ischemia under specific conditions. Catecholamine levels were not measured as part of the current study. However, arterial, coronary venous, and interstitial norepinephrine levels in anesthetized pigs have been well established (11, 12) and support the contention that an ambient level of α-adrenergic activation is present. Nevertheless, HOE-642 produced no observable changes in pH_i during ischemia. This finding might indicate that alternative modes of cytosolic buffering or H^+ extrusion predominate during myocardial ischemia in situ.

A delay in cellular alkalinization by HOE-642 occurs despite the circumstances in situ, which tend to promote rapid H^+ extrusion during reperfusion. Some of the contributing factors are absent in the simpler buffer-perfused heart system. They include not only the previously addressed α-adrenergic agonism but also the entry of the powerful blood-borne buffer system, which rapidly increases the sarcolemmal pH gradient (14) and theoretically stimulates NHE during reperfusion. The rapidity of pH_i normalization with reperfusion in this pig model can be defined by comparison with apparent rates obtained from similar reperfusion protocols in perfused hearts. Evaluation of a study by Hartmann and Decking (6), who employed HO-E642 in isolated hearts, indicate that the realkalinization occurs three to four times faster in the heart in situ. Inhibition of this rapid process highlights the potency and perhaps the specificity that HOE-642 exhibits as it operates under these complex conditions.

PCr depletion rates provide indexes for ATP utilization during ischemia, whereas PCr repletion during reperfusion represents a measure of mitochondrial function (14, 15). Similar to results from a prior study (16) in situ, our data could not establish a link between NHE activation and mitochondrial function. The level of ATP retention generally corresponds to myocardial viability, is related to purine loss, and is thus altered by HOE-642 in this model of myocardial stunning. Others (6, 19) have noted an HOE-642-associated reduction in high-energy phosphate loss during more prolonged periods of ischemia in perfused hearts. Extension of ischemic time in the pig model reduces the PCr peak to the extent that pH_i and high-energy phosphate content analysis in situ are impossible. Thus this study was limited to examination of relatively brief global ischemic periods, which induced myocardial dysfunction, as evidenced by increased diastolic stiffness and decreased pressure-rate product after reperfusion.

Previously, with the use of a similar porcine model in situ to study hypothermic circulatory arrest and reperfusion, we (16) demonstrated that a reduction in cellular realkalinization rate through alkaline cardioplegia proffered superior systolic and diastolic postischemic function. However, these relationships were not totally reproducible in the current experiments. NHE-1 inhibition and delayed realkalinization reduced postischemic diastolic stiffness but did not alter global systolic performance parameters. These data corroborate results obtained by Klein et al. (9), who similarly demonstrated HOE-642-induced improvement in global and regional diastolic relaxation parameters after reperfusion in the porcine myocardium in situ without effecting systolic function. The specific relationship between NHE-1 inhibition and diastolic compliance after ischemic insult remains a subject of conjecture. Ladilov and co-authors (10) have shown that specific NHE-1 inhibition during reoxygenation of cardiomyocytes prolongs cytosolic acidosis, attenuates Ca^{2+} oscillations, and reduces hypercontracture. These mechanisms of hypercontracture have been linked to diastolic compliance in the intact heart (21).

Sodium flux. Intracellular Na^+ accumulation during ischemia and reperfusion presumably induces reversal of the Na^+/Ca^{2+} exchanger in situ. This action promotes Ca^{2+} overload, currently considered a principal source of myocardial injury after ischemia (8). This

study did not measure relative intracellular Na^+ content during ischemia and reperfusion. A study (6) in isolated perfused hearts demonstrated that NHE inhibition ameliorates Na^+ entry during both ischemia and reperfusion. While Na^+ entry appears to relate to the H^+ efflux rate during reperfusion, attenuation of the Na^+ influx during ischemia in the perfused heart model is not accompanied by concomitant changes in pH_i. Balschi (2) confirmed the pairing of H^+ extrusion with Na^+ accumulation during ischemia and early reperfusion in the pig heart in situ. However, the effect of NHE inhibition on Na^+ entry during reperfusion in situ remains unknown. This action may proffer substantial protection on the heart yet requires confirmation in situ.

In conclusion, this study represents the first analysis of the NHE inhibitor effect on H^+ flux in heart under the complex conditions that exist in situ. The results of this study confirm that HOE-642 alters myocardial H^+ flux during reperfusion. In this specific model with a relatively short global ischemic period, the rate of intracellular realkalinization relates to the level of postischemic diastolic performance; however, a causative link between these two has not been proven.

This work was funded in part by National Heart, Lung, and Blood Institute Grant R01-HL-60666, awarded to M. A. Portman. HOE-642 was provided by Dr. Wolfgang Scholz, Hoechst.

REFERENCES

1. **Aye NN, Xue YX, and Hashimoto K.** Antiarrhythmic effects of cariporide, a novel Na^+-H^+ exchange inhibitor, on reperfusion ventricular arrhythmias in rat hearts. *Eur J Pharmacol* 339: 121–127, 1997.
2. **Balschi JA.** ^{23}Na NMR demonstrates prolonged increase of intracellular sodium following transient regional ischemia in the in situ pig heart. *Basic Res Cardiol* 94: 60–69, 1999.
3. **Buerke M, Rupprecht HJ, vom Dahl J, Terres W, Seyfarth M, Schultheiss HP, Richardt G, Sheehan FH, and Drexler H.** Sodium-hydrogen exchange inhibition: novel strategy to prevent myocardial injury following ischemia and reperfusion. *Am J Cardiol* 83: 19G–22G, 1999.
4. **Dhein S, Krusemann K, Engelmann F, and Gottwald M.** Effects of the type-1 Na+/H+-exchange inhibitor cariporide (Hoe 642) on cardiac tissue. *Naunyn Schmiedebergs Arch Pharmacol* 357: 662–670, 1998.
5. **Erhardt LR.** GUARD During Ischemia Against Necrosis (GUARDIAN) trial in acute coronary syndromes. *Am J Cardiol* 83: 23G–25G, 1999.
6. **Hartmann M and Decking UK.** Blocking Na(+)-H+ exchange by cariporide reduces Na(+)-overload in ischemia and is cardioprotective. *J Mol Cell Cardiol* 31: 1985–1995, 1999.
7. **Hendryx M, Mubagwa K, Verdonck F, Overloop K, Van-Hecke P, Vanstapel F, VanLommel A, Verbeken E, Lauweryns J, and Flameng W.** New Na^+-H^+ exchange inhibitor HOE 694 improves postischemic function and high energy phophate resynthesis and reduces $Ca2^+$ overload in isolated perfused rabbit heart. *Circulation* 89: 2787–2798, 1994.
8. **Karmazyn M.** Mechanisms of protection of the ischemic and reperfused myocardium by sodium-hydrogen exchange inhibition. *J Thromb Thrombolysis* 8: 33–38, 1999.
9. **Klein HH, Bohle RM, Pich S, Lindert-Heimberg S, Wollenweber J, Schade-Brittinger C, and Nebendahl K.** Time-dependent protection by Na^+/H^+ exchange inhibition in a regionally ischemic, reperfused porcine heart preparation with low residual blood flow. *J Mol Cell Cardiol* 30: 795–801, 1998.
10. **Ladilov YV, Siegmund B, and Piper HM.** Protection of reoxygenated cardiomyocytes against hypercontracture by inhibition of Na^+/H^+ exchange. *Am J Physiol Heart Circ Physiol* 268: H1531–H1539, 1995.
11. **Lameris TW, de Zeeuw S, Alberts G, Boomsma F, Duncker DJ, Verdouw PD, Veld AJ, and van Den Meiracker AH.** Time course and mechanism of myocardial catecholamine release during transient ischemia in vivo. *Circulation* 101: 2645–2650, 2000.
12. **Lameris TW, van Den Meiracker AH, Boomsma F, Alberts G, de Zeeuw S, Duncker DJ, Verdouw PD, and Veld AJ.** Catecholamine handling in the porcine heart: a microdialysis approach. *Am J Physiol Heart Circ Physiol* 277: H1562–H1569, 1999.
13. **Miura T, Ogawa T, Suzuki K, Goto M, and Shimamoto K.** Infarct size limitation by a new Na(+)-H+ exchange inhibitor, Hoe 642: difference from preconditioning in the role of protein kinase C. *J Am Coll Cardiol* 29: 693–701, 1997.
14. **Portman MA, Panos AL, Xiao Y, Anderson DL, Alfieris GM, Ning XH, and Lupinetti FM.** Influence of cardioplegia pH on cellular energy metabolism and H^+ ion flux during neonatal hypothermic circulatory arrest and reperfusion. *J Thorac Cardiovasc Surg* 114: 601–608, 1997.
15. **Portman MA, Standaert TA, and Ning XH.** The relation of myocardial oxygen consumption and function to high energy phosphate utilization during graded hypoxia and reoxygenation in sheep in vivo. *J Clin Invest* 95: 2134–2132, 1995.
16. **Portman MA, Xiao Y, Broers BGE, and Ning XH.** Hypoxic pH_i and function modulation by Na^+/H^+ exchange and α-adrenoreceptor inhibition in heart in vivo. *Am J Physiol Heart Circ Physiol* 272: H2664–H2670, 1997.
17. **Rehring TF, Shapiro JI, Cain BS, Meldrum DR, Cleveland JC, Harken AH, and Banerjee A.** Mechanisms of pH preservation during global ischemia in preconditioned rat heart: roles for PKC and NHE. *Am J Physiol Heart Circ Physiol* 275: H805–H813, 1998.
18. **Russ U, Balser C, Scholz W, Albus U, Lang HJ, Weichert A, Schölkens BA, and Gögelein H.** Effects of the Na^+/H^+-exchange inhibitor Hoe 642 on intracellular pH, calcium and sodium in isolated rat ventricular myocytes. *Pflugers Arch* 433: 26–34, 1996.
19. **Scholz W, Albus U, Counillon L, Gögelein H, Lang HJ, Linz W, Weichert A, and Scholkens BA.** Protective effects of HOE642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. *Cardiovasc Res* 29: 260–268, 1995.
20. **Sugiyama A, Aye NN, Sawada N, and Hashimoto K.** Cariporide, a highly selective Na^+/H^+ exchange inhibitor, suppresses the reperfusion-induced lethal arrhythmias and overshoot phenomenon of creatine phosphate in situ rat heart. *J Cardiovasc Pharmacol* 33: 116–121, 1999.
21. **Weiss RG, Lakatta EG, and Gerstenblith G.** Effects of amiloride on metabolism and contractility during reoxygenation in perfused rat hearts. *Circ Res* 66: 1012–1022, 1990.