Na^+/H^+ Exchange

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Contents

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Inhibition of Na^+/H^+ Exchange

Purpose and Rationale

Na^+/H^+ exchange was first described by Murer et al. (1976) in a study of intestinal and renal brush-border vesicles. The plasma membrane Na^+/H^+ exchanger is an ubiquitous pH-regulating cellular ion transport system. It is driven by the Na^+ gradient and extrudes protons from the cytosol in exchange for extracellular Na^+ ions (Aronson 1985; Frelin et al. 1988; Fliegel and Dyck 1995; Orlowski and Grinstein 1997; Wakabayshi et al. 1997; Dibrov and Fliegel 1998). Six mammalian Na^+/H^+ exchangers, NHE1, NHE2, NHE3, NHE4, NHE5 (Attaphitaya et al. 1999; Szabo et al. 2000), and NHE6, have been described (Tse et al. 1994; Orlowski 1999; Counillon and Pouysségur 2000).

In cardiac tissue the exchanger has a major role in the control of intracellular pH. At the onset of cardiac ischemia and during reperfusion, Na^+/H^+ exchange is excessively activated by low intracellular pH. Since the deleterious Na^+ influx in this condition was found to originate mainly from Na^+/H^+ exchange (Frelin et al. 1984; Schömig et al. 1988), the exchanger seems to be responsible for an increase of cytosolic sodium in ischemic cells. The accumulation of intracellular Na^+ causes an activation of Na^+/K^+ ATPase (Frelin et al. 1984; Rasmussen et al. 1989) which in turn increases ATP consumption.

During ischemia the aerobic metabolism of glucose terminates in lactic acid. A vicious circle leads to a further decrease of intracellular pH and to a further activation of Na^+/H^+ exchange, resulting in energy depletion, cellular Na^+ overload, and, finally due to the coupling of Na^+ and Ca^{2+} transport via Na^+/Ca^{2+} exchange, cellular Ca^{2+} overload (Lazdunski et al. 1985; Tani and Neely 1990; Scholz and Albus 1993). Especially in ischemic cardiac tissue, where Na^+/H^+ exchange is the predominant pH-regulating ion transport system (Weissberg et al. 1989), these pathological events can lead to increased excitability and precipitation of cellular death. Therefore, it is desirable to find potent and well-tolerated inhibitors of Na^+/H^+ exchange which should be able to interrupt this vicious cycle, to conserve cellular energy stores and to diminish cellular excitability and necrosis during cardiac ischemia. Such effects have been found with relatively weak inhibitors of Na^+/H^+ exchange at high toxic doses, such as amiloride and ethyl isopropyl amiloride (Scholz et al. 1992).

The myocardial Na^+/H^+ exchanger is regarded as a therapeutic target for the prevention of myocardial ischemic and reperfusion injury and attenuation of postinfarction heart failure (Karmazyn et al. 2001).

More potent Na^+/H^+ exchange inhibitors showed beneficial effects on ischemia/reperfusion injury (see sections "Coronary Artery Ligation, Reperfusion Arrhythmia and Infarct Size in Rats" and "Ventricular Arrhythmia After Coronary Occlusion," chapter "▶ Cardiovascular Analysis In Vivo") in rats (Aye et al. 1997; Myers et al. 1998; Aihara et al. 2000), dogs (Gumina et al. 1998, 2000), and pigs (Portman et al. 2001). Heart hypertrophy and heart failure after myocardial infarction are reduced (Yoshida and Karmazyn 2000; Kusumoto et al. 2001). Ischemia-induced apoptosis in isolated rat hearts is attenuated by sodium–hydrogen exchange inhibitors (Chakrabarti et al. 1997).

Linz and Busch (2003) demonstrated the effects of NHE1 inhibition from protection during acute ischemia/reperfusion to prevention of myocardial remodeling.

References and Further Reading

Aihara K, Hisa H, Sato T, Yoneyama F, Sasamori J, Yamaguchi F, Yoneyama S, Mizuno Y, Takahashi A, Nagai A, Kimura T, Kogi K, Sato S (2000) Cardioprotective effect of TY-12533, a novel Na^+-H^+ exchange inhibitor on ischemia/reperfusion injury. Eur J Pharmacol 404:221–229

Aronson PS (1985) Kinetic properties of the plasma membrane Na^+-H^+ exchanger. Annu Rev Physiol 47:545–560

Attaphitaya S, Park K, Melvin JE (1999) Molecular cloning and functional expression of a rat

Na^+/H^+ exchanger (NHE5) highly expressed in brain. J Biol Chem 274:4383–4388

Aye NN, Xue YX, Hashimoto K (1997) Antiarrhythmic effects of, a novel Na^+/H^+ exchange inhibitor, on reperfusion ventricular arrhythmias in rat hearts. Eur J Pharmacol 339:121–127

Chakrabarti S, Hoque ANE, Karmazy M (1997) A rapid ischemia-induced apoptosis in isolated rat hearts and its attenuation by the sodium-hydrogen exchange inhibitor HOE 642 (cariporide). J Mol Cell Cardiol 29:3169–3174

Counillon L, Pouysségur J (2000) The expanding family of eukaryotic Na^+-H^+ exchangers. J Biol Chem 275:1–4

Dibrov P, Fliegel L (1998) Comparative molecular analysis of Na^+/H^+ exchangers: a unified model for Na^+/H^+ antiport? FEBS Letters 424:1–5

Fliegel L, Dyck JRB (1995) Molecular biology of the cardiac sodium/hydrogen exchanger. Cardiovasc Res 29:155–159

Frelin C, Vigne P, Lazdunski M (1984) The role of Na^+/H^+ exchange system in cardiac cells in relation to the control of the internal Na^+ concentration. J Biol Chem 259:8880–8885

Frelin C, Vigne P, Ladoux A, Lazdunski M (1988) The regulation of intracellular pH in cells from vertebrates. Eur J Biochem 174:3–14

Gumina RJ, Mizumura T, Beier N, Schelling P, Schultz JJ, Gross GJ (1998) A new sodium/hydrogen exchange inhibitor, EMD 85131, limits infarct size in dogs when administered before or after coronary artery occlusion. J Pharmacol Exp Ther 286:175–183

Gumina RJ, Daemmgen J, Gross GJ (2000) Inhibition of the Na^+/H^+ exchanger attenuates phase 1b ischemic arrhythmias and reperfusion-induced ventricular fibrillation. Eur J Pharmacol 396:119–124

Karmazyn M, Sostaric JV, Gan XT (2001) The myocardial Na^+/H^+ exchanger: a therapeutic target for the prevention of myocardial ischaemic and reperfusion injury and attenuation of postinfarction heart failure. Drugs 61:375–389

Kusumoto K, Haist JV, Karmazyn M (2001) Na^+/H^+ exchange inhibition reduces hypertrophy and heart failure after myocardial infarction in rats. Am J Physiol (Heart Circ Physiol) 280: H738–H745

Lazdunski M, Frelin C, Vigne P (1985) The sodium/hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulation internal concentrations of sodium and internal pH. J Mol Cell Cardiol 17:1029–1042

Linz WJ, Busch AE (2003) NHE-1 inhibition: from protection during acute ischaemia/reperfusion to prevention of myocardial remodelling. Naunyn Schmiedebergs Arch Pharmacol 368:239–246

Murer H, Hopfer U, Kinne R (1976) Sodium/proton antiport in brush-border-membranes isolated from rat small intestine and kidney. Biochem J 154:597–604

Myers ML, Farhangkhoee P, Karmazyn M (1998) Hydrogen peroxide induced impairment of postischemic ventricular function is prevented by the sodium-hydrogen exchange inhibitor HOE &42 (cariporide). Cardiovasc Res 40:290–296

Orlowski J (1999) Na^+/H^+ exchangers. Molecular diversity and relevance to the heart. Ann N Y Acad Sci 874:346–353

Orlowski J, Grinstein S (1997) Na^+/H^+ exchangers in mammalian cells. J Biol Chem 272:22373–22776

Portman MA, Panos AL, Xiao Y, Anderson DL, Ning X-H (2001) HOE-642 (cariporide) alters pHi and diastolic function after ischemia during reperfusion in pig hearts in situ. Am J Physiol (Heart Circ Physiol) 280:H830–H834

Rasmussen HH, Cragoe EJ, Ten Eick RE (1989) Na^+-dependent activation of Na^+-K^+ pump in human myocardium during recovery from acidosis. Am J Physiol 256:H431–H439

Scholz W, Albus U (1993) Na^+/H^+ exchanger exchange and its inhibition in cardiac ischemia and reperfusion. Basic Res Cardiol 88:443–455

Schömig A, Kurz T, Richardt G, Schömig E (1988) Neuronal sodium homeostasis and axoplasmic amine concentration determine

calcium-independent noradrenaline release in normoxic and ischemic rat heart. Circ Res 63:214–226

Szabo EZ, Numata M, Shull GE, Orlowski J (2000) Kinetic and pharmacological properties of a human brain Na^+/H^+ exchanger isoform 5 stably expressed in Chinese hamster ovary cells. J Biol Chem 275:6302–6307

Tani M, Neely JR (1990) Na^+ accumulation increases Ca^{+2} overload and impairs function in anoxic rat heart. J Mol Cell Cardiol 22:57–72

Tse CM, Levine SA, Yun CHC, Brant SR, Nath S, Pouysségur J, Donowitz M (1994) Molecular properties, kinetics and regulation of mammalian Na^+/H^+ exchangers. Cell Physiol Biochem 4:282–300

Wakabayshi S, Shigekawa M, Pouysségur J (1997) Molecular physiology of vertebrate Na^+/H^+ exchangers. Physiol Rev 77:51–74

Weissberg PL, Little PJ, Cragoe EJ, Bobik A (1989) The pH of spontaneously beating cultured rat hearts is regulated by an ATP-calmodulin-dependent Na^+/H^+ antiport. Circ Res 64:676–685

Yoshida H, Karmazyn M(2000) Na^+/H^+ exchange inhibition attenuates hypertrophy and heart failure in 1-wk postinfarction rat myocardium. Am J Physiol (Heart Circ Physiol) 278: H300–H304

Inhibition of Na^+/H^+ Exchange in Thrombocytes

Purpose and Rationale

The inhibition of Na^+/H^+ exchange has been studied in platelets by measuring the optical density after osmotic cell swelling (Rosskopf et al. 1991).

Procedure

About 5 ml blood is withdrawn by venipuncture from human donors or from the vena jugularis externa of Beagle dogs or from the aorta of anesthetized Wistar rats (weighing 250–350 g). Coagulation is inhibited by 0.8 ml citrate acid dextrose (65 mM citric acid, 11 mM glucose, 85 mM trisodium citrate). Platelet-rich plasma (PRP) is obtained by centrifugation of whole blood at 90 g for 10 min at room temperature. Platelet count is measured, e.g., with a Casey 1 multichannelyser (Schärfe System, Reutlingen, Germany).

Each of the experiments is performed with 10–50 µl PRP containing 20×10^6 platelets in a volume of 100 µl with saline. To activate Na^+/H^+ exchange in the platelets by intracellular acidification, 500 µl propionate buffer (135 mM Na propionate, 1 mM HCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, 20 mM HEPES, pH 6.7, 22 °C) is added to the PRP/NaCl solution. Swelling of the platelets results in a decrease of optical density which can be measured with an aggregometer, e.g., with a Turbitimer (Behringwerke, Marburg, Germany). The system is activated photometrically by the addition of the propionate buffer to the cuvette. The experiments are performed with and without the addition of the Na^+/H^+ exchange inhibitor to be tested. The inhibitors are added in concentrations between 10^{-4} and 10^{-8} mol/l. 5-(*N*-Ethyl-isopropyl)amiloride (EIPA) is used as standard. During the experiments all solutions are kept at 22 °C in a temperature-controlled water bath.

Evaluation

Results are given as means ± SD. Student's *t*-test is employed for statistical evaluation. IC_{50} values are calculated from dose–response curves.

References and Further Reading

Rosskopf D, Morgenstern E, Scholz W, Osswald U, Siffert W (1991) Rapid determination of the elevated Na^+-K^+ exchange in platelets of patients with essential hypertension using an optical swelling assay. J Hypertens 9:231–238

Inhibition of Na^+/H^+ Exchange in Cholesterol-Activated Rabbit Erythrocytes

Purpose and Rationale

The inhibition of Na^+/H^+ exchange has been studied in cholesterol-activated rabbit erythrocytes by flame photometry of sodium (Scholz et al. 1992, 1993).

Procedure

White rabbits (New Zealand strain, Ivanovas) are fed with a rabbit standard chow with 2 % cholesterol for 6 weeks to increase the Na^+/H^+ exchange (Scholz et al. 1990) and to make the erythrocytes suitable for measurement of sodium influx via Na^+/H^+ exchange by flame photometry. Blood is drawn from the ear artery of the rabbits and coagulation prevented with 25 IU/ml potassium heparin. The hematocrit of the samples is determined in duplicate by centrifugation. Aliquots of 100 µl are taken to measure the initial sodium content of the erythrocytes.

To determine the amiloride-sensitive sodium influx into erythrocytes, 100 µl of each blood sample is added to 5 ml of buffer made hyperosmolar by sucrose (140 mM NaCl, 3 mM KCl, 150 mM sucrose, 0.1 mM ouabain, 20 mM tris(hydroxymethyl)aminomethane, pH 7.4) and incubated for 60 min at 37 °C. Subsequently, the erythrocytes are washed three times in ice-cold $MgCl_2$–ouabain solution (112 mM $MgCl_2$, 0.1 mM ouabain).

For determination of intracellular sodium content, the cells are hemolyzed in distilled water, the cell membranes are centrifuged, and the sodium concentration of the hemolysate is measured by flame photometry. Net influx of sodium into the erythrocytes is calculated from the difference between the initial sodium content and the sodium content after incubation. Amiloride-sensitive sodium influx is calculated from the difference between sodium content of erythrocytes incubated with and without amiloride (3×10^{-4} M). Each experiment is done with the erythrocytes from six different animals. In each case, the comparison of Na^+ contents is based on erythrocytes from the same animal. Doses between 10^{-4} and 10^{-7} M of the inhibitor are tested.

Evaluation

Statistical analysis of the data obtained is performed with Student's *t*-test for paired groups. IC_{50} values are calculated from dose–response curves.

References and Further Reading

Scholz W, Albus U, Hropot M, Klaus E, Linz W, Schölkens BA (1990) Zunahme des Na^+/H^+-Austausches an Kaninchenerythrozyten unter atherogener Diät. In: Assmann G, Betz E, Heinle H, Schulte H (eds) Arteriosklerose – Neue Aspekte aus Zellbiologie und Molekulargenetik. Epidemiologie und Klinik. Vieweg, Braunschweig/Wiesbaden, pp 296–302

Scholz W, Albus U, Linz W, Martorana P, Lang HJ, Schölkens BA (1992) Effects of Na^+/H^+ exchange inhibitors in cardiac ischaemia. J Mol Cell Cardiol 24:731–740

Scholz W, Albus U, Lang HJ, Linz W, Martorana PA, Englert HC, Schölkens BA (1993) Hoe 694, a new Na^+/H^+ exchange inhibitor and its effects in cardiac ischemia. Br J Pharmacol 109:562–568

Sodium Influx into Cultured Cardiac Myocytes

Purpose and Rationale

The inhibition of Na^+/H^+ exchange has been studied in cultured cardiac myocytes (Scholz et al. 1992).

Procedure

Rat myocardial cells are isolated from hearts of neonatal rats by trypsin digestion. The cells are cultured in 35 mm dishes and grown to confluence in Dulbecco's minimum essential medium (DMEM, GIBCO) in an atmosphere containing 10 % CO_2.

After confluence, the cells are used for measurement of $^{22}Na^+$ influx. The cells are washed twice with Krebs–Ringer solution buffered with HEPES/Tris (KRB) in which sodium chloride has been replaced by choline chloride (choline chloride 130 mM, $CaCl_2$ 1.5 mM, KCl 5 mM, $MgCl_2$ 1 mM, HEPES 20 mM, pH 7.0 with Tris) and then incubated for 20 min at 37 °C in the same buffer with added 0.1 % bovine serum albumin (BSA) and 10 mM/l glucose. The culture dishes are then incubated for another 10 min with Na^+ propionate for cytosolic acidification and stimulation of Na^+/H^+ exchange. The compounds are dissolved in 500 µl/dish KRB in which 50 % of the sodium chloride has been replaced by choline chloride containing additionally 2 µCi/ml $^{22}Na^+$ bicarbonate and 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA). After the stimulation period, sodium influx is terminated by washing the cells twice with ice-cold stop solution (0.1 mM $MgCl_2$, 10 mM Tris, pH 7.0). Subsequently, the cells are lysed with 250 µl trichloroacetic acid and scraped from the dishes. Radioactivity is determined in a Packard gamma counter. Doses between 3×10^{-4} and 10^{-8} mM/l of standard and new compounds are tested. Six dishes are used for each concentration of test compounds.

Evaluation

Mean values ± SD are compared with Student's *t*-test. IC_{50} values are calculated from dose–response curves.

References and Further Reading

Scholz W, Albus U, Linz W, Martorana P, Lang HJ, Schölkens BA (1992) Effects of Na^+/H^+ exchange inhibitors in cardiac ischaemia. J Mol Cell Cardiol 24:731–740

Inhibition of Na^+/H^+ Exchange into Cultured Aortic Endothelial Cells

Purpose and Rationale

The inhibition of Na^+/H^+ exchange has been studied in endothelial cells (Scholz and Albus 1993) by measuring the $^{22}Na^+$ influx.

Procedure

Bovine aortic endothelial cells (BAEC) are isolated by dispase digestion form bovine aorta obtained from animals killed at the local slaughter house. The cells are cultured in 35 mm dishes and grown to confluence in Dulbecco's minimum essential medium (DMEM, GIBCO) in an atmosphere with 10 % CO_2. Three days after confluence, the cells are used for measurement of $^{22}Na^+$ influx. The cells are washed twice with Krebs–Ringer solution buffered with HEPES/Tris (KRB) in which sodium chloride has been replaced by choline chloride (choline chloride 130 mM, CaCl2 1.5 mM, KCl 5 mM, $MgCl_2$ 1 mM, HEPES 20 mM, pH 7.0 with Tris) and then incubated for 20 min at 37 °C in the same buffer with added 0.1 % bovine serum albumin (BSA) and 10 mM glucose. To stimulate Na^+/H^+ exchange, the culture dishes are incubated for another 10 min with 500 µl/dish KBR in which all sodium chloride has been replaced by 65 mM each of choline chloride and Na^+ propionate or with KBR in which 50 % of the sodium chloride has been replaced by choline chloride for unstimulated controls. In addition, the buffer contains 2 µCi/ml $^{22}Na^+$ and the test compounds or the standard. After the stimulation period, the sodium influx is terminated by washing the cells twice with ice-cold stop solution (0.1 mM $MgCl_2$, 10 mM Tris, pH 7.0). Subsequently, the cells are lysed with 250 µl trichloroacetic acid and scraped from the dishes. Radioactivity is determined in a Packard gamma counter. Doses between 10^{-5} and 10^{-7} mM/l of standard and new compounds are tested. Six dishes are used for each concentration of test compounds.

Evaluation

Mean values ± SD are compared with Student's *t*-test. IC_{50} values are calculated from dose–response curves.

Modifications of the Method

Ewart et al. (1997) studied lipoprotein lipase activity in cultured rat cardiomyocytes in the presence of insulin and dexamethasone.

References and Further Reading

Benos DJ, Cunningham S, Baker RR, Beason KB, Oh Y, Smith PR (1992) Molecular characteristics of amiloride-sensitive sodium channels. Rev Physiol Biochem Pharmacol 120:31–113

Ewart HS, Carroll R, Severson DL (1997) Lipoprotein lipase activity in rat cardiomyocytes is stimulated by insulin and dexamethasone. Biochem J 327:439–442

Frelin C, Vigne P, Lazdunski M (1985) The role of Na^+/H^+ exchange system in the regulation of internal pH in cultured cardiac cells. Eur J Biochem 149:1–4

Frelin C, Vigne P, Breittmayer JP (1990) Mechanism of the cardiotoxic action of palytoxin. Mol Pharmacol 38:904–909

Jean T, Frelin C, Vigne P, Lazdunski M (1986) The Na^+/H^+ exchange system in glial cell lines. Properties and activation by an hyperosmotic shock. Eur J Biochem 160:211–219

Scholz W, Albus U (1993) Na^+/H^+ exchange and its inhibition in cardiac ischemia and reperfusion. Basic Res Cardiol 88:443–455

NHE Activity Measured by Intracellular pH in Isolated Ventricular Myocytes

Purpose and Rationale

Changes of the intracellular pH of cultured bovine endothelial cells have been fluorometrically monitored using the pH-dye 2′,7′-*bis*(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) by Kitazono et al. (1988). This method has been used to study the activity of inhibitors of Na^+/H^+ exchange (Scholz et al. 1995).

Procedure

For preparation of isolated rat ventricular muscular cells (Yazawa et al. 1990), hearts of male Wistar rats are dissected, mounted on a Langendorff apparatus and perfused first at 37 °C for 3 min with Tyrode solution adjusted to pH 7.4, second for 5–7 min with nominally calcium-free Tyrode solution, and finally with calcium-free Tyrode solution containing 0.12–0.2 mg/ml collagenase (Sigma type I). After 15–20 min collagenase treatment, the heart is washed with storage solution (composition in mmol/L: KOH 70, L-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 20, HEPES 10, and EGTA 0.5, pH 7.4). The ventricles are cut into small pieces, and myocytes are dispersed by gently shaking and finally by filtration through a nylon mesh (365 µm). Thereafter, the cells are washed twice by centrifugation at 600–1,000 rpm for 5 min and kept at 4 °C until use. For the pH recovery experiment, the cells are loaded with the membrane-permeable acetoxymethyl ester (AM) form of the fluorescent indicator 2′,7′-*bis*(carboxyethyl)-5(6)-carboxyfluorescein (BCECF). BCECF-AM is dissolved in DMSO and diluted to a 1.25 µM storage solution. Cardiomyocytes are loaded in this solution for 30 min at room temperature and are then centrifuged and resuspended in storage solution. The measurements are performed in bicarbonate-free NaCl solution (NaCl 140, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1, glucose 10, and HEPES 10 mM/L, pH 7.4) at 34 °C using an apparatus according to Nitschke et al. (1991). The pH-dependent signal of BCECF is obtained by illuminating at 490 and 437 nm and dividing the emitted light signals (520–560 nm). The background signal, determined by closing the shutter, is subtracted from the total signal. The autofluorescence determined by illuminating unloaded cells can be ignored. In order to investigate the function of the Na^+/H^+ exchange system, the intracellular pH (pH_i) of the cells is decreased by the NH_4Cl prepulse technique, and the rate of return to resting pH_i is determined. Test compounds are dissolved in the incubation medium. For each test concentration, the recovery of pH_i is first recorded in control NaCl solution.

Evaluation

Data are analyzed by fitting a straight line to the initial (5 min) data points of the pH

recovery curve. For statistical presentation, the slopes of the linear curves are demonstrated. All reported data are presented as means ± SEM. Statistical comparisons are made using either a paired or unpaired *t*-test.

Modifications of the Method

The pH-sensitive fluorescence dye C-SNARF-1 (= carboxy-seminaphthorhodafluor-1) was used by Yasutake et al. (1996), Shipolini et al. (1997), and Yokoyama et al. (1998).

Fischer et al. (1999) tested new drugs for the Na^+/H^+ exchanger in Chinese hamster ovary cells which are enriched with the NHE1 isoform of the Na^+/H^+ antiporter. The Na^+/H^+ exchanger was stimulated with NaCl, and the rate of extracellular acidification was quantified with the cytosensor.

References and Further Reading

Fischer H, Seelig A, Beier N, Raddatz P, Seelig J (1999) New drugs for the Na^+/H^+ exchanger. Influence of Na^+ concentration and determination of inhibition constants with a microphysiometer. J Membr Biol 1:39–45

Kitazono T, Takeshige K, Cragoe EJ, Minakami S (1988) Intracellular pH changes of cultured bovine aortic endothelial cells in response to ATP addition. Biochem Biophys Res Commun 152:1304–1309

Nitschke R, Fröbe U, Greger R (1991) Antidiuretic hormone acts via V_1 receptor in intracellular calcium in the isolated perfused rabbit cortical thick ascending limb. Pflügers Arch 417:622–632

Scholz W, Albus U, Counillon L, Gögelein H, Lang H-J, Linz W, Weichert A, Schölkens BA (1995) Protective effects of HOE642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. Cardiovasc Res 29:260–268

Shipolini AR, Yokoyama H, Galiñanes M, Edmondson SJ, Hearse DJ, Avkiran M (1997) Na^+/H^+ exchanger activity does not contribute to protection by ischemic preconditioning in the isolated rat heart. Circulation 96:3617–3625

Yasutake M, Haworth RS, King A, Avkiran M (1996) Thrombin activates the sarcolemmal Na^+/H^+ exchanger: evidence for a receptor-mediated mechanism involving protein kinase C. Circ Res 79:705–715

Yazawa K, Kaibara M, Ohara M, Kamayama M (1990) An improved method for isolating cardiac myocytes useful for patch-clamp studies. Jpn J Physiol 40:157–163

Yokoyama M, Yasutake M, Avkiran M (1998) α_1-adrenergic stimulation of sarcolemmal Na^+/H^+ exchanger activity in rat ventricular myocytes: evidence for selective mediation by the α_{1A}-adrenoceptor subtype. Circ Res 82:1078–1985

NHE Subtype Specificity

Purpose and Rationale

Molecular identification of mammalian Na^+/H^+ exchanger subtypes has been pioneered by Pouysségour and coworkers (Sardet et al. 1989) who used genetic complementation of fibroblast cell lines that lack all endogenous NHEs. Schwark et al. (1998) studied an inhibitor of Na^+/H^+ exchanger subtype 3 in various cell types.

Procedure

cDNAs for the NHE subtypes human NHE1, rabbit NHE2, rat NHE3 (Pouysségour) or cloned by reverse transcription-polymerase chain reaction from human kidney mRNA are used. These cDNAs are cloned into the mammalian expression vector pMAMneo and transferred into the NHE-deficient mouse fibroblast cell line LAP1. Cells expressing the NHE subtypes are selected by the acid load survival method (Sardet et al. 1989). Clonal cell lines for each subtype are used for intracellular pH (pH_i) recovery after acid load. For studies of pH_i recovery (Faber et al. 1996), cells are scraped off the culture dishes, washed, and incubated with 5 μmol/l

BCECF-AM [2′,7′-*bis*(2-carboxyethyl)-5(6)-carboxyfluorescein-acetoxymethyl ester] for 20 min at 37 °C in a buffer containing 20 mM NH_4Cl. The cells are then washed to remove extracellular dye and resuspended in the loading buffer without BCECF-AM. Intracellular acidification is induced by addition of 975 µl NH_4Cl-free and HCO_3^--free solution (the so-called recovery medium: HCO_3^--free to inhibit the Na^+-dependent Cl^-/HCO_3^- exchanger of LAP1 cells) to a 25 µl aliquot of cells (≅25,000 cells). The pH_i recovery is recorded with a dual-grating DeltaScan single-photon counting fluorometer (Photon Technology International, South Brunswick, NJ, USA) with excitation wavelength of 505 and 440 nm and an emission wavelength of 535 nm. The measurement time varies between subtypes (120 s for NHE1, 300 s for NHE2, 180 s for NHE3). The inhibitors are first dissolved in DMSO, diluted in recovery medium, and added in a volume of 975 µl to this medium.

A cloned opossum kidney cell line (Helmle-Kolb et al. 1990) is used additionally. Cells are grown as a monolayer in growth medium (1:1 mixture of nutrient mixture Ham F12 and Dulbecco's modified medium Eagle with 10 % fetal calf serum). For subcultivation and pH-recovery experiments, the cells are detached from the surface of the culture vessels with trypsin–EDTA solution (2.5 g trypsin + 0.2 g EDTA per liter in Dulbecco's phosphate-buffered saline) and suspended in growth medium. Measurement time in pH_i recovery experiments is 400 s.

Porcine renal brush-border membrane vesicles (BBMV) prepared by a Mg^{2+} precipitation technique are loaded with 150 mmol/l NaCl, 5 mmol/l HEPES/Tris, pH 7.0, and preincubated for 10 min at 37 °C with various concentrations of NHE inhibitors. Intravesicular acidification through Na^+/H^+ exchange is started by diluting BBMV into Na^+-free buffer (150 mmol/l tetramethylammonium chloride, 5 mmol HEPES/Tris, pH 7.0) containing the appropriate concentrations of the NHE inhibitors and the fluorescent ΔpH indicator acridine orange (12 µmol/l). The fluorescence changes of acridine orange are recorded continuously by a Hitachi F-2000 spectrofluorometer at 495 nm excitation and 525 nm emission wavelength. The initial acridine orange fluorescence quenching in controls (no inhibitor) is set to 100 %.

Evaluation

Values are presented as means ± SD (four measurement per concentration). The IC_{50} values and Hill coefficients are calculated using the SigmaPlot software. Statistical significance is calculated by means of the distribution-independent *H*-test and nonparametric *U*-test. $P < 0.05$ is considered as significant.

Modifications of the Method

Counillon et al. (1993) and Scholz et al. (1995) determined the NHE subtype specificity of Na^+/H^+ antiporters by their ability to inhibit initial rates of amiloride-sensitive $^{22}Na^+$ uptake in fibroblast cell lines separately expressing the NHE1, NHE2, and NHE3 isoforms.

Ko et al. (2004) determined the inhibitory effects of flavonoids on phosphodiesterase isozymes from guinea pig and their structure–activity relationships.

References and Further Reading

Counillon LT, Scholz W, Lang HJ, Pouysségour J (1993) Pharmacological characterization of stably transfected Na^+/H^+ antiporter isoforms using amiloride analogs and a new inhibitor exhibiting anti-ischemic properties. Mol Pharmacol 44:1041–1045

Faber S, Lang HJ, Hropot M, Schölkens BA, Mutschler E (1996) A novel screening assay of the Na^+-dependent $Cl^-/HCO3^-$ exchanger (NCBE) and its inhibitors. Cell Physiol Biochem 6:39–49

Helmle-Kolb C, Montrose MH, Stange G, Murer H (1990) Regulation of Na^+/H^+ exchange in opossum kidney cells by parathyroid hormone, cyclic AMP and phorbol esters. Pflügers Arch 415:461–470

Ko WC, Shih CM, Lai YH, Chen JH, Huang HL (2004) Inhibitory effects of flavonoids on phosphodiesterase isozymes from guinea pig and their structure–activity relationships. Biochem Pharmacol 68:2087–2094

Sardet C, Franchi A, Pouysségour J (1989) Molecular cloning, primary structure, and expression of the human growth factor-activable Na^+/H^+ antiporter. Cell 56:271–280

Scholz W, Albus U, Counillon L, Gögelein H, Lang H-J, Linz W, Weichert A, Schölkens BA (1995) Protective effects of HOE642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. Cardiovasc Res 29:260–268

Schwark JR, Jansen HW, Lang HJ, Krick W, Burckhardt G, Hropot M (1998) S3226, a novel inhibitor of Na^+/H^+ exchanger subtype 3 in various cell types. Pflügers Arch 436:797–800

References and Further Reading

Inhibition of Na^+/H^+ Exchange

Aihara K, Hisa H, Sato T, Yoneyama F, Sasamori J, Yamaguchi F, Yoneyama S, Mizuno Y, Takahashi A, Nagai A, Kimura T, Kogi K, Sato S (2000) Cardioprotective effect of TY-12533, a novel Na^+-H^+ exchange inhibitor on ischemia/reperfusion injury. Eur J Pharmacol 404:221–229

Aronson PS (1985) Kinetic properties of the plasma membrane Na^+-H^+ exchanger. Annu Rev Physiol 47:545–560

Attaphitaya S, Park K, Melvin JE (1999) Molecular cloning and functional expression of a rat Na^+/H^+ exchanger (NHE5) highly expressed in brain. J Biol Chem 274:4383–4388

Aye NN, Xue YX, Hashimoto K (1997) Antiarrhythmic effects of cariporide, a novel Na^+/H^+ exchange inhibitor, on reperfusion ventricular arrhythmias in rat hearts. Eur J Pharmacol 339:121–127

Chakrabarti S, Hoque ANE, Karmazy M (1997) A rapid ischemia-induced apoptosis in isolated rat hearts and its attenuation by the sodium-hydrogen exchange inhibitor HOE 642 (cariporide). J Mol Cell Cardiol 29:3169–3174

Counillon L, Pouysségur J (2000) The expanding family of eukaryotic Na^+-H^+ exchangers. J Biol Chem 275:1–4

Dibrov P, Fliegel L (1998) Comparative molecular analysis of Na^+/H^+ exchangers: a unified model for Na^+/H^+ antiport? FEBS Lett 424:1–5

Fliegel L, Dyck JRB (1995) Molecular biology of the cardiac sodium/hydrogen exchanger. Cardiovasc Res 29:155–159

Frelin C, Vigne P, Lazdunski M (1984) The role of Na^+/H^+ exchange system in cardiac cells in relation to the control of the internal Na^+ concentration. J Biol Chem 259:8880–8885

Frelin C, Vigne P, Ladoux A, Lazdunski M (1988) The regulation of intracellular pH in cells from vertebrates. Eur J Biochem 174:3–14

Gumina RJ, Mizumura T, Beier N, Schelling P, Schultz JJ, Gross GJ (1998) A new sodium/hydrogen exchange inhibitor, EMD 85131, limits infarct size in dogs when administered before or after coronary artery occlusion. J Pharmacol Exp Ther 286:175–183

Gumina RJ, Daemmgen J, Gross GJ (2000) Inhibition of the Na^+/H^+ exchanger attenuates phase 1b ischemic arrhythmias and reperfusion-induced ventricular fibrillation. Eur J Pharmacol 396:119–124

Karmazyn M, Sostaric JV, Gan XT (2001) The myocardial Na^+/H^+ exchanger: a therapeutic target for the prevention of myocardial ischaemic and reperfusion injury and attenuation of postinfarction heart failure. Drugs 61:375–389

Kusumoto K, Haist JV, Karmazyn M (2001) Na^+/H^+ exchange inhibition reduces hypertrophy and heart failure after myocardial infarction in rats. Am J Physiol (Heart Circ Physiol) 280:H738–H745

Lazdunski M, Frelin C, Vigne P (1985) The sodium/hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulation internal concentrations of sodium and internal pH. J Mol Cell Cardiol 17:1029–1042

Linz WJ, Busch AE (2003) NHE-1 inhibition: from protection during acute ischaemia/reperfusion to prevention of myocardial remodelling. Naunyn Schmiedebergs Arch Pharmacol 368:239–246

Murer H, Hopfer U, Kinne R (1976) Sodium/proton antiport in brush-border-membranes isolated from rat small intestine and kidney. Biochem J 154:597–604

Myers ML, Farhangkhoee P, Karmazyn M (1998) Hydrogen peroxide induced impairment of postischemic ventricular function is prevented by the sodium-hydrogen exchange inhibitor HOE &42 (cariporide). Cardiovasc Res 40:290–296

Orlowski J (1999) Na^+/H^+ exchangers. Molecular diversity and relevance to the heart. Ann N Y Acad Sci 874:346–353

Orlowski J, Grinstein S (1997) Na^+/H^+ exchangers in mammalian cells. J Biol Chem 272:22373–22776

Portman MA, Panos AL, Xiao Y, Anderson DL, Ning X-H (2001) HOE-642 (cariporide) alters pHi and diastolic function after ischemia during reperfusion in pig hearts in situ. Am J Physiol (Heart Circ Physiol) 280: H830–H834

Rasmussen HH, Cragoe EJ, Ten Eick RE (1989) Na^+-dependent activation of Na^+-K^+ pump in human myocardium during recovery from acidosis. Am J Physiol 256:H431–H439

Scholz W, Albus U (1993) Na^+/H^+ exchanger exchange and its inhibition in cardiac ischemia and reperfusion. Basic Res Cardiol 88:443–455

Schömig A, Kurz T, Richardt G, Schömig E (1988) Neuronal sodium homeostasis and axoplasmic amine concentration determine calcium-independent noradrenaline release in normoxic and ischemic rat heart. Circ Res 63:214–226

Szabo EZ, Numata M, Shull GE, Orlowski J (2000) Kinetic and pharmacological properties of a human brain Na^+/H^+ exchanger isoform 5 stably expressed in Chinese hamster ovary cells. J Biol Chem 275:6302–6307

Tani M, Neely JR (1990) Na^+ accumulation increases Ca^{+2} overload and impairs function in anoxic rat heart. J Mol Cell Cardiol 22:57–72

Tse CM, Levine SA, Yun CHC, Brant SR, Nath S, Pouysségur J, Donowitz M (1994) Molecular properties, kinetics and regulation of mammalian Na^+/H^+ exchangers. Cell Physiol Biochem 4:282–300

Wakabayshi S, Shigekawa M, Pouysségur J (1997) Molecular physiology of vertebrate Na^+/H^+ exchangers. Physiol Rev 77:51–74

Weissberg PL, Little PJ, Cragoe EJ, Bobik A (1989) The pH of spontaneously beating cultured rat hearts is regulated by an ATP-calmodulin-dependent Na^+/H^+ antiport. Circ Res 64:676–685

Yoshida H, Karmazyn M (2000) Na^+/H^+ exchange inhibition attenuates hypertrophy and heart failure in 1-wk postinfarction rat myocardium. Am J Physiol (Heart Circ Physiol) 278:H300–H304

Inhibition of Na+/H+ Exchange in Thrombocytes

Rosskopf D, Morgenstern E, Scholz W, Osswald U, Siffert W (1991) Rapid determination of the elevated Na^+-K^+ exchange in platelets of patients with essential hypertension using an optical swelling assay. J Hypertens 9:231–238

Inhibition of Na^+/H^+ Exchange in Cholesterol Activated Rabbit Erythrocytes

Scholz W, Albus U, Hropot M, Klaus E, Linz W, Schölkens BA (1990) Zunahme des Na^+/H^+-Austausches an Kaninchenerythrozyten unter atherogener Diät. In: Assmann G, Betz E, Heinle H, Schulte H (eds) Arteriosklerose – Neue Aspekte aus Zellbiologie und Molekulargenetik. Epidemiologie und Klinik. Vieweg, Braunschweig/Wiesbaden, pp 296–302

Scholz W, Albus U, Linz W, Martorana P, Lang HJ, Schölkens BA (1992) Effects of Na^+/H^+ exchange inhibitors in cardiac ischaemia. J Mol Cell Cardiol 24:731–740

Scholz W, Albus U, Lang HJ, Linz W, Martorana PA, Englert HC, Schölkens BA (1993) Hoe 694, a new Na^+/H^+ exchange inhibitor and its effects in cardiac ischemia. Br J Pharmacol 109:562–568

Sodium Influx into Cultured Cardiac Myocytes

Scholz W, Albus U, Linz W, Martorana P, Lang HJ, Schölkens BA (1992) Effects of Na^+/H^+ exchange inhibitors in cardiac ischaemia. J Mol Cell Cardiol 24:731–740

Inhibition of Na^+/H^+ Exchange into Cultured Aortic Endothelial Cells

Benos DJ, Cunningham S, Baker RR, Beason KB, Oh Y, Smith PR (1992) Molecular characteristics of amiloride-sensitive sodium channels. Rev Physiol Biochem Pharmacol 120:31–113

Ewart HS, Carroll R, Severson DL (1997) Lipoprotein lipase activity in rat cardiomyocytes is stimulated by insulin and dexamethasone. Biochem J 327:439–442

Frelin C, Vigne P, Lazdunski M (1985) The role of Na^+/H^+ exchange system in the regulation of internal pH in cultured cardiac cells. Eur J Biochem 149:1–4

Frelin C, Vigne P, Breittmayer JP (1990) Mechanism of the cardiotoxic action of palytoxin. Mol Pharmacol 38:904–909

Jean T, Frelin C, Vigne P, Lazdunski M (1986) The Na^+/H^+ exchange system in glial cell lines. Properties and activation by an hyperosmotic shock. Eur J Biochem 160:211–219

Scholz W, Albus U (1993) Na^+/H^+ exchange and its inhibition in cardiac ischemia and reperfusion. Basic Res Cardiol 88:443–455

NHE Activity Measured by Intracellular pH in Isolated Ventricular Myocytes

Fischer H, Seelig A, Beier N, Raddatz P, Seelig J (1999) New drugs for the Na^+/H^+ exchanger. Influence of Na^+ concentration and determination of inhibition constants with a microphysiometer. J Membr Biol 1:39–45

Kitazono T, Takeshige K, Cragoe EJ, Minakami S (1988) Intracellular pH changes of cultured bovine aortic endothelial cells in response to ATP addition. Biochem Biophys Res Commun 152:1304–1309

Nitschke R, Fröbe U, Greger R (1991) Antidiuretic hormone acts via V_1 receptor in intracellular calcium in the isolated perfused rabbit cortical thick ascending limb. Pflügers Arch 417:622–632

Scholz W, Albus U, Counillon L, Gögelein H, Lang H-J, Linz W, Weichert A, Schölkens BA (1995) Protective effects of HOE642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. Cardiovasc Res 29:260–268

Shipolini AR, Yokoyama H, Galiñanes M, Edmondson SJ, Hearse DJ, Avkiran M (1997) Na^+/H^+ exchanger activity does not contribute to protection by ischemic preconditioning in the isolated rat heart. Circulation 96:3617–3625

Yasutake M, Haworth RS, King A, Avkiran M (1996) Thrombin activates the sarcolemmal Na^+/H^+ exchanger: evidence for a receptor-mediated mechanism involving protein kinase C. Circ Res 79:705–715

Yazawa K, Kaibara M, Ohara M, Kamayama M (1990) An improved method for isolating cardiac myocytes useful for patch-clamp studies. Jpn J Physiol 40:157–163

Yokoyama M, Yasutake M, Avkiran M (1998) α_1-adrenergic stimulation of sarcolemmal Na^+/H^+ exchanger activity in rat ventricular myocytes: evidence for selective mediation by the α_{1A}-adrenoceptor subtype. Circ Res 82:1078–1985

NHE Subtype Specificity

Counillon LT, Scholz W, Lang HJ, Pouysségour J (1993) Pharmacological characterization of stably transfected Na^+/H^+ antiporter isoforms using amiloride analogs and a new inhibitor exhibiting anti-ischemic properties. Mol Pharmacol 44:1041–1045

Faber S, Lang HJ, Hropot M, Schölkens BA, Mutschler E (1996) A novel screening assay of the Na^+-dependent $Cl^-/HCO3^-$ exchanger (NCBE) and its inhibitors. Cell Physiol Biochem 6:39–49

Helmle-Kolb C, Montrose MH, Stange G, Murer H (1990) Regulation of Na^+/H^+ exchange in opossum kidney cells by parathyroid hormone, cyclic AMP and phorbol esters. Pflügers Arch 415:461–470

Ko WC, Shih CM, Lai YH, Chen JH, Huang HL (2004) Inhibitory effects of flavonoids on phosphodiesterase isozymes from guinea pig and their structure–activity relationships. Biochem Pharmacol 68:2087–2094

Sardet C, Franchi A, Pouysségour J (1989) Molecular cloning, primary structure, and expression of the human growth factor-activable Na^+/H^+ antiporter. Cell 56:271–280

Scholz W, Albus U, Counillon L, Gögelein H, Lang H-J, Linz W, Weichert A, Schölkens BA (1995) Protective effects of HOE642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. Cardiovasc Res 29:260–268

Schwark JR, Jansen HW, Lang HJ, Krick W, Burckhardt G, Hropot M (1998) S3226, a novel inhibitor of Na^+/H^+ exchanger subtype 3 in various cell types. Pflügers Arch 436:797–800