研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Tsutsumi S, Aburatani H, et al.	Two distinct gene expression signatures in pediatric acute lymphoblastic leukemia with *MLL* rearrangements.	Cancer Research	63(16)	4882-4887	2003
Sekoguchi E, Aburatani H, et al.	A novel mitochondrial carnitine-acylcarnitine translocase induced by partial hepatectomy and fasting.	J Biol Chem	278	38796 – 38802	2003
Ono R, Aburatani H, et al.	Identification of a large novel imprinted gene cluster on mouse proximal chromosome 6.	Genome Res	13(7)	1696-1705	2003
Satoh T, Aburatani H, et al.	Role of heme oxygenase-1 protein in the neuroprotective effects of cyclopentenone prostaglandin derivatives under oxidative stress.	Eur J Neurosci	17(11)	2249-2255	2003
Shimizu H, Aburatani H, et al.	Characterization of the mouse Abcc12 gene and its transcript encoding an ATP-binding cassette transporter, an orthologue of human ABCC12.	Gene	310	17-28	2003
Kashima T, Aburatani H, et al.	Overexpression of cadherins suppresses pulmonary metastasis of osteosarcoma in vivo.	Int J Cancer	104(2)	147-154	2003
Midorikawa Y, Aburatani H, et al.	Glypican-3, overexpressed in hepatocellular carcinoma, modulates FGF2 and BMP-7 signaling..	Int J Cancer	103(4)	455-465	2003

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Ohtsuka M, Komuro I, et al.	Role of Na^+-Ca^{2+} exchanger in myocardial ischemia/reperfusion injury: evaluation using a heterozygous Na^+-Ca^{2+} exchanger knockout mouse model.	Biochem Biophys Res Commun	314	849-853	2004
Miyauchi H, Komuro I, et al.	Akt negatively regulates the in vitro lifespan of human endothelial cells via a p53/p21-dependent pathway.	EMBO J	23	212-220	2004
Matsuura K, Komuro I, et al.	Adult cardiac Sca-1 positive cells differentiate into beating cardiomyocytes.	J Biol Chem	279	11384-11391	2004
Ohsawa Y, Komuro I, et al.	Overexpression of P104L mutant caveolin-3 in mice develops hypertrophic cardiomyopathy with enhanced contractility in association with increased endothelial nitric oxide synthase activity.	Hum Mol Genet	13	151-157	2004
Ohtsuka M, Komuro I, et al.	Cytokine therapy prevents left ventricular remodeling and dysfunction after myocardial infarction through neovascularization.	FASEB J	18	851-853	2004
Toko H, Komuro I, et al.	Angiotensin II Type 1a Receptor Is Involved in Cell Infiltration, Cytokine Production, and Neovascularization in Infarcted Myocardium.	Arterioscler Thromb Vasc Biol	24	664-670	2004
Akazawa H, Komuro I, et al.	A novel LIM protein Cal promotes cardiac differentiation by association with CSX/NKX2-5.	J Cell Biol	164	395-405	2004

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Kasai H, Komuro I, et al.	Direct measurement of Ca^{2+} concentration in the SR of living cardiac myocytes.	Biochem Biophys Res Commun	314	1014-1020	2004
Zou Y, Komuro I, et al.	Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II.	Nat Cell Biol	6	499-506	2004
Ogihara T, Komuro I, et al.	Oxidative stress induces insulin resistance by activating the nuclear factor-kappaB pathway and disrupting normal subcellular distribution of phosphatidylinositol 3-kinase.	Diabetologia	47	794-805	2004
Funabashi N, Komuro I, et al.	Images in cardiovascular medicine. Double aortic arch with a compressed trachea demonstrated by multislice computed tomography.	Circulation	110	e68-e69	2004
Hayashi D, Komuro I, et al.	Atrial natriuretic peptide inhibits cardiomyocyte hypertrophy through mitogen-activated protein kinase phosphatase-1.	Biochem Biophys Res Commun	322	310-319	2004
Funabashi N, Komuro I, et al.	New method of measuring coronary diameter by electron-beam computed tomographic angiography using adjusted thresholds determined by calibration with aortic opacity.	Circ J	68	769-777	2004

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Akazawa H, Komuro I, et al.	Diphtheria toxin-induced autophagic cardiomyocyte death plays a pathogenic role in mouse model of heart failure.	J Biol Chem	279	41095-41103	2004
Ikeda Y, Komuro I, et al.	Vasorin, a transforming growth factor beta-binding protein expressed in vascular smooth muscle cells, modulates the arterial response to injury in vivo.	Proc Natl Acad Sci U S A	101	10732-10737	2004
Matsuura K, Komuro I, et al.	Cardiomyocytes fuse with surrounding non-cardiomyocyres and re-enter the cell cycle.	J Cell Biol	167	351-363	2004
Naito, A.T, Komuro I, et al.	Steroid-responsive thromboangiitis obliterans.	Lancet	364	1098	2004
Iwanaga K, Komuro I, et al.	Effects of G-CSF on cardiac remodeling after acute myocardial infarction in swine.	Biochem Biophys Res Commun	325	1353-1359	2004
Iwamoto T, Komuro I, et al.	Salt-sensitive hypertension is triggered by Ca(2+) entry via Na(+)/Ca(2+) exchanger type-1 in vascular smooth muscle.	Nat Med	10	1193-1199	2004
Minamino T, Komuro I, et al.	Vascular cell senescence and vascular aging.	J Mol Cell Cardiol	36	175-183	2004
Minamino T, Komuro I, et al.	Akt-induced Cellular Senescence: Implication for Human Disease.	Cell Cycle	3	449-451	2004
Komuro I, Ohtsuka M.	Forefront of Na+/Ca2+ exchanger studies: role of Na+/Ca2+ exchanger--lessons from knockout mice.	J Pharmacol Sci	96	23-26	2004

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Horii T, Suma H, et al.	Residual fibrosis affects a long-term result of left ventricular volume reduction surgery for dilated cardiomyopathy in a rat experimental study.	Eur J Cardiothorac Surg	26	1174-1179	2004
Athanasuleas CL, Suma H, et al.	Surgical ventricular restoration in the treatment of congestive heart failure due to post-infarction ventricular dilation.	J Am Coll Cardiol	44	1439-1445	2004
Fujioka S, Suma H, et al.	Evidence of viral infection in the myocardium of American and Japanese patients with idiopathic dilated cardiomyopathy.	Am J Cardiol	94	602-605	2004
Ueyama K, Suma H, et al.	Development of biologic coronary artery bypass grafting in a rabbit model: revival of a classic concept with modern biotechnology.	J Thorac Cardiovasc Surg	127	1608-1615	2004
Takita J, Aburatani H, et al.	Gene expression profiling and identification of novel prognostic marker genes in neuroblastoma	Genes Chromosomes Cancer	40	120-132	2004

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Matsuo K, Aburatani H, et al.	NFAT rescues osteoclarstogenesis in precursors lacking c-Fos.	J Biol Chem	279	26475-26480	2004
Hippo Y, Aburatani H, et al.	Identification of Soluble Amino Terminal Fragment of Glypican-3 as a Serological Maker for Early Stage Hepatocellular Carcinoma.	Cancer Research	64	2418-2423	2004
Joo A, Aburatani H, et al.	STAT3 MITF cooperatively induce cellular transformation through upregulation of c-fos expression	Oncogene	23	726-734	2004

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Harada M, Komuro I, et al.	G-CSF prevents cardiac Remodeling after myocardial infarction by activating Jak/Stat in cardiomyocytes	Nat Med	11	305-311	2005
Naito AT, Komuro I, et al.	Phosphatidylinositol 3-Kinase-Akt Pathway Plays a Critical Role in Early Cardiomyogenesis by Regulating Canonical Wnt Signaling.	Circ Res	97	144-151	2005
Hasegawa R, Komuro I, et al.	Effect of mental stress on coronary flow velocity reserve in healthy men.	Am J Cardiol	96	137-140	2005
Akazawa H, Komuro I,et al.	Cardiac transcription factor Csx/Nkx2-5: Its role in cardiac development and diseases.	Pharmacol Ther	107	252-268	2005
Saegusa N, Komuro I, et al.	Kir6.2-deficient mice are susceptible to stimulated ANP secretion: K(ATP) channel acts as a negative feedback mechanism?	Cardiovasc Res	67	60-68	2005
Yokoyama M, Komuro I, et al.	Plasma low-density lipoprotein reduction and structural effects on coronary atherosclerotic plaques by atorvastatin as clinically assessed with intravascular ultrasound radio-frequency signal analysis: a randomized prospective study.	Am Heart J	150	287 e1-e7	2005
Urano A, Komuro I, et al.	Infertility with defective spermiogenesis in mice lacking AF5q31, the target of chromosomal translocation in human infant leukemia.	Mol Cell Biol	25	6834-6845	2005

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Daimon M, Komuro I, et al.	Physiologic assessment of coronary artery stenosis without stress tests: noninvasive analysis of phasic flow characteristics by transthoracic Doppler echocardiography.	J Am Soc Echocardiogr	18	949-955	2005
Nagai T, Komuro I, et al.	Promotion of cardiac regeneration by cardiac stem cells.	Circ Res	97	615-617	2005
Ueda M, Komuro I, et al.	Pulmonary vein morphology before and after segmental isolation in patients with atrial fibrillation.	Pacing Clin Electrophysiol	28	944-953	2005
Funabashi N, Komuro I, et al.	Large collateral conus branch to the left anterior descending branch of the coronary artery in a subject with angina pectoris demonstrated by multislice computed tomography.	Int J Cardiol	103	105-106	2005
NiitsumaY, Komuro I, et al.	Atherosclerotic right internal thoracic arterial aneurysm demonstrated by multislice computed tomography.	Int J Cardiol	106	270-272	2006
Funabashi N, Komuro I, et al.	Patency of gastroepiploic arterial graft to left circumflex branch with distal portion of the anastomotic site demonstrated by multislice computed tomography.	Int J Cardiol	107	130-131	2006

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻号	ページ	出版年
Ge X, Aburatani H, et al.	Interpreting expression profiles of cancers by genome-wide survey of breadth-of-expression in normal tissues.	Genomics	86	127-141	2005
Kano M, Aburatani H, et al.	A meta-clustering analysis indicates distinct pattern alteration between two series of Gene Expression profiles for induced ischemic tolerance in rats.	Physiological Genomics	21	274-283	2005
Komura D, Aburatani H, et al.	Multidimensional support vector machines for visualization of gene expression data.	Bioinformatics	21	439-444	2005
Muraoka H,Negoro N, Terasaki F, et al.	Re-entry circuit in ventricular tachycardia due to focal fatty-fibrosis in patients with myotonic dystrophy.	Internal Med	44	129-135	2005

0022-3565/03/3041-284–293$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 2003 by The American Society for Pharmacology and Experimental Therapeutics
JPET 304:284–293, 2003

Vol. 304, No. 1
39024/1032441
Printed in U.S.A.

Attenuation of Ischemia/Reperfusion-Induced Renal Injury in Mice Deficient in Na^+/Ca^{2+} Exchanger

JUNJI YAMASHITA, SATOMI KITA, TAKAHIRO IWAMOTO, MASAYA OGATA, MASANORI TAKAOKA, NAOKO TAZAWA, MITSUNORI NISHIKAWA, KOJI WAKIMOTO, MUNEKAZU SHIGEKAWA, ISSEI KOMURO, and YASUO MATSUMURA

Department of Pharmacology, Osaka University of Pharmaceutical Sciences, Osaka, Japan (J. Y., M. O., M. T., N. T., M. N., Y. M.); Department of Molecular Physiology, National Cardiovascular Center Research Institute, Osaka, Japan (S.K., T.I., M.S.); Discovery Research Laboratory, Tanabe Seiyaku Co. Ltd., Osaka, Japan (K.W.); and Third Department of Internal Medicine, Chiba University School of Medicine, Chiba, Japan (I.K.)

Received May 15, 2002; accepted October 1, 2002

ABSTRACT

Using Na^+/Ca^{2+} exchanger (NCX1)-deficient mice, the pathophysiological role of Ca^{2+} overload via the reverse mode of NCX1 in ischemia/reperfusion-induced renal injury was investigated. Because $NCX1^{-/-}$ homozygous mice die of heart failure before birth, we used $NCX1^{+/-}$ heterozygous mice. NCX1 protein in the kidney of heterozygous mice decreased to about half of that of wild-type mice. Expression of NCX1 protein in the tubular epithelial cells and Ca^{2+} influx via NCX1 in renal tubules were markedly attenuated in the heterozygous mice. Ischemia/reperfusion-induced renal dysfunction in heterozygous mice was significantly attenuated compared with cases in wild-type mice. Histological renal damage such as tubular necrosis and proteinaceous casts in tubuli in heterozygous mice were much less than that in wild-type mice. Ca^{2+} deposition in necrotic tubular epithelium was observed more markedly in wild-type than in heterozygous mice. Increases in renal endothelin-1 content were greater in wild-type than in heterozygous mice, and this reflected the difference in immunohistochemical endothelin-1 localization in necrotic tubular epithelium. When the preischemic treatment with KB-R7943 was performed, the renal functional parameters of both $NCX1^{+/+}$ and $NCX1^{+/-}$ acute renal failure mice were improved to the same level. These findings strongly support the view that Ca^{2+} overload via the reverse mode of Na^+/Ca^{2+} exchange, followed by renal endothelin-1 overproduction, plays an important role in the pathogenesis of ischemia/reperfusion-induced renal injury.

Renal ischemia is characterized by the depletion of ATP and the development of intracellular acidosis, which alter cellular ionic homeostasis. In particular, elevated intracellular Ca^{2+} concentration causes cellular injury during ischemia and leads to irreversible renal damage during reperfusion (Schrier et al., 1987). An increase in the intracellular Na^+ concentration has been shown to correlate with Ca^{2+} overload. The accumulation of intracellular Na^+ concentration, which is caused by inhibition of the Na^+/K^+ ATPase activity because of decreased ATP production (Cross et al., 1995) and activation of the Na^+/H^+ exchange because of intracellular acidosis (Scholz et al., 1993), has been shown to activate the Na^+/Ca^{2+} exchanger (NCX1) and subsequently to cause Ca^{2+} overload. Therefore, the NCX1 plays a crucial role in cellular injury during ischemia and in cell death during reperfusion. In the last decade, the NCX1 has been cloned and the structure/function relationship intensively studied. In addition, many investigators have studied the pathophysiological significance of NCX1 in the abnormality of the circulatory system (Philipson and Nicoll, 2000).

The role of NCX1 in ischemia/reperfusion injury has been demonstrated using the selective NCX1 inhibitor KB-R7943. This compound has been reported to be a selective and potent inhibitor of the Ca^{2+} influx mode of Na^+/Ca^{2+} exchange in cardiomyocytes, smooth muscle cells, and NCX1-transfected fibroblasts (Iwamoto et al., 1996). Similar inhibitory effects of KB-R7943 on the reverse mode of NCX1 were observed in

This work was supported by Grant-in-Aid for Scientific Research 12670098 (to Y.M.) and 12670102 (to T.I.) from the Ministry of Education, Science and Culture of Japan, and a Grant from the Cardiovascular Research Foundation (to T.I.).

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

DOI: 10.1124/jpet.102.039024.

ABBREVIATIONS: NCX1, Na^+/Ca^{2+} exchanger; KB-R7943, 2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiourea methanesulfonate; ARF, acute renal failure; ET-1, endothelin-1; PBS, phosphate-buffered saline; BUN, blood urea nitrogen; Uosm, urinary osmolarity; FENa, fractional excretion of sodium; Ccr, creatinine clearance; KHB, Krebs-Henseleit buffer; $[Ca^{2+}]_i$, intracellular calcium concentration; BSS, balanced salt solution; $[Ca^{2+}]_o$, extracellular calcium concentration; RIA, radioimmunoassay; DMEM, Dulbecco's modified Eagle's medium; LDH, lactate dehydrogenase; Pcr, plasma creatinine concentration; UF, urine flow; SK&F96365, 1-[-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenyl]-1*H*-imidazole hydrochloride; ABT-627, [2*R*-(4-methoxyphenyl)-4*S*-(1,3-benzodioxol-5-yl)-1-(*N*,*N*-di(*n*-butyl)aminocarbonyl-methyl)-pyrrolidine-3*R*-carboxylic acid]; A-192621, [2*R*-(4-propoxyphenyl)-4*S*-(1,3-benzodioxol-5-yl)-1-(*N*-(2,6-diethylphenyl)aminocarbonyl-methyl)-pyrrolidine-3*R*-carboxylic acid].

guinea pig cardiac ventricular cells (Watano et al., 1996). Furthermore, KB-R7943 efficiently improved the ischemia/reperfusion-induced injury both in isolated rat perfused heart and in anesthetized rat heart, thereby suggesting that a selective Na^+/Ca^{2+} exchange inhibitor has beneficial effects against myocardial ischemia/reperfusion injury (Nakamura et al., 1998; Ladilov et al., 1999). In the kidney, we first demonstrated the protective effects of KB-R7943 on ischemia/reperfusion-induced acute renal failure (ARF), and therefore suggested that Ca^{2+} overload via the reverse mode of NCX1 plays an important role in the pathogenesis of this renal disease (Yamashita et al., 2001).

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide (Yanagisawa et al., 1988) that has been implicated as a mediator of cardiac, vascular, and renal diseases associated with regional and systemic vasoconstriction (Rubanyi and Polokoff, 1994). This peptide is produced in various tissues, including endothelial cells, smooth muscle cells, and renal tubular epithelial cells and acts through activation of G protein-coupled ET_A and ET_B receptors (Rubanyi and Polokoff, 1994). A potential contribution of ET-1 to the pathology of ischemic ARF has been suggested based on findings indicating that renal ET-1 mRNA expression, ET-1 content, and its affinity for ET receptors are elevated in the postischemic kidney (Firth and Ratcliffe, 1992; Wilhelm et al., 1999). ET_A-selective or nonselective ET_A/ET_B-receptor antagonists and ET-converting enzyme inhibitors are known to attenuate the ischemia/reperfusion-induced impairment of renal function (Gellai et al., 1995; Kuro et al., 2000; Matsumura et al., 2000). Taken together, it seems likely that renal ET-1 overproduction and its ET_A receptor-mediated actions are closely related to the pathogenesis of ischemic ARF.

The purpose of this study was to determine the pathological role of Na^+/Ca^{2+} exchange in the ischemia/reperfusion-induced ARF, using recently produced NCX1-deficient mice (Wakimoto et al., 2000). Homozygous NCX1-deficient mice ($NCX1^{-/-}$) died between embryonic days 9 and 10 (Wakimoto et al., 2000). Their hearts did not beat and cardiac myocytes showed apoptosis. Therefore, we used $NCX1^{+/-}$ heterozygous mice, which were subjected to the renal ischemia followed by reperfusion, and impairment of renal function, histological damage, and changes in renal ET-1 content were compared with those in $NCX1^{+/+}$ wild-type mice. We report here that $NCX1^{+/-}$ heterozygous mice exhibit an attenuated ischemia/reperfusion-induced renal dysfunction and cell injury, and a lowered ET-1 overproduction in the postischemic kidney, indicating that the Na^+/Ca^{2+} exchange mechanism and renal ET-1 system play an important role in the pathogenesis of postischemic ARF.

Materials and Methods

Animals. The generation of the NCX1-knockout mice has been described in detail previously (Wakimoto et al., 2000). Briefly, we cloned the NCX1 gene from a 129/SV mouse genomic library. The targeting vector was constructed by insertion of the neo cassette into the 3-kilobase pair *Xba*I-*Xho*I fragment containing exon 2 of NCX1 gene. The diphtheria toxin-A fragment gene was ligated to the 3′ position of the targeting vector for negative selection. The A3-1 embryonic stem cell line was transfected with the linearized targeting vector by electroporation. After G418 selection, homologous recombinants were identified by polymerase chain reaction and confirmed by Southern blot hybridization. Targeted embryonic stem cells were aggregated with eight cells from C57BL/6J (B6) mice, and chimeric blastocysts were implanted into the uterus of pseudopregnant ICR mice. Chimeric male mice were then mated to female B6 mice to confirm the germline transmission.

Surgery and Experimental Design. Male B6 mice ($NCX1^{+/-}$ and $NCX1^{+/+}$ mice; 15–20 g) were housed in a light-controlled room with a 12-h light/dark cycle, and access to food and water was ad libitum. Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences. Two weeks before the study, the right kidney was removed through a small flank incision made after pentobarbital anesthesia (50 mg/kg i.p.). After a 2-week recovery period, to induce ischemic ARF, these mice were anesthetized with pentobarbital (50 mg/kg i.p.), and the left kidney was exposed through a small flank incision. The left renal artery and vein were occluded for 45 min with a nontraumatic clamp. At the end of the ischemic period, the clamp was released and blood reperfused. In some animals, KB-R7943 (10 mg/kg) or its vehicle (a mixture of 15% ethanol, 15% polyethylene glycol 400, and 70% saline) was administered as a slow bolus injection at 1 ml/kg into the external jugular vein, 5 min before the occlusion.

In sham-operated control animals, the left kidney was treated identically, except for clamping. Animals exposed to 45-min ischemia were housed in metabolic cages at 24 h after reperfusion; 24-h urine samples were taken and blood samples were drawn from the aorta at the end of urine collection period. The plasma was separated by centrifugation. These samples were used for measurements of renal functional parameters. The kidneys were excised and examined using a light microscope.

In separate experiments, left kidneys were obtained 24 h after reperfusion to determine NCX1 protein expression and ET-1 content.

Western Blotting. Tissue homogenate preparation, SDS-polyacrylamide gel electrophoresis, and immunoblotting were performed as described previously (Yamashita et al., 2001). Immunoblot analysis was performed with anti-NCX1 polyclonal antibody at 1:300 dilution with PBS (Iwamoto et al., 1998). Protein was measured with the bicinchoninic acid assay reagent (Pierce Chemical, Rockford, IL). The immunoblots were visualized using the enhanced chemiluminescence detection system (Amersham Biosciences, Inc., Piscataway, NJ).

Blood and Urine Measurements. Blood urea nitrogen (BUN) and creatinine levels in plasma and urine were determined using commercial kits, the BUN-test-Wako and Creatinine-test-Wako (Wako Pure Chemicals, Osaka, Japan), respectively. Urinary osmolality (Uosm) was measured by freezing point depression (Fiske, MA). Urine and plasma sodium concentrations were determined using a flame photometer (205D; Hitachi, Hitachinaka, Japan). Fractional excretion of sodium (FENa, %) was calculated from the formula $FENa = UNaV/(PNa \times Ccr) \times 100$, where UNaV is urinary excretion of sodium, PNa is the plasma sodium concentration, and Ccr is creatinine clearance.

Histological Studies. Histological studies were done as described previously (Yamashita et al., 2001). Histopathological changes were analyzed for tubular necrosis and proteinaceous casts, as suggested by Solez et al. (1974). Tubular necrosis and proteinaceous casts were graded as follows: no damage (− or 0), mild (± or 1, unicellular, patchy isolated damage), moderate (+ or 2, damage less than 25%), severe (++ or 3, damage between 25 and 50%), and very severe (+++ or 4, more than 50% damage). Evaluations were made in a blind manner.

Using von Kossa method, the amount of black reaction products indicated as Ca^{2+} deposition in necrotic tubular epithelium was also determined by microscopic observation.

Primary Culture of Proximal and Distal Tubular Cells. Proximal and distal tubular cells were prepared from $NCX1^{+/+}$ and $NCX1^{+/-}$ mice with a modification of methods described previously (Gesek et al., 1987). Briefly, mice were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and the left kidney was perfused with ice-cold modified Krebs-Henseleit buffer (KHB) through the thoracic aorta after ligation of the aorta and vena cava above the renal

vessels. Modified KHB contains the following: 118 mM NaCl, 4.0 mM KCl, 1.0 mM KH_2PO_4, 27.2 mM $NaHCO_3$, 1.25 mM $CaCl_2$, 1.20 mM $MgCl_2$, 5.0 mM glucose, and 10 mM HEPES. The kidney was removed and the cortex was cut into 1-mm-thick slices, being incubated for 40 min at 37°C in atmosphere of 95% O_2/5% CO_2. The slices were then washed with KHB and transferred to an ice-cold solution. Nephron segments were isolated from the cortex region under microscope. Proximal tubules (segments 1–3) just after the glomerulus and distal convoluted tubules just after the thick ascending limb were excised. These isolated tubules were then explanted for 4 to 5 days on 35-mm dishes in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Measurement of $[Ca^{2+}]_i$ in Proximal and Distal Tubular Cells. $[Ca^{2+}]_i$ was monitored using Fluo-3/acetoxymethyl ester as a fluorescent Ca^{2+} indicator. Cells in 35-mm dishes were loaded with 4 μM Fluo-3/acetoxymethyl ester for 40 min at 37°C in 1 ml of balanced salt solution [BSS; 10 mM HEPES-Tris (pH 7.4), 146 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM glucose]. Loaded cells were then washed twice with BSS. Cells were exposed to Ca^{2+}, Mg^{2+}-free BSS containing 0.2 mM EGTA for 10 min and then to BSS containing 2 mM Ca^{2+}. KB-R7943 (10 μM) was pretreated for 10 min before the repletion of $[Ca^{2+}]_o$. Fluorescence signals from single tubular cells with excitation at 488 nm were monitored by confocal laser scanning microscope system (MRC1024; Bio-Rad, Hercules, CA). The fluorescence intensity of individual cells (F) was normalized to that (F_0) before adding 2 mM Ca^{2+}.

Renal ET-1 Assay. ET-1 was extracted from the kidney, as described elsewhere (Fujita et al., 1995). Briefly, kidneys were weighed and homogenized for 60 s in 8 ml of ice-cold organic solution (chloroform/methanol, 2:1, including 1 mM *N*-ethylmaleimide). The homogenates were left overnight at 4°C and then 0.4 ml of distilled water was added after which the homogenates were centrifuged at 1500*g* for 30 min and the resultant supernatant was stored. Aliquots of the supernatant were diluted 1/10 with a 0.09% trifluoroacetic acid solution and applied to Sep-Pak C18 cartridges. The sample was eluted with 3 ml of 63.3% acetonitrile and 0.1% trifluoroacetic acid in water. Eluates were dried in a centrifugal concentrator and the dried residue was reconstituted in assay buffer for radioimmunoassay (RIA). The clear solution was subjected to RIA. The recovery of ET-1 was approximately 80%. RIA for tissue ET-1 was done, as described previously (Matsumura et al., 1990b).

Immunohistochemistry. Excised left kidneys were preserved in phosphate-buffered 10% formalin, after which the kidneys were chopped into small pieces, embedded in paraffin wax, and cut at 3 μm. Tissue sections were incubated for 30 min at 37°C with anti-ET-1 polyclonal antibody (Peptide Institute, Inc., Osaka, Japan) or with anti-NCX1 polyclonal antibody (Iwamoto et al., 1998) at 1:2000 and 1:300 dilution with PBS, respectively. After washing with PBS, the sections were further incubated with goat anti-rabbit biotinylated secondary antibody (Nichirei, Tokyo, Japan) at 37°C for 10 min and then the streptavidin-horseradish peroxidase (Nichirei) was applied for 5 min. The complex was visualized with 3,3-diamonobenzidine.

Hypoxia and Reoxygenation in LLC-PK_1. LLC-PK_1 (American Type Culture Collection, Manassas, VA), a porcine kidney cell line, was grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 50 μg/ml streptomycin, and 50 U/ml penicillin at 37°C in a CO_2 incubator (95% air, 5% CO_2). When the cells cultured in 24-well plates became confluent, the culture medium was changed to DMEM without glucose and serum and the cells were exposed to the hypoxic condition using an Anaero Pack Pouch (Mitsubishi Bas Chemical Co., Inc., Tokyo, Japan), in which the oxygen concentration was less than 1% within 1 h after the exposure. After 6 h of hypoxia, the cells were put in a CO_2 incubator for 1 h in the DMEM to which glucose was added at the beginning of reoxygenation. After the exposure of the cells to hypoxia and reoxygenation, lactate dehydrogenase (LDH) activity in the culture supernatant for 7 h was measured with a commercial kit (Wako Pure Chemicals). KB-R7943 (10 μM) was added to the medium at the beginning of hypoxia and/or reoxygenation. LDH release was expressed as a percentage of total cellular LDH activity.

Statistical Analysis. Values are mean ± S.E.M. For statistical analysis, we used one-way analysis of variance followed by Bonferroni's or Dunnett's multiple comparison tests. Histological data were analyzed using the Kruskal-Wallis nonparametric test combined with the Steel-type multiple comparison test. For all comparisons, differences were considered significant at $P < 0.05$.

Results

Expression and Localization of NCX1 Protein. To justify the use of $NCX1^{+/-}$ heterozygous and $NCX1^{+/+}$ wild-type mice, NCX1 protein expression in the kidney of these animals was examined. As shown in Fig. 1, NCX1 protein level in renal tissues of $NCX1^{+/-}$ mice was about half of that seen in $NCX1^{+/+}$ mice. On the other hand, protein levels of Na^+/K^+-ATPase, sarcoplasmic reticulum Ca^{2+}-ATPase (type 2) and L-type voltage-dependent Ca^{2+} channel did not differ between $NCX1^{+/-}$ and $NCX1^{+/+}$ mice (data not shown). In addition, an immunohistochemical study clearly indicated that a staining for NCX1 protein expression was much more intense in tubular epithelial cells of renal cortex of $NCX1^{+/+}$ wild-type mice than in those of $NCX1^{+/-}$ mice (Fig. 2).

$[Ca^{2+}]_i$ Rise Evoked by the Reverse Mode of Na^+/Ca^{2+} Exchange in Cultured Renal Tubular Cells. $[Ca^{2+}]_o$ reple-

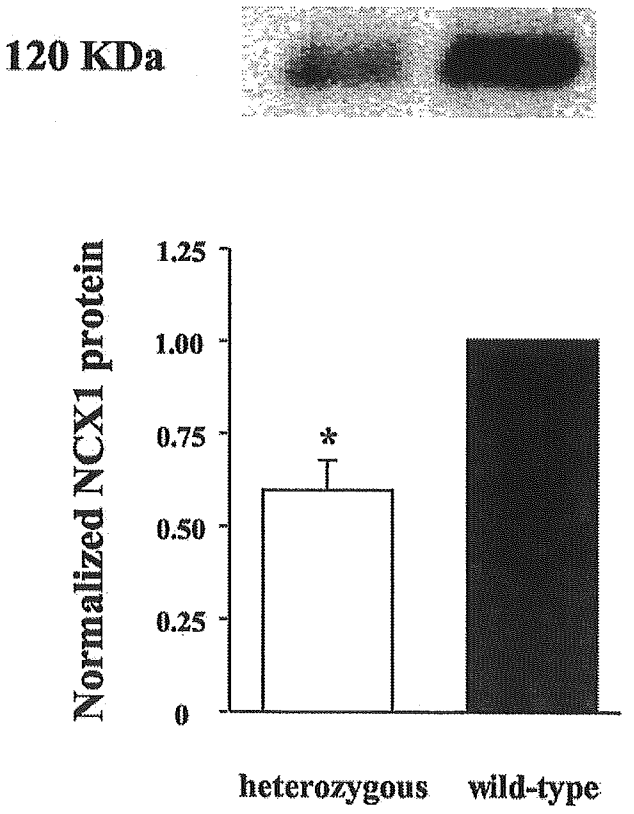

Fig. 1. NCX1 protein expression in renal tissues of $NCX1^{+/+}$ wild-type and $NCX1^{+/-}$ heterozygous mice. Each column and bar represents the mean ± S.E.M. ($n = 6$). *, $P < 0.01$, compared with wild-type mice.

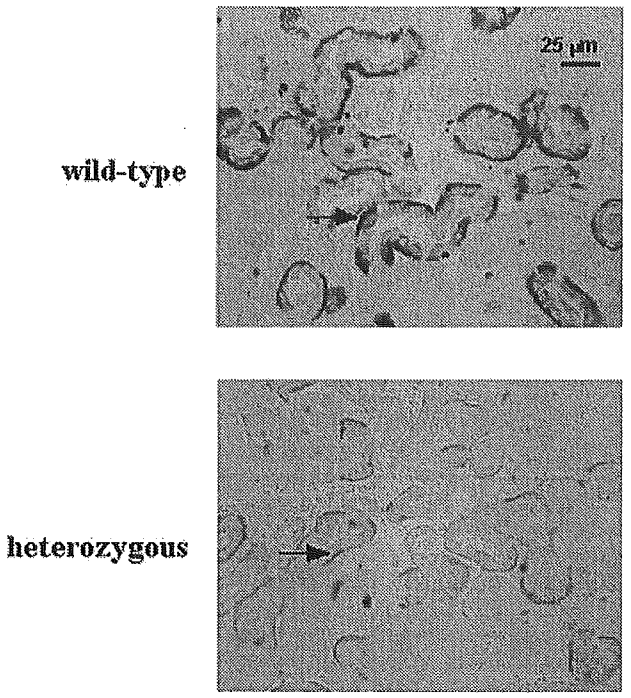

Fig. 2. Immunohistochemistry for NCX1 protein in the renal cortex of $NCX1^{+/+}$ wild-type and $NCX1^{+/-}$ heterozygous mice. Arrows indicate NCX1 protein expression in tubular epithelial cells. The protein expression was much more intense in $NCX1^{+/+}$ than in $NCX1^{+/-}$ mice.

tion after a period of $[Ca^{2+}]_o$ depletion is known to cause Ca^{2+} overloading via Na^+/Ca^{2+} exchange in cardiomyocytes or neuronal cells, a process called the Ca^{2+} paradox (Chapman and Tunstall, 1987). To assess the functional difference of Na^+/Ca^{2+} exchange in renal tubules between $NCX1^{+/+}$ and $NCX1^{+/-}$ mice, we examined $[Ca^{2+}]_i$ rise evoked by the Ca^{2+} paradox in cultured renal tubular cells using Ca^{2+} indicator Fluo-3. When distal tubular cells of $NCX1^{+/+}$ mice were exposed with a Ca^{2+}, Mg^{2+}-free buffer for 10 min and then placed in a buffer containing 2 mM Ca^{2+}, $[Ca^{2+}]_i$ markedly increased. In the proximal tubular cells, $[Ca^{2+}]_i$ rise was also observed, although being significantly smaller than that in distal tubular cells (Fig. 3). These $[Ca^{2+}]_i$ responses were blocked over 90% by pretreatment with KB-R7943 (10 μM), an inhibitor for the reverse mode of Na^+/Ca^{2+} exchange, but not significantly affected by verapamil (10 μM), L-type Ca^{2+} channel blocker, or SK&F96365 (50 μM), a blocker of store-operated Ca^{2+} channels (data not shown). In $NCX1^{+/-}$ mice, on the other hand, the $[Ca^{2+}]_i$ elevations in both tubular cells induced by 2 mM Ca^{2+} were markedly down-regulated compared with the case of $NCX1^{+/+}$ mice.

Renal Function after Ischemia/Reperfusion. As shown in Fig. 4, renal functional parameters of mice subjected to 45-min ischemia showed a marked deterioration, as measured 48 h after reperfusion. Compared with each sham-operated control animals, both $NCX1^{+/-}$ and $NCX1^{+/+}$ mice exhibited increases in BUN, plasma creatinine concentration (Pcr), urine flow (UF) and FENa, and decreases in Ccr, and Uosm. However, ischemia/reperfusion-induced changes in renal functional parameters of $NCX1^{+/-}$ mice were considerably small, compared with cases in $NCX1^{+/+}$ mice (BUN: $NCX1^{+/-}$, 78.0 ± 8.8 versus $NCX1^{+/+}$, 142.6 ± 7.4 mg/dl; Pcr, $NCX1^{+/-}$, 0.80 ± 0.10 versus $NCX1^{+/+}$, 1.34 ± 0.05 mg/dl; Ccr, $NCX1^{+/-}$, 2.15 ± 0.23 versus $NCX1^{+/+}$, 0.95 ± 0.13 ml min^{-1} kg^{-1}; UF, $NCX1^{+/-}$, 88.1 ± 10.4 versus $NCX1^{+/+}$, 102.6 ± 10.7 μl min^{-1} kg^{-1}; Uosm, $NCX1^{+/-}$, 663 ± 73 versus $NCX1^{+/+}$, 501 ± 18 mOsM/kg; FENa, $NCX1^{+/-}$, 1.05 ± 0.16 versus $NCX1^{+/+}$, 1.93 ± 0.12%). On the other hand, there were no significant differences in renal functional parameters between $NCX1^{+/+}$ and $NCX1^{+/-}$ sham-operated control mice.

Histological Renal Damage after Ischemia/Reperfusion. Histological examination revealed severe lesions in the kidney of $NCX1^{+/+}$ mice (48 h after the ischemia/reperfusion). These changes were characterized by tubular necrosis (Fig. 5b, outer zone outer stripe of medulla) and proteinaceous casts in tubuli (Fig. 5f, inner zone of medulla). In $NCX1^{+/-}$ mice, histologically evident damage was significantly less than that seen in $NCX1^{+/+}$ mice (Figs. 5, d and h; Table 1).

Ca^{2+} Deposition after Ischemia/Reperfusion. Figure 6 shows light micrographs of Ca^{2+} deposition demonstrated by von Kossa method in the kidney subjected to 45-min ischemia followed by reperfusion. Ca^{2+} deposition in medullary tubular epithelium of kidney of $NCX1^{+/+}$ mice was more evident compared with the case of $NCX1^{+/-}$ mice.

Effects of KB-R7943 on the Ischemia/Reperfusion-Induced Renal Dysfunction. To further evaluate the possible involvement of NCX1 in the ischemia/reperfusion-induced renal injury, the effect of pharmacological blockade of NCX1 was examined. As shown in Fig. 7, preischemic treatment with KB-R7943 improved the renal functional parameters of both $NCX1^{+/+}$ and $NCX1^{+/-}$ ARF mice to the same level.

Effects of KB-R7943 on the Hypoxia/Reoxygenation-Induced Injury in LLC-PK_1. LLC-PK_1 cells, derived from pig kidney, have characteristics of proximal tubules. We evaluated the effect of KB-R7943 on the hypoxia/reoxygenation-induced cell injury in LLC-PK_1. Hypoxia/reoxygenation technique is known as in vitro model system of ischemia/reperfusion-induced renal injury. As shown in Fig. 8, an enhanced LDH release from the cells exposed to hypoxia followed by reoxygenation was markedly suppressed by the treatment with KB-R7943 during the hypoxia. Similar suppressive effect of KB-R7943 was also observed by the addition at the beginning of reoxygenation.

Renal ET-1 Content after Ischemia/Reperfusion. To confirm the contribution of ET-1 to ischemia/reperfusion-induced renal injury both in $NCX1^{+/-}$ and $NCX1^{+/+}$ mice, we measured renal ET-1 content at 24 h after reperfusion. As shown in Fig. 9, renal ET-1 content was significantly increased by the ischemia/reperfusion, both in $NCX1^{+/+}$ and $NCX1^{+/-}$ mice, compared with that seen in each sham mice. However, ischemia/reperfusion-induced changes in renal ET-1 content of $NCX1^{+/-}$ mice were considerably small, compared with cases in $NCX1^{+/+}$ mice ($NCX1^{+/-}$, 0.71 ± 0.06 versus $NCX1^{+/+}$, 1.26 ± 0.23 ng/g tissue).

Immunohistochemical Analysis. To determine the localization of renal ET-1 peptide expression after the ischemia/reperfusion, an immunohistochemical study was done. As clearly indicated in Fig. 10, a staining for ET-1 peptide expression was intense in tubular lumen containing necrotic cells, and it was more prominent in $NCX1^{+/+}$ than in $NCX1^{+/-}$ mice.

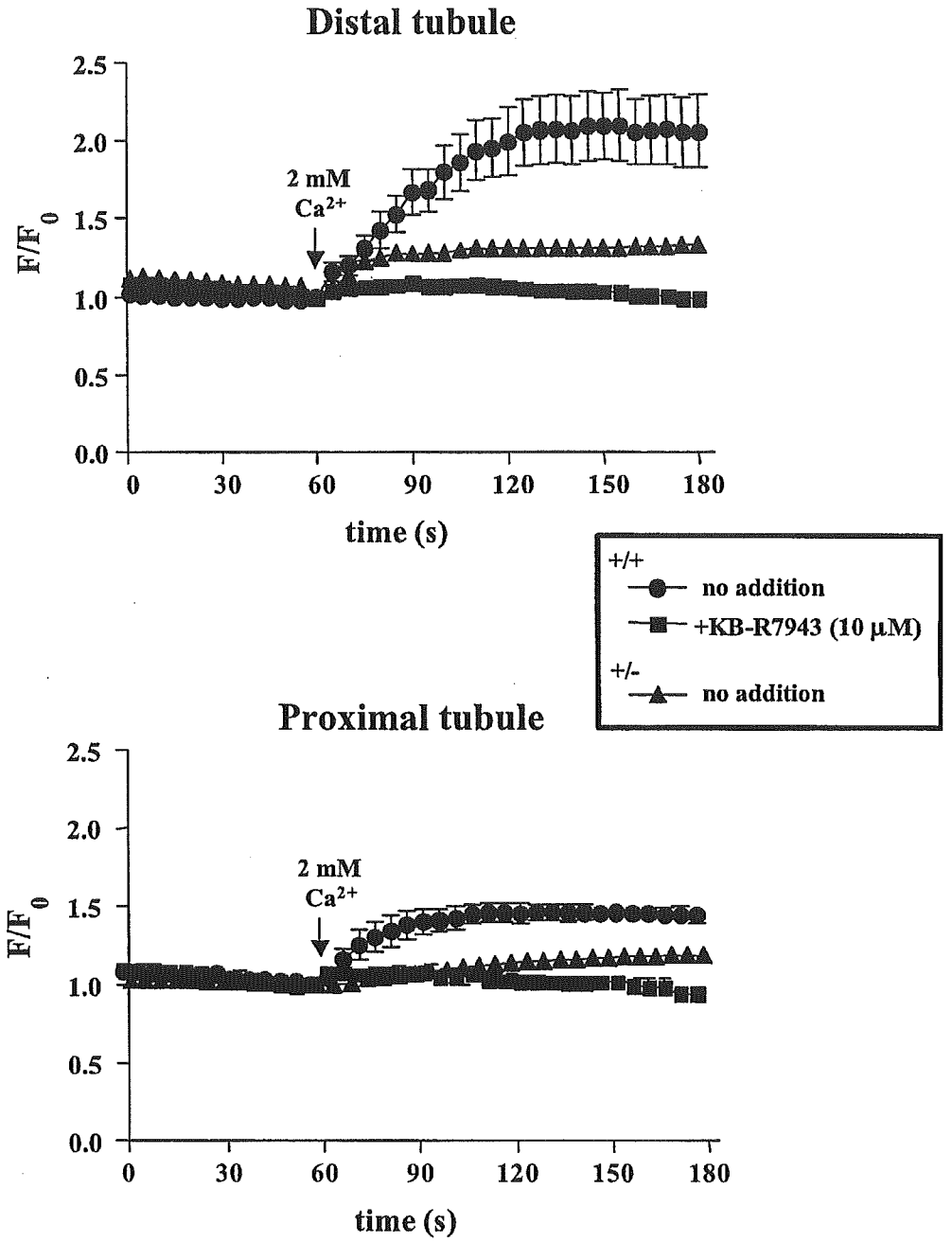

Fig. 3. $[Ca^{2+}]_i$ elevations evoked by the Ca^{2+} paradox in cultured proximal and distal tubular cells from $NCX1^{+/+}$ wild-type and $NCX1^{+/-}$ heterozygous mice. Fluo-3-loaded cells were exposed to a Ca^{2+}, Mg^{2+}-free BSS containing 0.2 mM EGTA for 10 min and then to BSS containing 2 mM Ca^{2+}, with or without 10 μM KB-R7943. KB-R7943 was pretreated for 10 min before the repletion of Ca^{2+}. The fluorescence intensity of individual cells was normalized to that (F_0) before adding 2 mM Ca^{2+}. Each point and bar represents the mean ± S.E.M.

Discussion

We investigated the pathological role of NCX1 in ischemia/reperfusion-induced renal injury using NCX1-knockout mice. Because $NCX1^{-/-}$ homozygous mice die of heart failure before birth (Wakimoto et al., 2000), we used $NCX1^{+/-}$ heterozygous mice, in which NCX1 protein expression in renal tissues was decreased to about half of those of $NCX1^{+/+}$ wild-type mice. Furthermore, expression of NCX1 protein in the tubular epithelial cells and Na^+/Ca^{2+} exchange activity of renal tubules were markedly attenuated in the heterozygous mice, thereby indicating the usefulness of these mice in examining the renal pathophysiological role of NCX1.

In the present study, the ischemia/reperfusion-induced renal dysfunction and histological damage was moderate in $NCX1^{+/-}$ mice compared with cases in $NCX1^{+/+}$ mice. His-

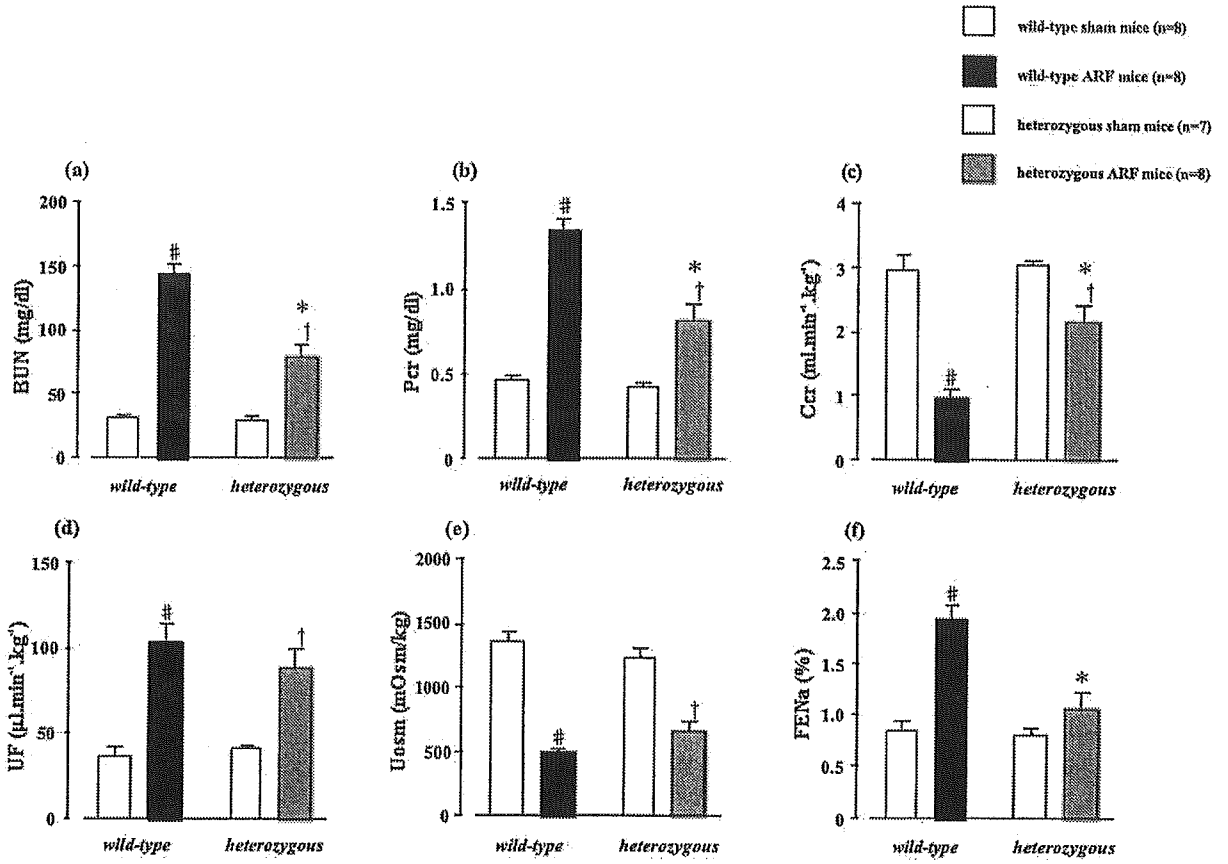

Fig. 4. Renal functional parameters of $NCX1^{+/+}$ wild-type and $NCX1^{+/-}$ heterozygous mice, with or without ARF. BUN (a), Pcr (b), Ccr (c), UF (d); Uosm (e), and FENa (f). Each column and bar represents the mean ± S.E.M. #, $P < 0.01$, compared with wild-type sham mice; †, $P < 0.01$, compared with heterozygous sham mice; *, $P < 0.01$, compared with wild-type ARF mice.

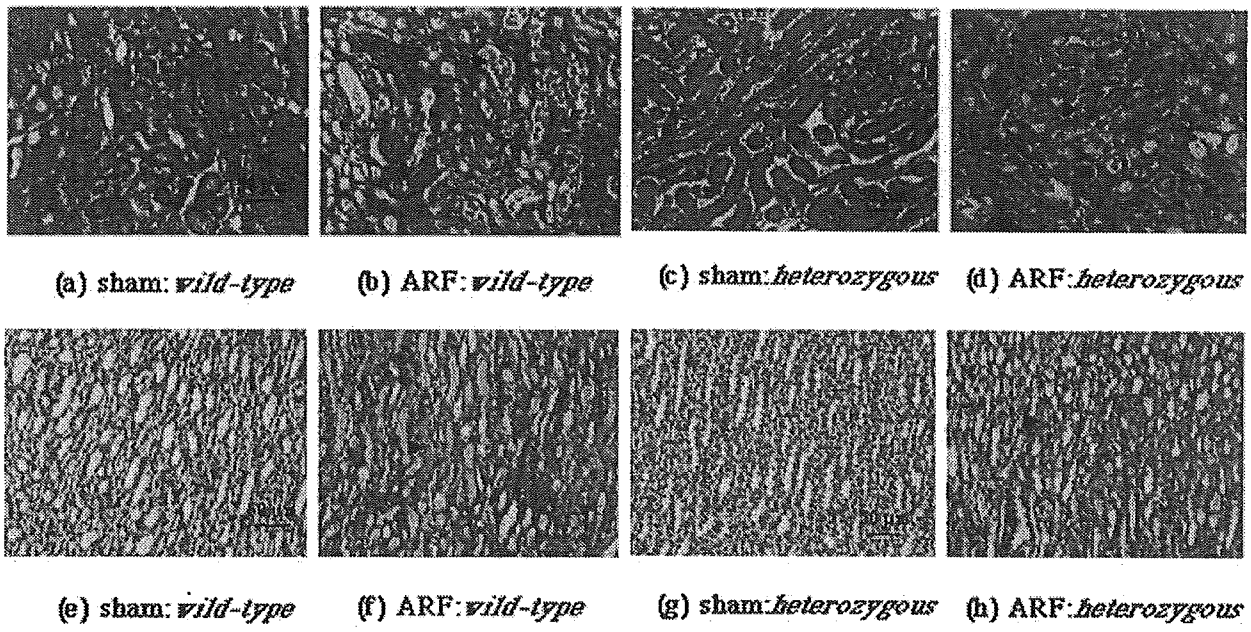

Fig. 5. Light microscopy of outer zone outer stripe of medulla (a–d) and inner zone of medulla (e–h) of the kidney of $NCX1^{+/+}$ wild-type and $NCX1^{+/-}$ heterozygous mice, with or without ARF. Arrows indicate tubular necrosis (b and d) and proteinaceous casts in tubuli (f and h) (hematoxyline and eosin staining).

TABLE 1

Histopathological changes in kidneys of wild-type and heterozygous mice, with or without ARF

Data are expressed as the number of animals with histopathological changes. Values in parentheses represent the mean ± S.E.M. of histopathological change/grade. Grades: no changes (− or 0), mild (± or 1), moderate (+ or 2), severe (++ or 3), very severe (+++ or 4).

	Wild-Type										Heterozygous									
	Sham Mice (n = 5)					ARF Mice (n = 6)					Sham Mice (n = 5)					ARF Mice (n = 6)				
Histopathological changes/grade	− (0	± 1	+ 2	++ 3	+++ 4)	− (0	± 1	+ 2	++ 3	+++ 4)	− (0	± 1	+ 2	++ 3	+++ 4)	− (0	± 1	+ 2	++ 3	+++ 4)
Tubular necrosis	5	0	0	0	0	0	0	0	4	2	5	0	0	0	0	0	3	3	0	0
									(3.33 ± 0.21[a])										(1.50 ± 0.22[b,c])	
Protein casts	5	0	0	0	0	0	0	0	2	4	5	0	0	0	0	0	2	2	2	0
									(3.67 ± 0.21[a])										(2.00 ± 0.37[b,c])	

ARF, acute renal failure.
[a] $P < 0.01$, compared with wild-type sham mice.
[b] $P < 0.01$, compared with heterozygous sham mice.
[c] $P < 0.01$, compared with wild-type ARF mice.

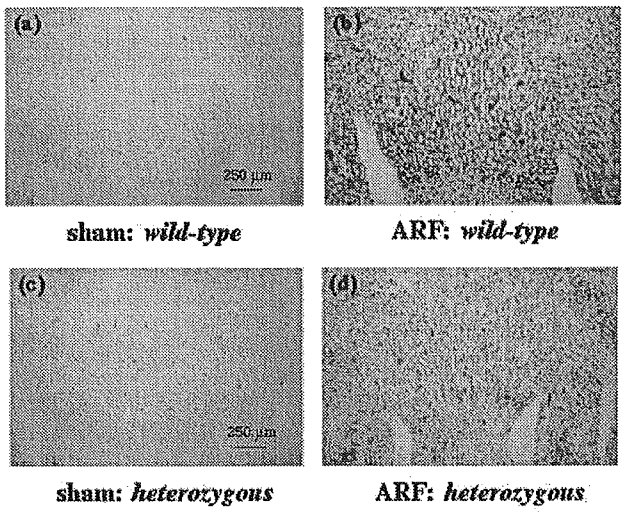

Fig. 6. Light microscopy of Ca^{2+} deposition in medulla of the kidney of $NCX1^{+/+}$ wild-type and $NCX1^{+/-}$ heterozygous mice, with or without ARF. Ca^{2+} deposition in medullary tubular epithelium of kidney of $NCX1^{+/+}$ ARF mice (b) was more evident compared with the case of $NCX1^{+/-}$ ARF mice (d) (von Kossa staining).

tochemically visualized Ca^{2+} deposition in medullary tubular epithelium of postischemic kidney of $NCX1^{+/-}$ mice was less evident than that seen in $NCX1^{+/+}$ mice. On the other hand, pharmacological blockade of NCX1 with KB-R7943 improved the renal dysfunction observed in both $NCX1^{+/+}$ and $NCX1^{+/-}$ ARF mice to the same level. An increment of ET-1 content in postischemic kidney of $NCX1^{+/-}$ mice was also less than that observed in $NCX1^{+/+}$ mice, and this difference reflected an immunohistochemical localization of ET-1 in tubular lumen-containing necrotic cells. These findings suggest that Ca^{2+} overload via the reverse mode of NCX1, followed by renal ET-1 overproduction, plays an important role in the pathogenesis of ischemia/reperfusion-induced renal injury.

In normal cardiac cells, NCX1 extrudes Ca^{2+} from sarcolemma to maintain the intracellular Ca^{2+} concentration at the diastolic level. In contrast, in ischemic cardiac cells where intracellular pH decreases, the intracellular Na^+ concentration rises through the Na^+/H^+ exchange system, which in turn increases the intracellular Ca^{2+} concentration through the Na^+/Ca^{2+} exchange system (Dennis et al., 1990). The Ca^{2+} overload via this system seems to contribute to the ischemia/reperfusion injury in the heart (Tani and Neely, 1989; Cross et al., 1998). This view may be applicable to the case of the pZostischemic ARF. Although the pathological mechanisms of Ca^{2+} overload in ischemic kidney have not been fully elucidated, there is substantial evidence indicating that increased cytosolic Ca^{2+} may be an important mediator of epithelial cell necrosis, which is a characteristic of ischemic ARF and that Ca^{2+} overload is a primary factor in certain types of cell injury (Wilson et al., 1984). In addition, a preischemic treatment with Ca^{2+} channel blockers has been known to exert a protective effect against the ischemia/reperfusion-induced renal injury (Goldfarb et al., 1983; Yamashita et al., 2001). Most recently, we found that KB-R7943, a selective and potent inhibitor of the Ca^{2+} influx mode of Na^+/Ca^{2+} exchange (Iwamoto et al., 1996; Watano et al., 1996), efficiently attenuated the ischemia/reperfusion-induced renal injury in both cases of pre- and postischemic treatments, thereby suggesting that Ca^{2+} overload via the reverse mode of the Na^+/Ca^{2+} exchange is a crucial factor in the pathology of postischemic renal insufficiency, and that an inhibitor of NCX1 may be an beneficial therapeutic agent for the postischemic ARF (Yamashita et al., 2001).

In the present study, using $NCX1^{+/-}$ heterozygous mice we confirmed the pathophysiological importance of Ca^{2+} handling via NCX1 in the ischemia/reperfusion-induced renal injury. The level of NCX1 protein expression in renal tissues of the $NCX1^{+/-}$ mice was about half of that seen in $NCX1^{+/+}$ wild-type mice. Ca^{2+} influx via Na^+/Ca^{2+} exchange, which is abolished by a selective NCX1 inhibitor KB-R7943, in proximal and distal tubular cells were much less potent in $NCX1^{+/-}$ mice than in $NCX1^{+/+}$ mice. In addition, we obtained the evidences that protein levels of Na^+/K^+-ATPase, sarcoplasmic reticulum Ca^{2+}-ATPase, and L-type voltage-dependent Ca^{2+} channel did not differ between $NCX1^{+/+}$ and $NCX1^{+/-}$ mice (T. Iwamoto, unpublished data). These findings seem to justify the usefulness of these animals in renal pathophysiological study. However, an attenuation of ischemia/reperfusion-induced renal injury observed in $NCX1^{+/-}$ mice was only partial. Thus, to elucidate more precisely the pathophysiological role of NCX1 in the postischemic ARF, further studies using $NCX1^{-/-}$ homozygous mice are needed. To attain this, adult $NCX1^{-/-}$ mice should be produced by the tissue (heart)-specific transgenic rescue. Furthermore, the rescued adult $NCX1^{-/-}$ mice may provide new information on the physiological role of NCX1 in regulatory mechanisms of renal function, although we observed no significant

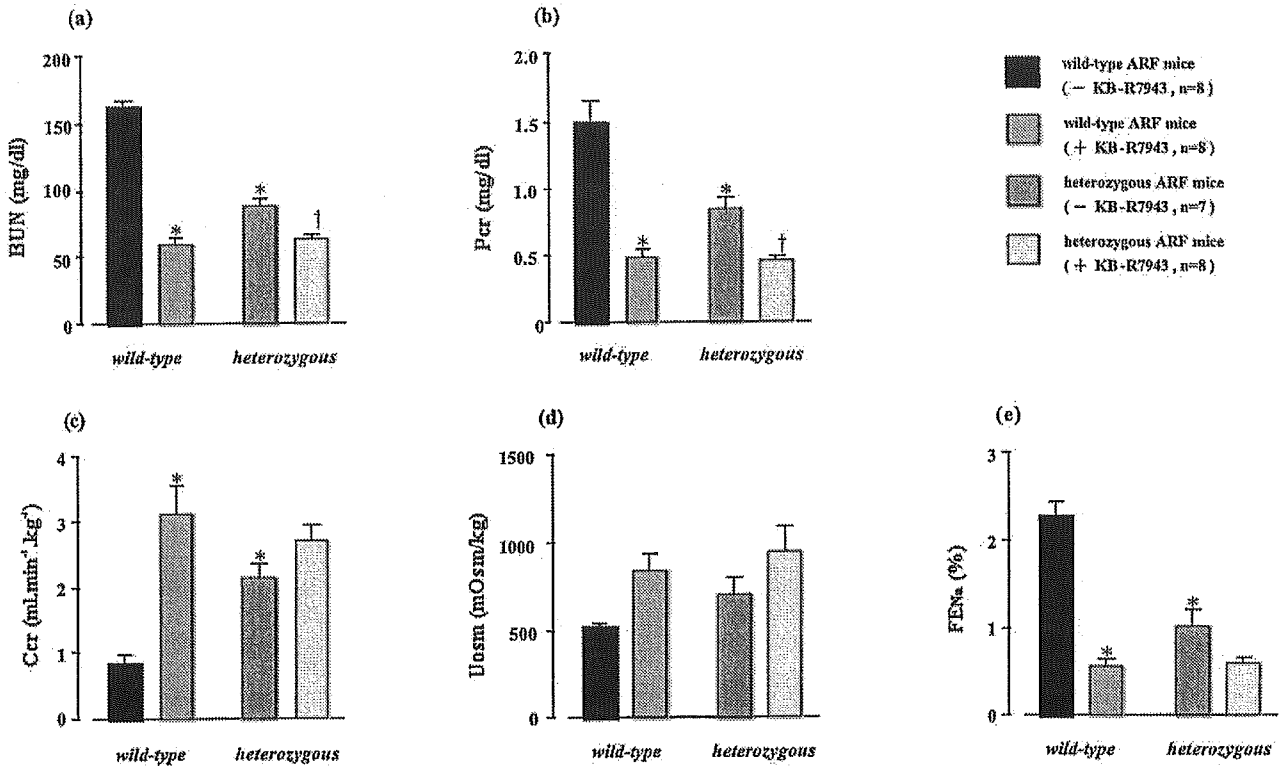

Fig. 7. Renal functional parameters of $NCX1^{+/+}$ wild-type and $NCX1^{+/-}$ heterozygous ARF mice, with or without KB-R7943 pretreatment. BUN (a), Pcr (b), Ccr (c), Uosm (d), and FENa (e). Each column and bar represents the mean ± S.E.M. *, $P < 0.01$, compared with wild-type ARF (−KB-R7943) mice; †, $P < 0.01$, compared with heterozygous ARF (−KB-R7943) mice.

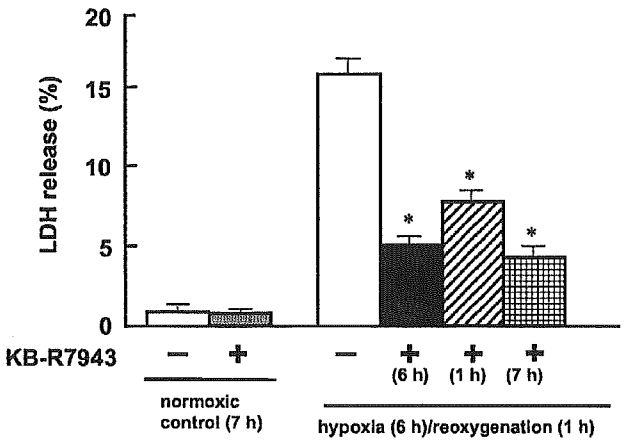

Fig. 8. Effects of KB-R7943 on the LDH release induced by hypoxia/reoxygenation in LLC-PK_1 cells. The cells were exposed to 7 h of normoxia or 6 h of hypoxia followed by 1 h of reoxygenation. KB-R7943 (10 μM) was added to the culture medium at the beginning of hypoxia and/or reoxygenation. Each column and bar represents the mean ± S.E.M. *, $P < 0.01$, compared with hypoxia and/or reoxygenation without KB-R7943.

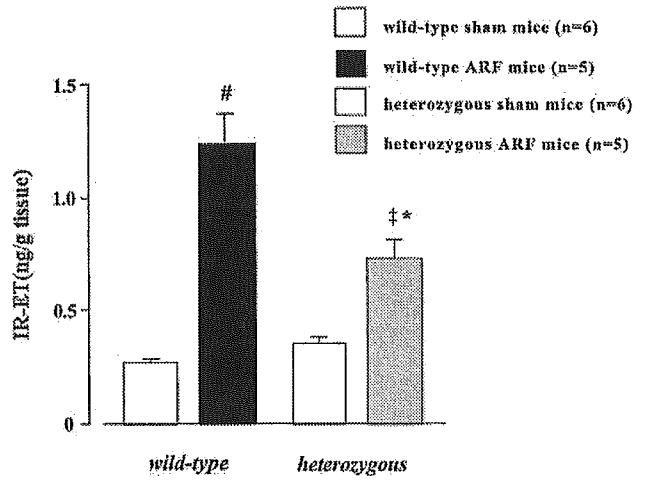

Fig. 9. Endothelin-1 content in the kidney of $NCX1^{+/+}$ wild-type and $NCX1^{+/-}$ heterozygous mice, with or without ARF. Each column and bar represents the mean ± S.E.M. #, $P < 0.01$, compared with wild-type sham mice; ‡, $P < 0.01$, compared with heterozygous sham mice; *, $P < 0.05$, compared with wild-type ARF mice. IR-ET, immunoreactive endothelin.

differences in renal function between $NCX1^{+/+}$ and $NCX1^{+/-}$ sham-operated control mice (Fig. 4). Alternatively, transgene technique using kidney-specific promotor to overexpress NCX1 protein and/or a conditional knockout technique would be also useful.

There is growing evidence that ET-1 is closely related to the development of the ischemic ARF. It has been demonstrated that ET-1 content (Shibouta et al., 1990; Matsumura et al., 2000) and ET-1 mRNA expression (Firth and Ratcliffe, 1992; Wilhelm et al., 1999) are elevated in renal tissues after ischemia/reperfusion. Our previous study has shown that daily oral administration of the ET_A-selective antagonist ABT-627, but not the ET_B-selective antagonist A-192621, had a beneficial effect on ischemia/reperfusion-induced renal

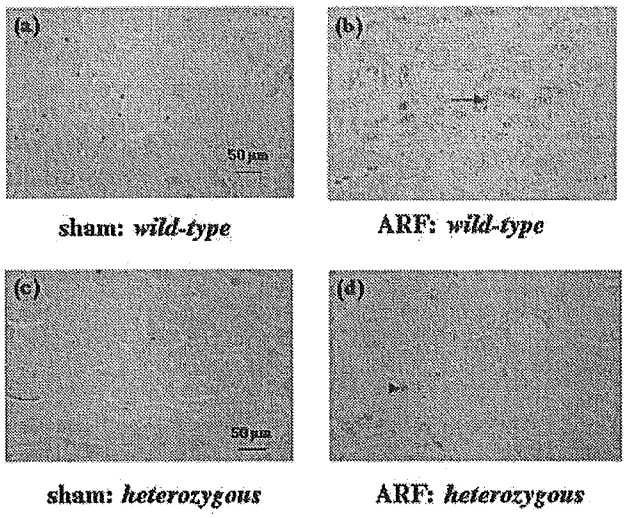

Fig. 10. Immunohistochemistry for endothelin-1 in medulla (outer zone outer stripe) of the kidney of $NCX1^{+/+}$ wild-type and $NCX1^{+/-}$ heterozygous mice, with or without ARF. Arrows indicate endothelin-1 peptide expression in lumens of necrotic tubular cells. The peptide expression was more intense in $NCX1^{+/+}$ (b) than in $NCX1^{+/-}$ mice (d).

dysfunction and tissue injury (Kuro et al., 2000). In addition, an ET-converting enzyme inhibitor, phosphoramidon (Matsumura et al., 1990a), was found to overcome ischemia/reperfusion-induced renal injury (Vemulapalli et al., 1993; Matsumura et al., 2000). Taken together, it seems likely that the up-regulation of renal ET-1 production and ET_A receptor-mediated actions are responsible for the pathogenesis of ischemic ARF. In the present study, there was an only moderate increment of ET-1 content in the postischemic kidney of $NCX1^{+/-}$ mice, compared with the case in $NCX1^{+/+}$ mice. In immunohistochemical study to determine the localization of ET-1 peptide in the postischemic kidney, an enhanced staining pattern was observed in necrotic tubular cells more markedly in $NCX1^{+/+}$ mice than in $NCX1^{+/-}$ mice. When the present study was in progress, similar localization pattern of ET-1 peptide in the kidney after the ischemia and 24 h of reperfusion was demonstrated by Wilhelm et al. (2001). Previously, Wilhelm et al. (1999) demonstrated increased ET-1 expression in the peritubular capillary network of the kidney after ischemia. Because this capillary bed is an extension of the efferent arteriole of the glomerulus and represents the primary blood supply of the tubules, it seems likely that ET-1 expressed at this site causes ongoing vasoconstriction in the peritubular capillary network and hypoxia in the neighboring tubules, and leads to tubular necrosis. Taken together, they hypothesized that ET-1 is first expressed in increased quantities in the peritubular capillary network shortly after the onset of renal ischemia and then transported across the basement membrane of the adjacent tubular epithelial cells, which are then sloughed off during the development of acute tubular necrosis. However, it remains obscure whether an overproduction of ET-1 in the ischemic kidney occurs in vascular endothelium, in tubular cells or in both, because ET-1 gene expression and the peptide production are up-regulated under the hypoxic condition, both in tubular cells and endothelial cells (Kourembanas et al., 1991; Ong et al., 1995).

One possible candidate for factors causing ET-1 overproduction in the kidney exposed to the ischemia/reperfusion may be an intracellular Ca^{2+} accumulation, which is an important mediator for the pathogenesis of ischemia/reperfusion injury of the kidney (Schrier et al., 1987). An increase of calcium entry has been known to induce the expression of ET-1 gene in endothelial cells (Rubanyi and Polokoff, 1994). We demonstrated that KB-R7943 efficiently improves the renal dysfunction and tissue injury induced by the ischemia/reperfusion, accompanying the suppression of increase in ET-1 content in the kidney after the ischemia/reperfusion (Yamashita et al., 2001). Taken together, ET-1 overproduction seems to be positioned down-stream to the Ca^{2+} overload, in the cascade of ischemia/reperfusion-induced renal injury, although ET-1 overproduction may result in the further increase of intracellular Ca^{2+} level.

The medullary thick ascending limb of the loop of Henle and the proximal tubule (pars recta), both situated in the outer medulla of the kidney, are the nephron segments that are most susceptible to ischemic injury (Brady et al., 2000). Also in our study, an ischemia/reperfusion produced a marked medullary tubular necrosis, in which ET-1 peptide was abundantly observed. On the other hand, NCX1 is known to be abundant in distal tubular portion, compared with proximal portion (Yu et al., 1992; Bourdeau et al., 1993). We also observed a marked Na^+/Ca^{2+} exchange activity in the distal tubules, compared with the case using proximal tubules. However, based on that proximal tubules are more sensitive to ischemic injury, NCX1 expressed in proximal portions may be more important in the ischemia/reperfusion-induced renal injury. It remains to be determined whether NCX1 protein is localized and functions in necrotic site of the nephron.

In separate experiments, we noted the ameliorative effect of KB-R7943 on hypoxia/reoxygenation-induced injury in $LLC\text{-}PK_1$ cells, which are derived from pig kidney and have characteristics of proximal tubules. Hypoxia/reoxygenation technique using $LLC\text{-}PK_1$ is known as in vitro model system of ischemia/reperfusion-induced renal tubular injury (Yonehana and Gemba, 1999). KB-R7943 was effective by the treatment not only during the hypoxia but also after the hypoxia, suggesting that Ca^{2+} influx via NCX1 during reoxygenation is more important in the hypoxia/reoxygenation-induced cell injury. This observation is in agreement with our previous report indicating that both pre- and postischemic treatments with KB-R7943 overcame the ischemia/reperfusion-induced renal injury (Yamashita et al., 2001).

In conclusion, $NCX1^{+/-}$ mice exhibited an attenuated development of the ischemia/reperfusion-induced renal injury. It seems most likely that Ca^{2+} overload via the reverse mode of Na^+/Ca^{2+} exchange, followed by renal ET-1 overproduction, plays an important role in the pathogenesis of ischemia/reperfusion-induced ARF. Taken together with the pharmacological evidence that an inhibitor of NCX1 could overcome the ischemia/reperfusion-induced renal injury in both cases of pre- and postischemic treatments, selective and potent inhibitors of NCX1 may be beneficial in the treatment of ischemic ARF in humans.

References

Bourdeau JE, Taylor AN, and Iacopino AM (1993) Immunocytochemical localization of sodium-calcium exchanger in canine nephron. *J Am Soc Nephrol* **4**:105–110.
Brady HR, Brenner BM, Clarkson MR, and Lieberthal W (2000) Acute renal failure,

in *Brenner and Rector's The Kidney*, 6th ed. (Brenner BN ed) pp 1201 1262, W.B. Saunders Company, Philadelphia.
Chapman RA and Tunstall J (1987) The calcium paradox of the heart. *Prog Biophys Mol Biol* **50**:67–96.
Cross HR, Lu L, Steenbergen C, Philipson KD, and Murphy E (1998) Overexpression of the cardiac Na^+-Ca^{2+} exchanger increases susceptibility to ischemia/reperfusion injury in male, but not female, transgenic mice. *Circ Res* **83**:1215–1223.
Cross HR, Radda GK, and Clarke K (1995) The role of Na^+/K^+ATPase activity during low flow ischemia in preventing myocardial injury; a 31P, 23Na and 87Rb NMR spectroscopic study. *Magn Reson Med* **34**:673–685.
Dennis SC, Coetzee WA, Cragoe EJ Jr, and Opie LH (1990) Effects of proton buffering and amiloride derivatives on reperfusion arrhythmias in isolated rat hearts. Possible evidence for an arrhythmogenic role of Na^+/H^+ exchange. *Circ Res* **66**:1156–1159.
Firth JD and Ratcliffe PJ (1992) Organ distribution of the three rat endothelin messenger RNAs and the effects of ischemia on renal gene expression. *J Clin Invest* **90**:1023–1031.
Fujita K, Matsumura Y, Kita S, Miyazaki Y, Hisaki K, Takaoka M, and Morimoto S (1995) Role of endothelin-1 and ET_A receptor in the maintenance of deoxycorticosterone acetate-salt-induced hypertension. *Br J Pharmacol* **114**:925–930.
Gellai M, Jugus M, Fletcher T, Nambi P, Ohlstein EH, Elliot JD, and Brooks DP (1995) Non peptide endothelin receptor antagonists V, Prevention and reversal of acute renal failure in the rat by SB 209670. *J Pharmacol Exp Ther* **275**:200–206.
Gesek FA, Wolff DW, and Strandhoy JW (1987) Improved separation method for rat proximal and distal renal tubules. *Am J Physiol* **22**:F358–F365.
Goldfarb D, Iaina A, Gavendo SS, Kapular S, and Eliahou HE (1983) Beneficial effect of verapamil in ischemic acute renal failure in the rat. *Proc Soc Exp Biol Med* **172**:389–392.
Iwamoto T, Pan Y, Nakamura TY, Wakabayashi S, and Shigekawa M (1998) Protein kinase *C*-dependent regulation of Na^+/Ca^{2+} exchanger isoforms NCX1 and NCX3 dose not require their direct phosphorylation. *Biochemistry* **37**:17230–17238.
Iwamoto T, Watano T, and Shigekawa M (1996) A novel isothiourea derivative selectively inhibits the reverse mode of Na^+/Ca^{2+} exchange in cells expressing NCX 1. *J Biol Chem* **271**:22391–22397.
Kourembanas S, Marsden PA, McQuillan LP, and Faller DV (1991) Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. *J Clin Invest* **88**:1054–1057.
Kuro T, Kohnou K, Kobayashi Y, Takaoka M, Opgenorth TJ, Wessale JL, and Matsumura Y (2000) Selective antagonism of ET_A but not ET_B receptor is protective against ischemic acute renal failure in rats. *Jpn J Pharmacol* **82**:307–316.
Ladilov Y, Haffner S, Balser-Schafer C, Maxeiner H, and Piper HM (1999) Cardioprotective effects of KB-R7943; a novel inhibitor of the reverse mode of Na^+/Ca^{2+} exchanger. *Am J Physiol* **276**:H1868–H1876.
Matsumura Y, Hisaki K, Takaoka M, and Morimoto S (1990a) Phosphoramidon, a metalloproteinase inhibitor, suppresses the hypertensive effect of big endothelin-1. *Eur J Pharmacol* **185**:103–106.
Matsumura Y, Ikegawa R, Takaoka M, and Morimoto S (1990b) Conversion of porcine big endothelin to endothelin by an extract from the porcine aortic endothelial cells. *Biochem Biophys Res Commun* **167**:203–210.
Matsumura Y, Kuro T, Kobayashi Y, Umekawa K, Ohashi N, and Takaoka M (2000) Protective effect of SM-19712, a novel and potent endothelin converting enzyme inhibitor, on ischemic acute renal failure in rats. *Jpn J Pharmacol* **84**:16–24.
Nakamura A, Harada K, Sugimoto H, Nakajima F, and Nishimura N (1998) Effects of KB-R7943, a novel Na^+/Ca^{2+} inhibitor, on myocardial ischemia/reperfusion injury. *Folia Pharmacol Jpn* **111**:105–115.
Ong ACM, Jowett TP, Firth JD, Burton S, Karet FE, and Fine LG (1995) An endothelin-1 mediated autocrine growth loop involved in human renal tubular regeneration. *Kidney Int* **48**:390–401.
Philipson KD and Nicoll DA (2000) Sodium-calcium exchange: a molecular perspective. *Annu Rev Physiol* **62**:111–133.
Rubanyi GM and Polokoff MA (1994) Endothelins: molecular biology, biochemistry, pharmacology, physiology and pathophysiology. *Pharmacol Rev* **46**:325–415.
Scholz W, Albus U, Lang HJ, Linz W, Martorana PA, Englert HC, and Scholkens BA (1993) Hoe 694, a new Na^+/H^+ exchange inhibitor and its effects in cardiac ischemia. *Br J Pharmacol* **109**:562–568.
Schrier RW, Arnold PE, Van Putten VJ, and Burke TJ (1987) Cellular calcium in ischemic acute renal failure: role of calcium entry blockers. *Kidney Int* **32**:313–321.
Shibouta Y, Suzuki N, Shino A, Matsumoto H, Terashita Z, Kondo K, and Nishikawa K (1990) Pathophysiological role of endothelin in acute renal failure. *Life Sci* **46**:1611–1618.
Solez K, Kramer EC, Fox JA, and Heptinstall RH (1974) Medullary plasma flow and intravascular leukocyte accumulation in acute renal failure. *Kidney Int* **6**:24–37.
Tani M and Neely JR (1989) Role of intracellular Na^+ in Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemic rat hearts: possible involvement of H^+-Na^+ and Na^+-Ca^{2+} exchange. *Circ Res* **65**:1045–1056.
Vemulapalli S, Chiu PJ, Chintala M, and Bernardino V (1993) Attenuation of ischemic acute renal failure by phosphoramidon in rats. *Pharmacology* **47**:3188–3193.
Wakimoto K, Kobayashi K, Kuro-O M, Yao A, Iwamoto T, Yanaka N, Kita S, Nishida A, Azuma S, Toyoda Y, et al. (2000) Targeted disruption of Na^+/Ca^{2+} exchanger gene leads to cardiomyocyte apoptosis and defects in heart beat. *J Biol Chem* **275**:36991–36998.
Watano T, Kimura J, Morita T, and Nakanishi H (1996) A novel antagonist, No. 7943, of the Na^+-Ca^{2+} exchange current in guinea-pig cardiac ventricular cells. *Br J Pharmacol* **119**:555–563.
Wilhelm SM, Simonson MS, Robinson AV, Stowe NT, and Schulak JA (1999) Endothelin up-regulation and localization following renal ischemia and reperfusion. *Kidney Int* **55**:1011–1018.
Wilhelm SM, Stowe NT, Robinson AV, and Schulak JA (2001) The use of the endothelin receptor antagonist, tezosentan, before or after renal ischemia protects renal function. *Transplantation* **71**:211–216.
Wilson DR, Arnold PE, Burke TJ, and Schrier RW (1984) Mitochondrial calcium accumulation and respiration in ischemic acute renal failure in the rat. *Kidney Int* **25**:519–526.
Yamashita J, Itoh M, Kuro T, Kobayashi Y, Ogata M, Takaoka M, and Matsumura Y (2001) Pre-ischemic or post-ischemic treatment with a novel Na^+/Ca^{2+} exchange inhibitor, KB-R7943, shows renal protective effects in rats with ischemic acute renal failure. *J Pharmacol Exp Ther* **296**:412–419.
Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, and Masaki T (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature (Lond)* **332**:411–415.
Yonehana T and Gemba M (1999) Ameliorative effect of adenosine on hypoxia-reoxygenation injury in LLC-PK_1, a porcine kidney cell line. *Jpn J Pharmacol* **80**:163–167.
Yu AS, Hebert SC, Lee SL, Brenner BM, and Lytton J (1992) Identification and localization of renal Na^+-Ca^{2+} exchanger by polymerase chain reaction. *Am J Physiol* **263**:F680–F685.

Address correspondence to: Dr. Yasuo Matsumura, Department of Pharmacology, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan. E-mail: matumrh@gly.oups.ac.jp

Reprinted from HEART, March 2003, Vol 89, No 3, p 257

IMAGES IN CARDIOLOGY

Multiple cystic aneurysms in aortitis demonstrated by three dimensional volume rendering images of multislice computed tomography

A 29 year old man presented with slight fever and pulsation at the left neck originating from a gradually expanding palpable mass. Multislice computed tomography (CT) (Aquilion, Toshiba, Tokyo, Japan) was performed with a 1 mm slice thickness, helical pitch 5.5, and 100 ml of iodinated contrast material (300 mg/ml) delivered intravenously at a rate of 3 ml/s. An aneurysm in the left common carotid artery (LCCA) with a mural thrombus was revealed. The aortic arch, proximal portion of the descending aorta (DA) and ascending aorta (AA) appeared to be separated, as if indicating dissection of the lumen. Three dimensional volume rendering images showed collateral arteries around the anterior region of the neck and a cystic lesion from the distal aortic arch to the proximal DA after which the peripheral part of the aorta heads rightward, then downward. In axial source images at this level, the lumen of the distal portion of the aortic arch and proximal portion of the DA appears separated. Cut plane volume rendering images show distal and proximal portions of the LCCA aneurysm. Stenosis and post-stenotic dilation in the proximal portion of the left subclavian artery (LSA) are observed. Multiple cystic lesions are shown at the inferior border of the aortic arch, which in the axial images (panel D, left) appeared as aortic dissection. Thus, three dimensional volume rendering images showed the presence of multiple cystic aneurysms, but not aortic dissections. CT and blood serum studies indicated inflammation and enabled the diagnosis of aortitis, and steroid therapy was started.

N Funabashi
N Komiyama
I Komuro
komuro-tky@umin.ac.jp