ELSEVIER

Biochimica et Biophysica Acta 1374 (1998) 56–62

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

NHE-1 is the sodium–hydrogen exchanger isoform present in erythroid cells

Rangaprasad Sarangarajan [a], Narinder Dhabia [a], Manoocher Soliemani [b], Nancy Baird [b], Clinton Joiner [a,*]

[a] *Division of Hematology/Oncology, Department of Pediatrics, Cincinnati Comprehensive Sickle Cell Center, Cincinnati, OH 45229, USA*
[b] *Division of Nephrology, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, USA*

Received 14 April 1998; accepted 9 July 1998

Abstract

Erythrocyte sodium hydrogen exchanger (NHE) represents one of a limited number of sodium entry pathway in erythrocytes. At least five NHE isoforms have been identified, differing in tissue specificity, regulatory characteristics, and pharmacological sensitivities. Although physiological characteristics of erythrocyte NHE suggest that the widely expressed NHE-1 isoform may be present, evidence is not conclusive and does not exclude the existence of other isoforms. In this study, Northern blot and reverse transcription–polymerase chain reaction (RT–PCR) analyses were used to test for five NHE isoforms in erythroid cells. Blood from patients with sickle cell disease was depleted of white blood cells (WBC) by passage through leukocyte filters and cellulose column. RT–PCR performed on WBC depleted reticulocyte RNA using a NHE-1 primer set yielded product a of expected size, the sequence of which was identical to the published human NHE-1 sequence. Northern blot analysis of the reticulocyte RNA using a 1.6 kb probe revealed a message of approximately 5.0 kb in size. RT–PCR analysis of rat kidney RNA using primers specific for NHE isoforms -2, -3, -4 and rat brain RNA using primer specific for NHE-5 isoform yielded products of expected size, whereas WBC depleted RNA under identical conditions yielded no products. These results identify the erythroid isoform of the sodium–hydrogen exchanger as NHE-1. 0005-2736/98/$ – see front matter © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Erythrocyte; Sodium–hydrogen exchanger; Sickle cell; Reticulocyte

1. Introduction

The sodium–hydrogen exchanger (NHE) exists in the plasma membrane of a variety of cells and is primarily involved in the regulation of intracellular pH and cell volume [1–3]. This electroneutral antiporter is inhibited by micromolar concentrations of amiloride [4,5,28]. To date five NHE isoforms have been cloned from various tissues [6–9]. NHE-1 is ubiquitously expressed [2,10], whereas NHE-2 isoform is expressed in small intestine, colon and stomach, with lower levels of expression in kidney, brain and skeletal muscle [7,11]. NHE-3 isoform is present in kidney, small intestines, testes, ovary, colon and brain but is absent in heart, lungs, liver and skeletal muscle [6,8,12]. NHE-4 isoform is also expressed in stomach [6] and some regions of the brain [32] whereas NHE-5 is present in brain, spleen and testis and is absent in kidneys [9]. Four of the five NHE

* Corresponding author. Comprehensive Sickle Cell Center, Children's Hospital Medical Center, 3333 Burnett Avenue, Cincinnati, OH 45229, USA. Fax: +1-513-636-5562;
E-mail: joinc0@chmcc.org

PII: S0005-2736(98)00130-8

isoforms (NHE-1, -2, -3 and -4) are present in kidney to varying degrees [7,11].

Activity of the NHE can be measured in erythrocytes [14,16], and may be involved in cell volume regulation in response to osmotic changes [13–15]. Increase in NHE activity is observed in normal young erythrocytes (reticulocytes) as well as sickle erythrocytes [17]. Human erythrocyte NHE activity is modulated by intracellular calcium, protein kinase C and insulin [27,28]. It has been suggested that the erythrocyte NHE is involved in counteracting the reduction of cell volume elicited by activation of the KCl cotransporter [17]. In dog erythrocytes, the swelling activated potassium–chloride cotransporter and shrinkage-activated NHE appear to be reciprocally regulated by changes in cell volume [16]. This may particularly be important in context of sickle cell disease, wherein increased KCl cotransporter activity in sickle erythrocyte contributes to cation depletion and cellular dehydration, which contributes to the process of erythrocyte sickling [18].

Several lines of evidence suggests the presence of the widely expressed NHE-1 isoform in erythrocytes. The dose–response curve of amiloride inhibition of red blood cell (RBC) NHE activity is compatible with that of NHE-1 [28] but is not conclusive. This is due to the fact that amiloride is relatively nonspecific in its ability to inhibit the activities of the various NHE isoforms. NHE activity in erythrocytes is modulated by intracellular calcium [29], and insulin and insulin-like growth factors [27]. These characteritics are consistent with, but not specific for, the NHE-1 isoform [30,31]. A polyclonal antibody to a synthetic peptide corresponding to putative NHE-1 external and cytosolic domains recognizes a protein in RBC membranes of the appropriate size [19], although the peptide contained sequences found in other NHE isoforms. Finally, Northern blot analysis of murine erythroleukemia cells reveals an RNA species specific for NHE-1 [20]. Although these studies are consistent with the presence of NHE-1 in the human erythrocyte, proof is lacking and the possibility that other isoforms might be present has not been excluded. To resolve these issues, the present study was designed to identify the erythrocyte NHE isoform by reverse transcription–polymerase chain reaction (RT–PCR) with NHE isoform-specific primers and by Northern blot analysis.

2. Materials and methods

2.1. Preparation of white blood cells (WBC)-depleted erythrocytes

Blood with high reticulocyte content was collected from otherwise healthy patients with sickle cell disease, after informed consent. Whole blood (approximately 60–80 ml) was centrifuged at $80\times g$ in a swinging bucket Beckman GPR centrifuge for 15 min. The plasma was aspirated and remaining blood centrifuged at 3000 rpm for an additional 4 min. Residual plasma was aspirated along with the buffy coat and the pellet resuspended in ice-cold HBS (140 mM NaCl, 20 mM Hepes, 0.1 mM EDTA, 10 mM glucose; pH 7.4). The blood suspension was passed through a high efficiency leukocyte removal filter (RCXL1 Filter, Pall Biomedical, Fajardo, PR) to remove WBC from the suspension. The blood was depleted further of WBCs by passing it through two columns in series, each containing a total of 4 g of a mixture of microcrystalline cellulose and α-cellulose (Sigma, St. Louis, MO) in a ratio of 3:1, respectively [21]. The entire suspension (the filtrate) was passed through the RCXL1 filter and cellulose column one more time. The blood filtrate thus obtained was centrifuged at 3000 rpm for 3 min, washed twice with ice-cold HBS and the erythrocyte pellet was used immediately for isolation of total RNA.

2.2. RNA isolation

Total RNA was isolated from blood and rat kidney by modification of the one-step procedure of Chomczynski and Sacchi [22] using TRI Reagent BD and Trizol reagent, respectively. RNA obtained from whole blood has been abbreviated as WB RNA, WBC-depleted filtered blood has been designated FB RNA, RK and RB RNA from rat kidney and rat brain, respectively.

2.3. Reverse transcription and polymerase chain reaction (RT–PCR)

cDNA was synthesized from total RNA using 200 U of SuperScript RNase H^- Reverse Transcriptase II (Gibco). Each 20 ml reaction mixture contained 5 mM $MgCl_2$, $1\times$PCR buffer, 1 mM each deoxy-

ribonucleotide, 2 U of ribonuclease (RNase) inhibitor and 2.5 μM Oligo(dT) 12–18 primer. Reverse transcription was carried out at 42°C for 30 min in a Perkin Elmer thermal recycler unless otherwise specified. PCR was performed (100 ml mixture containing 2 mM $MgCl_2$, 1×PCR buffer, 1 mM each deoxyribonucleotide, 20 mM each of forward and reverse primers and 2.5 U of *Taq* polymerase; 95°C, 1 min; 60°C, 1 min; 72°C, 2 min; 72°C, 15 min extension) using NHE isoform-specific primers. The nucleotide positions and expected product sizes are described in Table 1. PCR products were analyzed by size fractionation on a 2% agarose gel stained with ethidium bromide. The reaction mixtures and experimental conditions used in RT–PCR for the WBC-specific marker HLA-DQα on WB RNA and FB RNA are the same as described above.

2.4. Subcloning and sequence analysis of PCR amplicons

PCR products were ethanol precipitated, resuspended and size fractionated on a 2% agarose gel stained with ethidium bromide. The PCR product of appropriate size obtained from FB RNA, corresponding to the expected size of the NHE-1 PCR product, was cut out of the agarose gel, eluted and ethanol-precipitated. The DNA was resuspended and subcloned into an *Eco*RI site of pCR2.1 vector using the Original TA Cloning kit (Invitrogen, San Diego, CA) according to manufacturer's protocol. After confirmation of the presence of the insert by restriction enzyme digestion and agarose gel electrophoresis, the insert was sequenced by automated sequencing at the University of Cincinnati Medical Center DNA Core facility. The sequence obtained was compared for similarity to the NHE isoforms in the GenBank database using BLAST software.

2.5. Gel electrophoresis and Northern blot analysis

Twenty μg of total RNA was denatured with formaldehyde, size fractionated by electrophoresis through a 1.2% agarose gels, and transferred onto a nylon membrane [23]. RNA was fixed on the membrane by UV crosslinking using a Stratagene Stratalinker 1800. RNA size markers (0.24–9.5 kb ladder, Gibco BRL, Gaithersburg, MD) were visualized with UV light after staining with ethidium bromide. Membranes were prehybridized overnight at 42°C in a prehybridizing solution containing 50% formamide, 5×SSPE, 5×Denhardt's solution, 0.1% SDS and 200 μg/ml of denatured salmon sperm DNA. Hybridization was carried out for 24 h in 50% formamide, 5×SSPE, 2×Denhardt's solution, 0.1% SDS and 100 μg/ml of denatured salmon sperm DNA. The rat NHE-1-specific cDNA restriction endonuclease fragment, a *Pst*I–*Pst*I fragment (nucleotides 478–1850) [24]. This NHE-1 isoform-specific cDNA restriction endonuclease fragment was radiolabeled (approximately 2×10^6 cpm/ml) and was added to the hybridization solution. The cDNA was radiolabeled with [α-^{32}P]dCTP using High Prime DNA Labeling kit according to the manufacturer's instructions. The blots were washed at room temperature twice for 30 min in 2×SSC, 0.5% SDS and once for

Table 1
Sodium/hydrogen exchanger primer positions and product sizes

Isoform	5′ (Sense) primer (5′–3′)	3′ (Antisense) primer (5′–3′)	Nucleotides	Product size	Reference
NHE-1	CAAGAGACGAAGCGCTCCATCAACG	ATCTGGTTCCAGGCTTCCTCGTAGG	1943–2406	463 bp	[6]
NHE-2	AGCTTCCCGAGAAGTTGC	TCGCACCAACCTTGGTGG	2213–2576	363 bp	[7]
NHE-3	ACAAGAAGGCAGCCAAGC	TTGGGAATCTGGACACG	2104–2458	354 bp	[6]
NHE-4	AGCCGTGGAATCCATGC	CCTCAAGGTGTTCTGGC	2228–2376	148 bp	[6]
NHE-5	AACATCTCTGGTCCCTGAG	CCTAGGGGCTACAAGTCCAGCCTGC	23–411	388 bp	[9]
HLA-DQα	GCTCTGATGCTGGGGTCCC	GGGCCCTTGGTGTCTGGAA	16–759	743 bp	[25]

Description of the nucleotide sequences and positions of the NHE isoform-specific 5′ primer (sense) and 3′ primer (antisense) sets used for RT–PCR analysis of the rat kidney, rat brain and filtered blood RNA for NHE isoforms and HLA-DQα. Corresponding to each of the primer sets, the figure listed under product size is the size of the expected nucleotide fragment for each NHE isoform. The sequences for the isoform-specific primers were obtained from literature cited (right column).

30 min in 0.1×SSC, 0.5% SDS. The blots were then examined by autoradiography.

3. Results and discussion

The identification of the erythroid NHE isoform by use of molecular biology techniques depends on the preparation of purified reticulocytes from whole blood free of mRNA species derived from WBC source. Blood with high reticulocyte counts from sickle cell patients was obtained and WBC were eliminated by use of a combination of high-efficiency leukocyte filters and cellulose columns. Reticulocyte preparations was tested for the presence of WBC by RT–PCR analysis using primers specific for the HLA-DQα cDNA. This gene is expressed only in WBC and not in erythroid cells [25], and is a sensitive marker for determination of leukocyte derived RNA contamination of reticulocyte preparations. Fig. 1 illustrates that the HLA-DQα mRNA was detected as a PCR product of expected size in whole blood preparations (WB RNA) containing WBC, but is absent in purified reticulocyte preparations (FB RNA), demonstrating that FB RNA was free of RNA from white blood cells. Sequence analysis of bands observed in lane marked FB RNA were PCR products, the sequences of which were unrelated to HLA-DQα (Fig. 1). Both WB and FB RNA showed the presence of a RT–PCR product of the expected size for hemoglobin (Fig. 2).

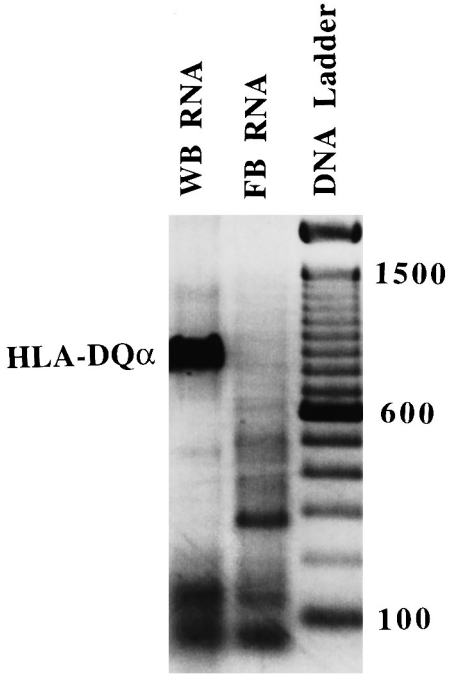

Fig. 1. RT–PCR analysis of whole blood and filtered blood RNA for HLA-DQα expression to verify complete removal of white blood cell in filtered blood preparations. PCR products were analyzed by size fractionation on 2% agarose gel by ethidium bromide staining. A band of expected size (947 bp) for HLA-DQα is present in lane containing whole blood (WB RNA) and is absent in filtered blood (FB RNA). The extreme right lane is the 0.1 kb DNA size marker, the values corresponding to the expected size of the DNA fragment.

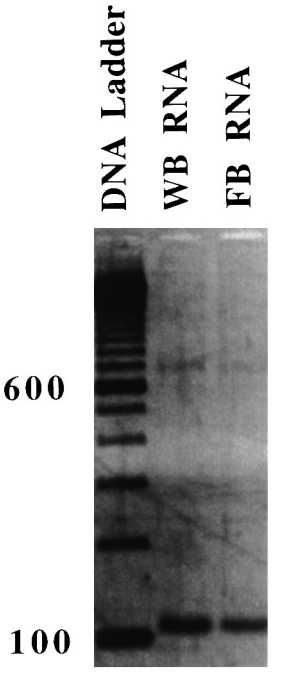

Fig. 2. RT–PCR analysis of filtered and whole blood for hemoglobin: RT–PCR was carried out as described in Section 2. PCR product of expected size (110 bp) for hemoglobin is obtained from both whole (WB RNA) and filtered (FB RNA) blood.

Table 1 lists the nucleotide sequences, positions of isoform-specific PCR primers and expected product size of various NHE isoforms. Electrophoretic analysis of the RT–PCR amplicon for FB RNA and RK RNA using NHE-1-specific primers is shown in Fig. 3. A product of expected size (463 bp) is identified in rat kidney, where this isoform is known to be expressed. A product of similar size is also identified in reticulocyte RNA (FB RNA). The 463 bp product obtained from FB RNA was subcloned and sequenced and found to be identical to expected

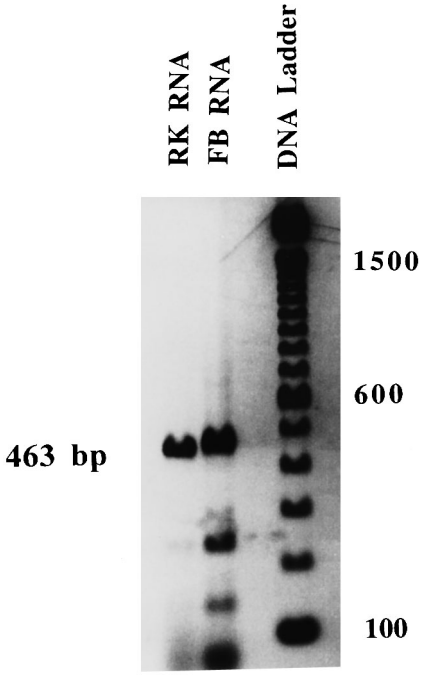

Fig. 3. RT–PCR analysis of filtered blood for presence of NHE-1 isoform. Ethidium bromide staining RT–PCR products size fractionated on 2% agarose gel revealed a 463 bp product from filtered blood (FB RNA). A 463 bp PCR product is also seen in rat kidney (RK) RNA used as a positive control. Nucleotide sequences of PCR products corresponding to approximately 120 bp and 220 bp FB RNA were unrelated to sodium–hydrogen exchanger and did not contain primer sequences.

NHE-1 sequence [26]. Northern blot analysis of total RNA isolated from reticulocytes and rat kidney is shown in Fig. 4. The probe was a 1.6 kb fragment of NHE-1 [24], and recognized an approximately 5.0 kb species in both RK and FB RNA preparations, consistent with published reports of NHE-1 transcript size [6]. Thus, by both RT–PCR and Northern analysis, the presence of mRNA for NHE-1 is demonstrable in reticulocytes, indicating the expression of this isoform in erythroid cells.

To determine the expression of other NHE isoforms in reticulocytes, FB RNA was used for RT–PCR in presence of isoform-specific primers listed in Table 1, with RK and RB RNA as positive controls. While RK RNA yielded PCR products of the expected size for NHE isoforms -2, -3 and -4, no products were obtained from FB RNA (Fig. 5). Likewise, RT PCR with NHE-5 primers yielded the expected 450 bp product with RB RNA, but not with FB RNA (Fig. 6).

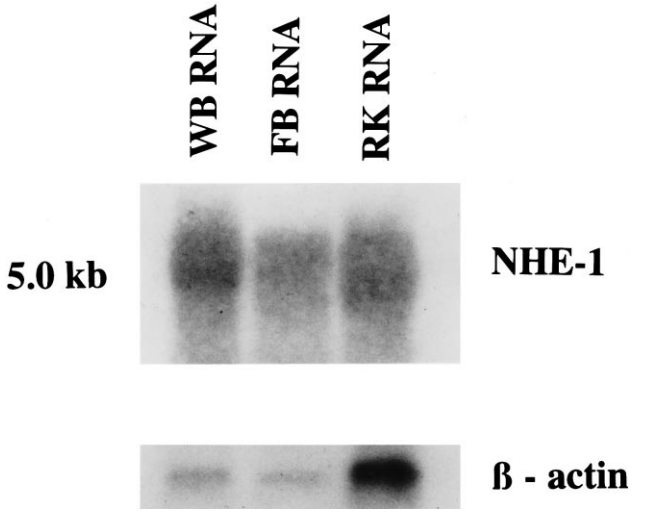

Fig. 4. Northern blot analysis of whole blood (WB), filtered blood (FB) and rat kidney (RK) RNA for NHE-1 using isoform-specific probe. The top panel is a 72-h exposure of autoradiograph describing message for NHE-1 approximately 5.0 kb in size. The blot was stripped and probed for β-actin as positive control. The bottom panel is an autoradiograph showing message for β-actin after overnight exposure.

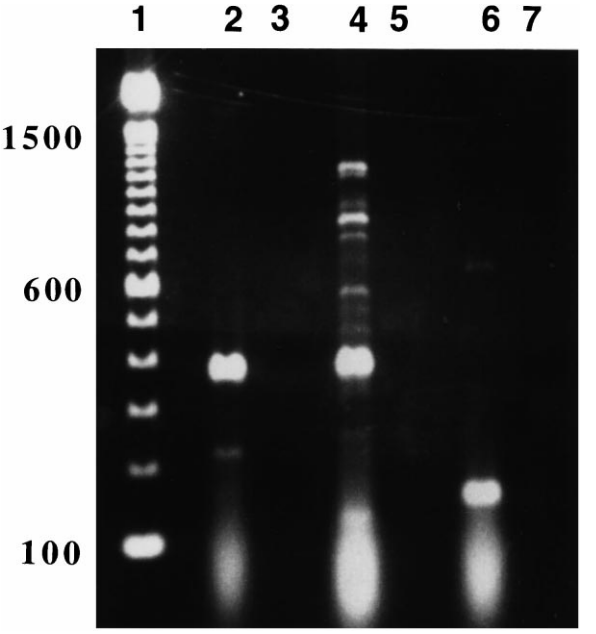

Fig. 5. RT–PCR analysis of filtered blood RNA to determine the presence of other NHE isoforms using primers specific for NHE-2, NHE-3 and NHE-4, respectively. Lanes 2, 4 and 6 correspond to RT–PCR products of expected sizes for NHE-2, NHE-3 and NHE-4 for rat kidney RNA used as positive control. No corresponding products are seen in lanes 3, 5 and 7 for filtered blood RNA.

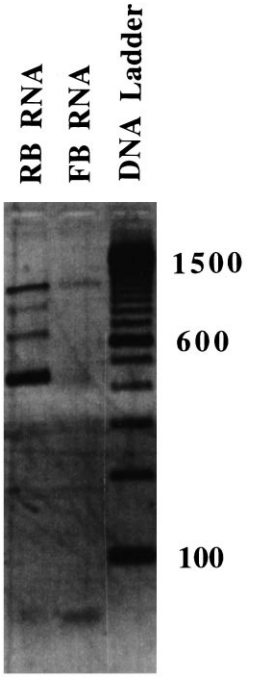

Fig. 6. RT–PCR of filtered blood (FB) RNA using primers specific for NHE-5 isoform. Ethidium bromide staining of agarose gel describes the PCR product of expected size for rat brain (RB) RNA, used as a positive control. All PCR products detected in RB RNA were subcloned and sequenced. Only the band corresponding to 388 bp is identical to NHE-5. FB RNA does not show any PCR product of expected size for NHE-5. A faint band seen in FB RNA corresponding 1100 bp is unrelated to NHE-5.

We conclude that reticulocytes express NHE-1, without evidence for expression of other known NHE isoforms. The conclusions are based on the evidence from the experiments designed specifically to detect the presence of various NHE isoform-specific mRNA expression in the reticulocytes. It is possible that other isoforms of the sodium–hydrogen exchanger are expressed earlier during the erythroid differentiation. The results presented in this study do not exclude the possibility that erythroid cells could express other NHE isoforms that have not yet been identified. However, the expression of NHE-1 in the later stages of differentiation, along with the indirect data that the physiological characteristics of the sodium–hydrogen exchanger activity in red blood cells are consistent with NHE-1, strongly suggest that NHE-1 is the functionally dominant isoform in human erythrocytes.

Acknowledgements

We would like to thank Greta Snipes and other nurses involved in collection of blood samples for the study; Moarong Jiang, Mary Palascak, Therese Nemeth, William Claussen and Zhaohui Wang for their assistance in the laboratory; Peaches Carter and Doreen Collins at the Comprehensive Sickle Cell Center for their assistance with the paperwork. This project was supported in part by HL 57614 (C.H.J.); DK46789 and DK52821 from NIDDK (M.S.); SW-95-05-YI (N.B.) from American Heart Association (Ohio Affiliate) and a Summer Undergraduate Fellowship (N.D.) from American Heart Association (Ohio Affiliate).

References

[1] L. Counillon, J. Pouyssegur, Structure–function studies and molecular regulation of the growth factor activatable sodium–hydrogen exchanger (NHE-1), Cardiovasc. Res. 29, (2) (1995) 147–154.
[2] R.L. Mahnensmith, P.S. Aronson, The plasma membrane sodium–hydrogen exchanger and its role in physiological and pathophysiological processes, Circ. Res. 56, (6) (1985) 773–788.
[3] S. Grinstein, M. Woodside, G.G. Goss, A. Kapus, Osmotic activation of the Na/H antiporter during volume regulation, Biochem. Soc. Trans. 22 (1994) 512–516.
[4] P.S. Aronson, M.A. Suhm, J. Nee, Mechanisms of active H^+ secretion in the proximal tubule, Am. J. Physiol. 245 (1983) F467–F659.
[5] D.J. Benos, Amiloride: A molecular probe of sodium transport in tissues and cells, Am. J. Physiol. 242 (1982) C131–C145.
[6] J. Orlowski, R.A. Kandasamy, G.E. Shull, Molecular cloning of putative members of the Na/H exchanger gene family: cDNA cloning, deduced amino acid sequence, and mRNA tissue expression of the rat Na/H exchanger NHE-1 and two structurally related proteins, J. Biol. Chem. 267, (13) (1992) 9331–9339.
[7] C.M. Tse, S.A. Levine, C.H.C. Yun, M.H. Montrose, P.J. Little, J. Pouyssegur, M. Donowitz, Cloning and expression of a rabbit cDNA encoding a serum-activated ethylisopropylamiloride-resistant epithelial Na/H exchanger isoform (NHE-2), J. Biol. Chem. 269, (16) (1993) 11917–11924.
[8] C.M. Tse, S.R. Brant, M.S. Walker, J. Pouyssegur, M. Donowitz, Cloning and sequencing of a rabbit cDNA encoding an intestinal and kidney-specific Na/H exchanger isoform (NHE-3), J. Biol. Chem. 267, (13) (1992) 9340–9346.
[9] C.A. Klanke, Y.R. Su, D.F. Callen, Z. Wang, P. Meneton,

N. Baird, R.A. Kandasamy, J. Orlowski, B.E. Otterund, M. Leppert, G.E. Shull, A.G. Menon, Molecular cloning and physical and genetic mapping of a novel human Na/H exchanger (NHE5/SLC9A5) to chromosome 16q22.1, Genomics 25 (1995) 615–622.

[10] J. Noel, J. Pouyssegur, Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na/H exchanger isoforms, Am. J. Phyisol. 268 (1995) C283–C296.

[11] Z. Wang, J. Orlowski, G.E. Shull, Primary structure and functional expression of a novel gastrointestinal isoform of the rat Na/H exchanger, J. Biol. Chem. 268 (1993) 11925–11928.

[12] S.R. Brant, C.H.C. Yun, M. Donowitz, C.M. Tse, Cloning, tissue distribution, functional analysis of the human Na/H exchanger isoform, NHE3, Am. J. Physiol. 38 (1995) C198–C206.

[13] M.L. Jennings, S.M. Douglas, P.E. McAndrew, Amiloride-sensitive sodium–hydrogen exchange in osmotically shrunken rabbit red blood cells, Am. J. Physiol. 251 (1986) C32–C40.

[14] N. Escobales, M. Canessa, Amiloride-sensitive Na^+ transport in human red cells: Evidence for a Na/H exchange system, J. Membr. Biol. 90 (1986) 21–28.

[15] P.M. Cala, Volume regulation by red blood cells mechanisms of ion transport between cells and mechanisms, Mol. Physiol. 4 (1983) 33–52.

[16] J.C. Parker, T.J. McManus, L.C. Starke, H.J. Gitelman, Coordinated regulation of Na/H exchange and [K-Cl] cotransport in dog red cells, J. Gen. Physiol. 96, (6) (1990) 1141–1152.

[17] M. Canessa, M.E. Fabry, S.M. Suzuka, K. Morgan, R.L. Nagel, Na^+/H^+ exchange is increased in sickle cell anemia and young normal red cells, J. Membr. Biol. 116 (1990) 107–115.

[18] C.H. Joiner, Cation transport and volume regulation in sickle red blood cells, Am. J. Physiol. 264 (1993) C251–C270.

[19] E.J. Weinman, D. Steplock, D. Corry, S. Shenolikar, Identification of the human NHE-1 form of Na–H exchanger in rabbit renal brush border membrane, J. Clin. Invest. 91 (1993) 2097–2102.

[20] E. Delpire, S.R. Gullans, Cell volume and K^+ transport during differentiation of mouse erythroleukemia cells, Am. J. Physiol. 235, (2) (1994) C515–C523.

[21] E. Beutler, C. West, K.G. Blume, The removal of leukocyte and platelets from whole blood, J. Lab. Clin. Med. 88 (1976) 328–333.

[22] P. Chomczynsky, N. Sacchi, Single-step RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, Anal. Biochem. 162 (1987) 156–159.

[23] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

[24] Z. Wang, H. Rabb, T. Craig, C. Burnham, G.E. Shull, M. Soleimani, Ischemic-reperfusion injury in the kidney: Overexpression of colonic H^+-K^+-ATPase and suppression of NHE-3, Kidney Int. 51 (1997) 1106–1115.

[25] D. Prati, B.D. Rawal, C. Dang, C. Capelli, G.N. Vyas, DNA enzyme immunoassay of the PCR-amplified HLA-DQ alpha gene for estimating residual leukocytes in filtered blood, Clin. Diagn. Lab. Immunol. 2, (2) (1995) 182–185.

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

[27] G. Ceolotto, P. Conlin, G. Clari, A. Semplicini, M. Canessa, Protein kinase C and insulin regulation of red blood cell Na^+/H^+ exchange, Am. J. Physiol. 272 (1997) C818–C826.

[28] N. Escobales, M. Canessa, Ca^{2+}-activated Na^+ fluxes in human red cells, J. Biol. Chem. 260, (22) (1985) 11914–11923.

[29] B. Bertrand, S. Wakabayashi, T. Ikeda, J. Pouyssegur, M. Shigekawa, The Na^+/H^+ exchanger isoform 1 (NHE1) is a novel member of the calmodulin-binding proteins. Identification and characterization of calmodulin-binding sites, J. Biol. Chem. 269, (18) (1994) 13703–13709.

[30] A. Klip, Action of insulin on Na^+/H^+ exchange, in: S. Griensteion (Ed.), Na^+/H^+ Exchange, CRC, Boca Raton, FL, 1986, pp. 285–303.

[31] D.W. Johnson, B.K. Brew, P. Poronnik, D.I. Cook, M.J. Field, A.Z. Gyory, C.A. Pollock, Insulin-like growth factor I stimulates apical sodium/hydrogen exchange in human proximal tubule cells, Am. J. Physiol. 272, (4) (1997) F484–F490.

[32] C. Bookstein, M.V. Musch, A. DePaoli, Y. Xie, K. Rabenau, M. Villereal, M.C. Rao, E.B. Chang, Characterization of the rat Na^+/H^+ exchanger isoform NHE-4 and localization in rat hippocampus, Am. J. Physiol. 271 (1996) C1629–C1638.