Molecular cloning, tissue distribution, and functional expression of the human Na$^+$/H$^+$ exchanger NHE2

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Malakooti, J aleh, Refka Y. Dahdal, Larry Schmidt, Thomas J. Layden, Pradeep K. Dudeja, and Krishnamurthy Ramaswamy. Molecular cloning, tissue distribution, and functional expression of the human Na$^+$/H$^+$ exchanger NHE2. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G383–G390, 1999.—In the present report, we describe the cloning of a human colonic cDNA that describes the full-length Na$^+$/H$^+$ exchanger (NHE) 2 coding region. The human NHE2 (hNHE2) cDNA encodes for a polypeptide of 812 amino acids with a 90% overall identity to both rabbit and rat NHE2 isoforms. In comparison with SLC9A2, recently reported as the human NHE2, the hNHE2 polypeptide is 115 amino acids longer in the NH$_2$-terminal end and shows only an 84% DNA nucleotide sequence identity. Northern blot analysis revealed that hNHE2 message has an uneven tissue distribution, with high levels in the skeletal muscle, colon, and kidney and lower levels in the testis, prostate, ovary, and small intestine. Protein expression studies with hNHE2 clone showed that a 75-kDa protein was expressed. Stable expression of transfected full-length hNHE2 cDNA in Na$^+$/H$^+$ exchange-deficient LAP1 cells exhibited Na$^+$-dependent pH recovery after an acid prepulse that was inhibited by 0.1 mM amiloride. These data indicate that this cDNA is the true human NHE2 cDNA and that the encoded protein is capable of catalyzing Na$^+$/H$^+$ exchange activity.

human colon; sodium transport

THE SODIUM/HYDROGEN EXCHANGERS (NHE) are highly conserved in all eukaryotes and participate in various cellular functions, such as maintenance of intracellular pH (pHi) and cell volume and vectorial Na$^+$ transport. At physiological conditions, these antiporters mediate the electroneutral exchange of extracellular Na$^+$ with intracellular H$^+$ with a stoichiometry of 1:1 (17, 27). Recent molecular cloning studies have demonstrated the existence of a Na$^+$/H$^+$ exchange gene family, of which at least six members, designated NHE1 to NHE6, have been cloned from different animal species (1, 2, 12, 18, 25, 26, 29). The NHE1 isoform is ubiquitously expressed in all tissue types (17, 19, 27). Unlike NHE1, NHE2, NHE3, and NHE4 isoforms exhibit tissue-specific expression (2, 19, 29). In polarized epithelial cells, NHE1 is localized to the basolateral membrane (30) and is involved in the housekeeping functions, whereas NHE2 and NHE3 are localized to the apical membrane and are suggested to be involved in vectorial sodium transport (26, 29). Recent functional and immunochemical studies have suggested basolateral membrane localization of the NHE4 isoform in the thick ascending limb and distal convoluted tubules of the rat renal cortex (3). Functional analysis in heterologous systems has confirmed the ability of NHE1, NHE2, and NHE3 cDNA clones to complement the Na$^+$/H$^+$ exchange-deficiency of the mutant cells that lack endogenous NHE activity (10, 19, 31, 33). In comparison to the other NHE isoforms, NHE4 exhibits distinct functional and pharmacological properties. For example, in a Na$^+$/H$^+$ exchange-deficient heterologous system, this isoform can only be activated in the presence of a coupling agent such as DIDS, and it is extremely resistant to amiloride (4).

Structurally, all NHE isoforms share the same topology and are known to have two distinct domains. Roughly the first 500 amino acids comprise the NH$_2$-terminal domain, which is highly hydrophobic with 10–12 potential membrane-spanning segments. The last 300 amino acids are highly hydrophilic and compose the COOH-terminal domain, suggested to be the cytoplasmic region of the protein (19, 25).

To date, the complete primary structure of the human NHE1 and NHE3 isoforms have been determined and their biochemical properties have been characterized (2, 25). Cloning of a human NHE2 (hNHE2) isoform from human liver, designated SLC9A2, has also been described (11). However, SLC9A2 shows significant sequence differences from the full-length rat and rabbit NHE2 and exhibits a 99% amino acid identity to a truncated version of the rat NHE2 isoform (5).

Our earlier transport studies utilizing human intestinal basolateral and brush-border membrane vesicles have indicated kinetic and other mechanistic differences for the transport of Na$^+$ from what has been reported in the rat and rabbit intestine, prompting us to investigate the characteristics of the hNHE isoforms at the molecular level (6, 7, 13, 20, 21, 34). Our recent studies using RNase protection and in situ hybridization demonstrated that the expression of NHE2 and NHE3 mRNA in the human gastrointestinal tract was different from the results obtained in the rat and rabbit tissues (8). In the present study, we demonstrate cloning of the full-length hNHE2 cDNA and its functional expression. We present data showing that our clone is the hNHE2 cDNA and is different from SLC9A2. The hNHE2 cDNA codes for a 75-kDa protein that is capable of catalyzing Na$^+$/H$^+$ exchange activity in LAP1 cells lacking endogenous NHE activity. Northern blot analysis of hNHE2 mRNA expression in various human tissues revealed that NHE2 mRNA was predomi-
nantly expressed in skeletal muscle, colon, and kidney and was present at lower levels in the testis, prostate, ovary, and small intestine.

**EXPERIMENTAL PROCEDURES**

Materials. Buffer reagents were purchased from Sigma Chemical (St. Louis, MO). Restriction endonucleases and other modifying enzymes were from either New England Biolabs (Beverly, MA), GIBCO BRL (Gaithersburg, MD), or Promega (Madison, WI). TA cloning kit, JM109 competent cell, and transcription/translation-coupled (TNT) wheat germ extract system were from Promega.

Molecular techniques. DNA manipulations, including restriction enzyme digestions, ligations, plasmid isolation, and Escherichia coli transformation, were carried out by standard methods (22).

RNA extraction and PCR analysis. Total RNA was extracted by the RNeasy method (Tel-Test, Friendswood, TX) according to the manufacturer’s directions. Poly(A)^+ mRNA was prepared by two successive passages through oligo(dt) cellulose column using the QuickPrep mRNA purification kit (Pharmacia Biotech, Piscataway, NJ). PCR was performed in a Perkin-Elmer Cetus DNA cyclr, with thermostable DNA polymerases Taq (Perkin-Elmer, Foster City, CA), Klen-Taq (Clontech, Palo Alto, CA), or rTth (Boehringer Mannheim, Indianapolis, IN) according to the manufacturers’ directions.

cDNA cloning. A Marathon-ready cDNA library of T84, a colonic adenocarcinoma cell line, was constructed with poly(A)^+ mRNA and an oligo(dt) primer with the use of a kit from Clontech. The hNHE2-specific primers were designed based on the nucleotide sequence of our previously cloned 595-bp partial hNHE2 cDNA (8). The sequences of the primers used for 5’ rapid amplification of cDNA ends (RACE) and 3’ RACE were 5’-TTGTTCTCGATTAACGAC-3’ and 5’-TACTGTCCTCATTGATCAT-3’, respectively. These oligonucleotides and Marathon anchor primers were used to amplify cDNA fragments from the T84 Marathon-ready library by RACE. PCR products were gel purified and cloned in pGEM-T, a TA cloning vector, and then were used to transform E. coli JM109. Sequence analysis of the 3’ RACE clones extended the 595-bp sequence to a poly(A)^+ tail, which was located 179-bp downstream from an in-frame translation termination codon. In the 5’ direction, the sequence was extended about 1.2 kb in several independent clones. However, no translation initiation codon was present at the NH2-terminal end of the open reading frame (ORF), and the 5’ termini of some clones diverged from sequences corresponding to hNHE2 ORF, suggesting that the cloned cDNA was missing the 5’ end. Attempt to extend the cDNA sequence further in the 5’ direction from our T84 Marathon-ready library was unsuccessful. Therefore, a human colonic cDNA library (Clontech) was screened by PCR to obtain the missing 5’ segment of the cDNA. This was accomplished by employing a sense primer from λgt11 donor site and an antisense primer that was designed based on one of the 5’ RACE clones obtained from the T84 library. Subsequent to sequencing the new clones, PCR primers were synthesized based on the extreme 5’ and 3’ ends of newly identified overlapping sequences and were used to amplify hNHE2 cDNA from a human normal colonic cDNA library, which was then cloned in pGEM-T PCR cloning vector.

DNA sequence analysis. The hNHE2 cDNA was sequenced from double-stranded templates by the dideoxyoligonucleotide chain termination method (23) with the use of the 35S-labeled dATP and Sequenase II kit (US Biochemical, Cleveland, OH).

The entire hNHE2 cDNA sequence was determined on both DNA strands.

Northern blot analysis. Commercially available human multiple-tissue Northern blots, [hMTN and hMTN II (Clontech)], each containing poly(A)^+ RNA (2 µgulane) from different human tissues, were used for Northern blot analysis. A 350-bp HindIII fragment from the 3’ end of the coding region of hNHE2 was radiolabeled with [α-32P]dCTP (3,000 Ci/mmol; ICN, Costa Mesa, CA) by random priming to a specific activity of 10^9 cpm/µg. Prehybridization and hybridization were performed at 68°C in ExpressHyb solution (Clontech) according to the manufacturer’s instructions, with a final probe concentration of 10^6 cpm/ml. The blots were washed for 40 min at room temperature in 2× standard saline citrate (SSC)-0.05% SDS and for 20 min at 50°C in 0.1× SSC-0.1% SDS and were visualized by X-ray autoradiography.

Construction of hNHE2 expression vectors. The plasmids used in these studies were all constructed in mammalian expression vector pRC-CMV (Invitrogen, Carlsbad, CA). To facilitate subcloning of hNHE2 cDNA, a Hind III restriction endonuclease site was introduced to sequences upstream from the ATG translation initiation codon by PCR. The PCR products were then digested with restriction enzymes Hind III and BamH I, gel purified, and cloned, along with the BamH I and Not I (Not I restriction endonuclease site was from the polylinker of pGEM-T cloning vector) fragments of hNHE2 cDNA in Hind III-Not I sites of pRC-CMV, resulting in plasmid pHNHE2. To construct a 5’-tagged hNHE2 expression vector, Flag epitope (Eastman Kodak) tag was added in frame between the first and second amino acids by PCR using a sense oligonucleotide: 5’-GTCAGCTTCTCGAGATGGAATCTCAAGGACGACGATGACAAAGAAACCACCTGAGCACG3’ that contained a Hind III restriction endonuclease site upstream from the ATG start codon (underlined), followed by 24 nucleotides coding for Flag peptide (shown in italic), which preceded the hNHE2 sequences (shown in bold) and antisense primer 5’-GATGAGTTGGAGAGGATCAGTACAGA-3’. The PCR products were then digested with restriction enzymes Hind III and BamH I, gel purified, and cloned, along with the BamH I and Not I fragments of hNHE2 cDNA, Hind III-Not I sites of pRC-CMV. The resulting plasmid was pHFPNHE2. Plasmid pEAP harboring hNHE1 cDNA (kindly provided by J. Pouyssegur) was used to construct a Flag-tagged NHE1 expression vector (pFPNHE1), as described for pFPNHE2 construction. All PCR products and ligation points of the new constructions were sequenced to confirm the fidelity of the cDNA inserts.

In vitro synthesis of human NHE2 protein. Proteins encoded by clones pHNHE2, pHNHE2, pFPNHE2, and pRC-CMV were synthesized in vitro using a T7-dependent TNT wheat germ extract system (Promega). Briefly, 1 µg of plasmid DNA was combined with 25 µl of wheat germ extract, 20 µM of amino acid mixture minus methionine, 40 units of RNase inhibitor, 10 units of T7 RNA polymerase, and 40 µCi of [35S]methionine (Amersham, Arlington Heights, IL) in a final volume of 50 µl. After a 1-h incubation at 30°C, the 2.5-µl aliquot from each sample was removed, mixed with 10 µl of sample buffer, boiled at 100°C for 3 min, and analyzed on a 10% SDS-polyacrylamide gel by PAGE. Gel was fixed in a solution of 20% methanol-10% acetic acid, dried, and exposed to X-ray film. A plasmid containing the luciferase gene was used as positive control (pLUC), and a reaction without the DNA template was performed to test the background incorporation of [35S]methionine.

Cell culture and transfection. The LAP1 mouse fibroblast cell line, which lacks endogenous Na^⁺/H^+ exchange activity (10), was used for transfection studies, and cells were grown...
based on our previously cloned 595-bp fragment, which
sensitivity to amiloride. The perfusion medium was then
colonic adenocarcinoma cell line T84 was utilized (see
(Fig. 1). As a template, a cDNA library from human
hNHE2 cDNA, a 5
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rabbit and rat NHE2 isoforms. To isolate the entire
clone showed a high degree of amino acid homology to
cDNA from a human stomach cDNA library (8). This
measurement was then performed in six-well plates seeded by 1 x 10^6 cells per plate with the use of LipofectAMINE (GIBCO BRL), with 1.5
μg of total plasmid DNA. After 48 h, transfectants were tested for survival in response to acid load as described (9) and stable transfectants were selected in media containing G418 (400 active U/ml) in a period of 1–2 wk and maintained in
media containing G418.

Measurement of Na+\!/H+ exchange activity. The Na+\!/H+ exchange activity was determined using 2',7'-bis(carboxy-
ethyl)-5(6)-carboxyfluorescein (BCECF) fluorescence to mea-
sure pH alterations in response to extracellular Na+. After
an ammonium chloride prepulse. Cells grown on slides were quickly
washed three times with Na+ medium containing (in mM) 120
NaCl, 1.7 CaCl2, 5 KCl, 1.0 MgCl2, 1.0 NaH2PO4, 36 mannitol, 5
dextrose, 10 HEPES, and Tris, pH 7.4. The cells were then loaded
with BCECF in the dark at 23°C for 15 min as described previously (16). Cells were washed two times in Na+ medium and
placed in a 2-ml cuvette at a 45° angle to the excitation
beams in a PTI Alpha scan spectrophotometer (Princeton, NJ). Ex-
citation wavelengths were 500 and 440 nm, and the emission
wavelength was 530 nm. To measure basal ex-
changer activity, the medium was changed to a Na+-free medium in which Na+ was replaced with choline and then to a
Na+-medium in the presence of 0.1 mM amiloride for 3 min. After these initial measurements, cells were acidified by
perfusing with 20 mM NH4Cl prepulse followed by incubation in
Na+-free buffer. This was followed by perfusion in a Na+-
containing medium with 0.1 mM amiloride to determine
sensitivity to amiloride. The perfusion medium was then
changed to a medium containing Na+ to determine the
Na+-dependent pH recovery as indicated by increased BCECF
fluorescence. A pH curve of BCECF fluorescence activity
ratios was then established at the end of each experiment
using the nigericin technique as described previously (16).

RESULTS

Cloning of the human NHE2 cDNA. We have previ-
ously described the cloning of a 595-bp partial NHE2
cDNA from a human stomach cDNA library (8). This
cDNA was a high degree of amino acid homology to
rat and rat NHE2 isoforms. To isolate the entire
hNHE2 cDNA, a 5' and 3' RACE protocol was employed (Fig. 1). As a template, a cDNA library from human
colon adenocarcinoma cell line T84 was utilized (see
EXPERIMENTAL PROCEDURES). Primers were designed
based on our previously cloned 595-bp fragment, which
extended the cDNA sequence at the 3' end to a poly(A)+
tail. In the 5' direction, the cDNA sequence was ex-
pressed about 1.2 kb in several independent clones; how-
ever, DNA sequence analysis revealed that at the 5' end all of the 1.2-kb fragments had either minimal
advances toward the translation initiation site or had
recombined with unrelated DNA sequences. Therefore,
we used DNA from a normal human colonic cDNA \lgt11
library to obtain the 5' region. This was accomplished
by PCR with the use of an antisense primer to one of the
5' RACE-generated clones and a sense primer from the
\lgt11 cloning site. A fragment that had overlapping
DNA sequences at the 3' end with hNHE2 sequence and contained an in-frame translation initiation codon
was obtained. Together, these clones generated a com-
posite cDNA of 2691 nucleotides. Next, a set of primers
derived from the 5' and 3' ends of the composite
sequence were used to amplify and clone the entire
hNHE2 cDNA from a normal human colonic cDNA
library.

DNA nucleotide sequence of hNHE2 cDNA and de-
duced amino acid sequence. The complete DNA nucleo-
tide sequence of hNHE2 cDNA and its deduced amino
sequence are shown in Fig. 2. The cDNA contains
90 bp of 5' untranslated region, 2436 bp of ORF, 162
bp of 3' untranslated region, and a 20-nucleotide
poly(A)+ tract. The extent of the transcription unit in
the 5' direction is uncertain. The ORF is capable of
encoding a polypeptide of 812 amino acid residues with
a calculated molecular weight of 91,413. The putative
ATG translation initiation site at bp 91 (Fig. 2) is in fair
agreement with Kozak's (14) consensus sequence, A/G
CCATGG, with a G at position +4 and a C at position
-3. There are two other in-frame ATG triplets at bp 149 and
217. Although nucleotide sequences surrounding
the ATG codon at bp 149 do not match Kozak's consensus
sequence, those of bp 217 are in complete agree-
ment. However, these codons are less likely to be used
as translation initiation sites because the first ATG at
bp 91 aligns well with both rabbit and rat NHE2
translation start codons, and the amino acid residues
downstream from this initiator triplet conform with the
structural features of other NHE family members. In
the 3' untranslated region, a potential polyadenylation
signal GATAAA was found 16 nucleotides upstream
from the poly(A)+ tract. Two independent clones, one
from the T84 library and the other from a human
colon cDNA library, both terminated at this poly(A)+
site.
Amino acid sequence alignment. A comparison of the amino acid sequences of hNHE2 with those of rabbit, rat, and SLC9A2 is shown in Fig. 3. In pairwise comparisons, hNHE2 showed a 92% identity to rabbit and a 90% identity to rat amino acid sequences. However, the amino acid sequence of hNHE2 differed in the NH₂-terminal end from the SLC9A2 sequence by an extension of 115 amino acid residues. When this region was excluded, the two polypeptides exhibited an 89% amino acid identity and the corresponding DNA region showed only an 84% DNA nucleotide homology (data not shown).

The alignment of NHE2 hydropathy plots from different species, as predicted by the algorithm of Kyte and Doolittle (15), revealed that human, rabbit, and rat NHE2 had virtually identical hydropathy profiles, all predicting 12 potential transmembrane domains, whereas the first two hydrophobic domains are missing in SLC9A2 (results not shown).

In an attempt to verify hNHE2 and SLC9A2, a cDNA pool from human normal liver (Clontech) was screened by PCR with the use of specific primers to hNHE2 and SLC9A2 (11). Although the DNA region corresponding to hNHE2 was readily amplified and subsequently cloned, SLC9A2-specific primers (5'-GCAGGGGCACTCGGGGGC-3' and 5'-GATGGGTCATGTTGGA-3') failed to generate any PCR products (data not shown). DNA nucleotide sequence analysis of the PCR-generated human liver cDNA clones were a complete match with our colonic hNHE2 cDNA sequence. Therefore, on the basis of these data, we conclude that our cloned hNHE2 cDNA represents the true homologue of the NHE2 isoform.

Tissue distribution of human NHE2. To determine tissue distribution and relative expression of hNHE2 mRNA, commercially available human multiple-tissue Northern blots (hMTN and hMTN, Clontech) were probed with hNHE2 cDNA. To minimize crosshybridization with other isoforms, the cDNA probe was chosen from the least-conserved 3' end region. Examination of mRNA distribution revealed a unique pattern of gene expression, with multiple forms present in different tissues (Fig. 4). The major mRNA detected was 5.2 kb, but additional bands at 3.0 kb and 6.5 kb were also...
Fig. 3. Amino acid comparisons among hNHE2 (HuNHE2.PEP), rabbit NHE2 (RbNHE2.PEP), rat NHE2 (RtNHE2.PEP), and SLC9A2. Amino acid residues conserved among all polypeptides are overlined. Putative transmembrane domains are underlined with asterisks. Deletions are indicated by dashes. Amino acid residues are numbered on right.

Fig. 4. Northern blot analysis. Northern blots of poly(A)+ RNA from different human tissues were probed with a 350-bp Hind III cDNA fragment from 3' end of hNHE2 cDNA. RNA size markers (in kb) are indicated in middle.
present in some tissues. The mRNA abundance varied considerably among different tissues. The expression was the highest in skeletal muscle, followed by colon and kidney, and was present at considerably lower levels in the testis, prostate, ovary, and small intestine (Fig. 4). The message was not detected in the heart, liver, thymus, leukocytes, or brain by this method. Two additional hMTN and hMTNII blots were screened with a different probe from the 3' end of the hNHE2 cDNA, and signals identical to the previous data were observed in all blots (data not shown).

Characterization of hNHE2 protein. To confirm that hNHE2 cDNA can indeed be translated to a protein of expected size, the full-length hNHE2 cDNA was cloned into a mammalian expression vector entirely (pFhNHE2) or with an additional Flag epitope sequence added at the 5' end (pFhNHE2). Proteins encoded by these clones and control plasmids pFhNHE1, pLuc, and empty vector pRC-CMV were synthesized in vitro using a T7-dependent TNT-wheat germ extract system. A protein of about 75 kDa was observed in reaction products of pFhNHE2 and phNHE2 (Fig. 5, lanes 1 and 2). The deduced amino acid sequence of the hNHE2 polypeptide predicts the presence of three potential N-glycosylation sites (Malakooti and Ramaswamy, unpublished data). However, Tse et al. (28) have shown that the rabbit NHE2 protein, despite containing three N-glycosylation consensus sequences, is only O-linked glycosylated, and on an SDS-PAGE migrates as an 85-kDa protein, whereas the unglycosylated form is 75 kDa. This suggests that the 75-kDa protein observed here might also be the unmodified form of the hNHE2 polypeptide.

The human NHE1 has both N- and O-linked glycosylations. Sardet et al. (24) have shown that the glycosylated NHE1 polypeptides migrate on SDS gels as 95- and 110-kDa proteins, whereas when expressed in Sf9 insect cells, an 85-kDa protein accounting for the unglycosylated form is detected. In our TNT expression system, pFhNHE1 also produced a protein of about 85 kDa (Fig. 5, lane2). These results suggest that all of the plasmids constructed in this study are capable of expressing polypeptides of the expected sizes for hNHE2 and hNHE1 isoforms.

Functional characterization of hNHE2 cDNA. To demonstrate that hNHE2 cDNA encoded a functional Na+/H+ exchanger, the pH (pHi) recovery from acid loads in response to external Na+ in stable LAP/pRC-CMV empty vector (A) and LAP/pFhNHE2 (B) transfectants is shown. Cells were grown on a coverslip, loaded with the pH-sensitive dye BCECF, and then were perfused with 120 mM Na+ medium containing HCO3−-free solution for 10 min at 15 ml/min, during which pHi remained constant. Addition of Na+ from perfusate resulted in a drop in pHi of cells. Removal of amiloride from Na+ medium allowed the pHi to return to its initial level. After a 5-min exposure to NH4Cl, there was a rapid drop in pHi, in absence of Na+, which was maintained in presence of Na+ medium containing amiloride. Removal of amiloride while Na+ remained in perfusate resulted in a rapid increase in pHi.
DISCUSSION

Increasing evidence indicates that the apical NHE3 isoform is involved in Na\(^+\) absorption in the mammalian intestine and kidney (1–3). To date, however, the physiological role of NHE2, the second apical isoform, is not well understood. Our current studies have focused on cloning the NHE2 isoform so as to fully characterize its function and regulation in the human intestine.

In this study, we have identified and characterized the cDNA for the human Na\(^+\)/H\(^+\) exchanger NHE2 isoform. The hNHE2 cDNA encodes a polypeptide of 812 amino acid residues that exhibits an overall 90% amino acid identity to the rat and rabbit NHE2 isoforms. As it is noted for other Na\(^+\)/H\(^+\) exchanger family members, the level of identities is greater in the NH\(_2\)-terminal region between transmembrane domains 2–12, with the transmembrane domain 1 exhibiting the least degree of homology. The COOH terminus, although highly divergent among different isoforms, exhibits high levels of identities (80%) among the NHE2 homologues of different species. This may indicate a similar mode of gene regulation among these species. Our studies comparing our cloned hNHE2 to the SLC9A2 isoform (11) clearly show that the cDNA reported here is the true human NHE2 homologue. Also, it should be emphasized that the SLC9A2 cDNA is almost 100% homologous to the rat NHE2 variant reported earlier (5).

Our earlier studies (8) with RNase protection and in situ hybridization demonstrated that the NHE2 and NHE3 mRNA levels in various intestinal tissues were different. For example, the message for NHE2 was expressed more in the distal colon compared with the ileum, whereas that of NHE3 was expressed more in the ileum. The Northern blot hybridization studies reported here also show higher expression of NHE2 in the colon compared with the small intestine, in which it is barely detectable. The mRNA overexpression seen in the skeletal muscle was unexpected. The abundance of the NHE2 mRNA in skeletal muscle tissues may suggest that the expression of this isoform is particularly important in muscle and may play a role other than transepithelial Na\(^+\) absorption in this tissue. Taking into consideration that hNHE3 is not expressed in the skeletal muscle (2), hNHE2 overexpression is interesting, and future studies are necessary to fully understand its function in the muscle. The origin of multiple transcripts observed in Northern blots of mRNA from different tissues may be speculated to be the result of 1) transcription termination at less-effective polyadenylation signals, 2) incomplete RNA processing, 3) alternative splicing, and 4) use of alternative promoters and/or transcription initiation sites. In our Northern blot assays a message corresponding to the size of our hNHE2 cDNA done (2.7 kb) was not detected. This may be because of the current lack of information on the transcription initiation site or a less-effective polyadenylation signal, GATAAA (Fig. 1), which is a 1-bp variant of the canonical polyadenylation signal sequence A\(_{99}\)A\(_{99}\)A\(_{99}\)T\(_{100}\)A\(_{99}\)A\(_{99}\)A\(_{98}\) (32). It is possible that rare species of mRNA may not be detectable under the conditions used for our Northern blot analysis. In fact, although hNHE2 was detected in the human liver RNA by RT-PCR, it was not observed by Northern blot analysis. This may also be the case for the lack of hNHE2 mRNA detection in small intestine, which was clearly detected earlier by RNase protection assay (8). The results of our Northern blot analysis, therefore, indicate that the hNHE2 mRNA is restricted in its tissue distribution predominantly to epithelial tissues, with the exception of the skeletal muscle, suggesting additional roles of NHE2 in specialized functions in these tissues.

Our functional expression studies show that the hNHE2 cDNA codes for a protein that shows characteristics of a Na\(^+\)/H\(^+\) exchanger. The NHE-deficient LAP1 cells, when transfected with hNHE2 cDNA, were capable of pH recovery from an acid load effected by ammonium chloride pulse. The pH recovery from an acid load was inhibited by amiloride at a concentration of 0.1 mM. Further studies with different amiloride analogs have to be carried out to fully characterize the human NHE2 and to compare the K\(_i\) values obtained for amiloride analogs with the other mammalian isoforms.

In summary, our studies demonstrate the successful cloning of the hNHE2 cDNA and its functional expression in NHE-deficient cells. The tissue distribution studies confirm our earlier studies of its expression in the colon, possibly indicating its major importance in colonic Na\(^+\) absorption. Future studies of its regulation by various stimuli, such as growth factors and hormones, in NHE2-transfected cells will be necessary to gain insights into the physiological role of this hNHE2 isoform.

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REFERENCES


G390 CLONING AND CHARACTERIZATION OF HUMAN NHE2 ISOFORM

Koste, J., and R. F. Doolittle.

Kozak, M.


Molecular cloning of Na+/H+ exchanger isoforms NHE1, NHE2, and NHE3 mRNA. Am. J. Physiol. 268 (Cell Physiol. 37): C283–C296, 1995.

Ghishan, F. K., S. M. Knobel, and M. Summar.


