Cloning and Functional Expression of a Human Kidney Na⁺:HCO₃⁻ Cotransporter*  

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Several modes of HCO₃⁻ transport occur in the kidney, including Na⁺-independent Cl⁻/HCO₃⁻ exchange (mediated by the AE family of Cl⁻/HCO₃⁻ exchangers), sodium-dependent Cl⁻/HCO₃⁻ exchange, and Na⁺:HCO₃⁻ cotransport. The functional similarities between the Na⁺-coupled HCO₃⁻ transporters and the AE isoforms (i.e. transport of HCO₃⁻ and sensitivity to inhibition by 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid) suggested a strategy for cloning the other transporters based on structural similarity with the AE family. An expressed sequence tag encoding part of a protein that is related to the known anion exchangers was identified in the GenBank™ expressed sequence tag data base and used to design an oligonucleotide probe. This probe was used to screen a human kidney cDNA library. Several clones were identified, isolated, and sequenced. Two overlapping cDNA clones were spliced together to form a 7.6-kilobase cDNA that contained the entire coding region of a novel protein. Based on the deduced amino acid sequence, the cDNA encodes a protein with a M₀ of 116,040. The protein has 29% identity with human brain AE3. Northern blot analysis reveals that the 7.6-kilobase mRNA is highly expressed in kidney and pancreas, with detectable levels in brain. Functional studies in transiently transfected HEK-293 cells demonstrate that the cloned transporter mediates Na⁺:HCO₃⁻ cotransport.

More than 85% of the filtered load of HCO₃⁻ is reabsorbed in the proximal tubule of the kidney (1, 2). This transepithelial flux is accomplished predominantly via a luminal membrane Na⁺/H⁺ exchanger and a basolateral Na⁺:HCO₃⁻ cotransporter (1–5). The Na⁺:HCO₃⁻ cotransporter (3–5) mediates an electrogenic process with an apparent stoichiometry of 3 HCO₃⁻ ions per Na⁺ ion (6–8). In addition to the Na⁺:HCO₃⁻ cotransporter and Cl⁻/HCO₃⁻ exchangers, a Na⁺-dependent Cl⁻/HCO₃⁻ exchange system has been described in the kidney, which exchanges Cl⁻ for Na⁺ and HCO₃⁻ (4). The Na⁺:HCO₃⁻ cotransporter, the Na⁺-dependent Cl⁻/HCO₃⁻ exchanger, and the electroneutral Cl⁻/HCO₃⁻ exchangers (AE isoforms) share similar pharmacological and functional properties, including sensitivity to inhibition by diisulfonic stilbenes and transport of HCO₃⁻ (3–5, 7–11). This suggested that the Na⁺-dependent HCO₃⁻ transporters might be related to the AE family of Cl⁻/HCO₃⁻ exchangers. In fact during the revision of this manuscript the sequence of an amphibian Na⁺:HCO₃⁻ cotransporter (NBC) (1) that is related to the AE family was reported (12). We report here the isolation and functional characterization of a human kidney NBC using a cloning strategy based on its similarity to the known AE isoforms.

**EXPERIMENTAL PROCEDURES**

**Cloning and Sequencing**—The GenBank™ nonredundant EST database was queried against the three known anion exchangers AE1, AE2, and AE3. A sequence with a score of 172, from a human pancreatic islet cell line (GenBank™ accession number W39298) was a close, but not an identical, match to rat AE3.

A sense-stranded oligonucleotide, 5'-AGG GAG CAA AGA GTC ACT GGA ACC-3', from W39298 was synthesized, biotinylated and used in the GeneTrapper™ cDNA positive selection system (LifeTechnologies, Inc.) to screen a SuperScript™ human (38-year-old Caucasian male) kidney cDNA library (LifeTechnologies, Inc.) directionally cloned in pCMVSPORT1. The GeneTrapper™ system uses streptavidin-linked magnetic beads to enrich for sequences complementary to the biotinylated oligonucleotide. After plating, the library was screened by hybridization with 32P-end-labeled oligonucleotide, and 21 positive clones were selected.

The three largest unique clones were chosen for sequencing. Two of the three clones (one of approximately 5 kb and another of approximately 1.6 kb) contained the EST sequence (W39298) and also contained sequences of perfect homology to each other. An open reading frame analysis suggested that the entire coding region was not contained between the two clones. Therefore, a second oligonucleotide was synthesized (which was 5'-to the EST sequence) for a second round of GeneTrapper™ cDNA sequence. The sequence of the second oligonucleotide was 5'-CAG GCC AAC AAG TCC AAA CCG AGG-3'. A polymerase chain reaction analysis was performed using the T7 and SP6 primers to reveal the size of the cDNA inserts of 48 randomly chosen clones. The largest (with a 3.5-kb insert) overlapped the 5-kb clone by 828 base pairs, included the EST clone W39298, and contained the remainder of the open reading frame (as well as 149 base pairs of the 5'-noncoding region). Two Sse83871 restriction sites, one in the region of overlap and another in the polylinker, allowed the construction of the full-length Na⁺:HCO₃⁻ cotransporter clone.

**Transient Transfection with the Cloned cDNA**—HEK-293 cells, grown for 24 h on fibronectin-coated glass coverslips (in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum) were transfected with 8 m g of the plasmid DNA (pCMVSPORT1) (13), and cells were studied 44–52 h after transfection.

**Intracellular pH Measurement**—Changes in intracellular pH (pHᵢ) were monitored in cells using BCECF (14, 15). HEK-293 cells were

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF007216.

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‡The abbreviations used are: NBC, sodium bicarbonate cotransporter; BCECF, 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein acetoxymethoxy ester; DIIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; pHᵢ, intracellular pH.

§This paper is available on line at http://www.jbc.org

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2 In previously published abstracts reporting the amphibian NBC (Romero, M. F., Hediger, M. A., Boulpaep, E. L., and Boron, W. F. (1996) PARSER 1996, AS9) and a closely related rat NBC (Romero, M. F., Hediger, M. A., and Boron, W. F. (1996) Am. Soc. Nephrol. 7, A0055), the authors stated that these proteins exhibited no substantial homology to existing proteins.
cloned transporter is not the Na\(^{+}\)/HCO_3\(^{-}\) cotransporter under basal conditions. A faint band can also be detected in human brain. The lower panel in Fig. 3 demonstrates the expression of GADPH in all lanes of the multiple tissue Northern blot, indicating that intact mRNA was present in all lanes.

**Functional Expression of the Cloned cDNA**—To determine the functional identity of the protein encoded by the cDNA, transiently transfected cells were assayed for the presence of Na\(^{+}\)/HCO_3\(^{-}\) cotransport, Na\(^{+}\)-dependent Cl\(^{-}\)/HCO_3\(^{-}\) exchange, or Na\(^{+}\)-independent Cl\(^{-}\)/HCO_3\(^{-}\) exchange. Depleting media (only Cl\(^{-}\) and sodium, control cells showed no pH recovery from the intracellular acidification (Fig. 4A). However, in transfected cells, switching from the Na\(^{+}\)-free solution (solution A, Table I) to the Na\(^{+}\)-containing solution (solution C) in the presence of HCO_3\(^{-}\) resulted in a rapid recovery from acidic pH (Fig. 4B), with the recovery of 0.225 pH (ΔpH) of 0.225 ± 0.035 in transfected cells versus almost 0 in nontransfected cells (n = 5). The recovery from cell acidification was observed only in the presence Na\(^{+}\) and HCO_3\(^{-}\) and was completely inhibited by 300 μM DIDS (Fig. 4B), consistent with the presence of Na\(^{+}\)/HCO_3\(^{-}\) cotransport. Depleting the intracellular Cl\(^{-}\) (18) by incubating the cells in Cl\(^{-}\)-free media (only Cl\(^{-}\) free solutions were used for the duration of the experiment) did not reduce the rate of Na\(^{+}\)-dependent HCO_3\(^{-}\) movement into acid-loaded cells (Fig. 4C), indicating that the cloned transporter is not the Na\(^{+}\)-dependent Cl\(^{-}\)/HCO_3\(^{-}\) exchanger (ΔpH was 0.225 ± 0.035 in chlorine-containing cells (n = 5) and 0.28 ± 0.018 in chlorine-depleted cells (n = 4)).
Transfected cells, switched from a Cl\(^{-}\)-containing solution (solution C) to Cl\(^{-}\)-free media (solution D), demonstrated little cell alkalinization (Fig. 4D), indicating that under physiological conditions, the cloned transporter does not function in Cl\(^{-}\)/HCO\(_3\)^{−} exchange mode.

To determine whether the cloned transporter can mediate HCO\(_3\)^{−}-dependent \(^{22}\)Na influx, HEK 293 cells were grown in 24-well plates, acidified with NH\(_4\) pulse in a manner similar to Fig. 4A and assayed for \(^{22}\)Na influx in the presence of HCO\(_3\)^{−}. The results showed that transfected cells mediated significant acid-stimulated DIDS-sensitive \(^{22}\)Na influx in the presence of HCO\(_3\)^{−}, whereas nontransfected cells had no DIDS-sensitive \(^{22}\)Na influx (7.15 ± 0.5 nmol/mg of protein/4 min in transfected cells versus 0.25 ± 0.1 nmol/mg of protein/4 min in nontransfected cells, \(p < 0.001, n = 4\)).

The Na\(^{+}\)-HCO\(_3\)^{−} cotransporter can mediate the movement of HCO\(_3\)^{−} out of or into the cell, depending on the ionic composition of experimental solutions (3–5). In the present study, switching the Na\(^{+}\)-containing solution to Na\(^{+}\)-free media did not result in significant cell acidification, as would have been the case if the transporter were functioning in efflux mode. Rather, the transporter functioned only in an uptake mode. Whether lack of efflux mode was caused by low intracellular Na\(^{+}\) concentration, decreased membrane potential, or other mechanisms is not clear at present.

**DISCUSSION**

The cDNA clone was identified by virtue of its significant homology with, but divergence from, the anion exchanger family. Both the homology with, and the divergence from AE3, are apparent in Fig. 2. Fig. 1 shows the nucleotide sequence and conceptual translation of the open reading frame of the Na\(^{+}\):HCO\(_3\)^{−} cotransporter. The close relationship between the size of the cDNA clone (7586 base pairs, not including the poly(A) tail) and the mRNA found in human kidney indicate that the full sequence has been obtained.

Tissue distribution studies show high expression levels in kidney and pancreas, with detectable levels in brain (upper panel). The blot was probed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to ensure the presence of intact RNA in each lane (lower panel).
and thus demonstrates the presence of Na\(^+\)-dependent transport process. The Cl\(^-\) dependence of this transporter is distinct from the Na\(^+\)-dependent Cl\(^-\)/HCO\(^3\)-cotransporter movement in kidney and other tissues is due to differences in the membrane potential, cellular ionic composition in these tissues, or whether it suggests the presence of other isoforms of this transporter remains to be determined.

In conclusion, a cDNA encoding a Na\(^+\)-HCO\(^3\)-cotransporter was cloned based on similarity of the anion exchangers to an expressed sequence tag. The Na\(^+\)-HCO\(^3\)-cotransporter cDNA encodes an mRNA of 7.6 kb and a protein molecular mass of 116 kDa. The Na\(^+\)-HCO\(^3\)-cotransporter mRNA is expressed in several tissues, including kidney, pancreas, and brain, indicating its pivotal role in cell pH regulation in mammalian tissues.

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