Cloning and characterization of a human electrogenic Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporter isoform (hhNBC)

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Choi, Inyeong, Michael F. Romero, Nassirah Khandoudi, Antoine Bril, and Walter F. Boron. Cloning and characterization of a human electrogenic Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporter isoform (hhNBC). Am. J. Physiol. 276 (Cell Physiol. 45): C576–C584, 1999.—Our group recently cloned the electrogenic Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporter (NBC) from salamander kidney and later from mammalian kidney. Here we report cloning an NBC isoform (hhNBC) from a human heart cDNA library. hhNBC is identical to human renal NBC (hkNBC), except for the amino terminus, where the first 85 amino acids in hhNBC replace the first 41 amino acids of hkNBC. About 50% of the amino acid residues in this unique amino terminus are charged, compared with ~22% for the corresponding 41 residues in hkNBC. Northern blot analysis, with the use of the unique 5′ fragment of hhNBC as a probe, shows strong expression in pancreas and expression in heart and brain, although at much lower levels. In Xenopus oocytes expressing hhNBC, adding 1.5% CO\(_{2}\)/10 mM HCO\(_{3}^{-}\) hyperpolarizes the membrane and causes a rapid fall in intracellular pH (pH\(_{i}\)), followed by a pH\(_{i}\) recovery. Subsequent removal of Na\(^{+}\) causes a depolarization and a reduced rate of pH\(_{i}\) recovery. Removal of Cl\(^{-}\) from the bath does not affect the pH\(_{i}\) recovery. The stilbene derivative DIDS (200 µM) greatly reduces the hyperpolarization caused by adding CO\(_{2}\)/HCO\(_{3}^{-}\). In oocytes expressing hhNBC, the effects of adding CO\(_{2}\)/HCO\(_{3}^{-}\) and then removing Na\(^{+}\) were similar to those observed in oocytes expressing hhNBC. We conclude that hhNBC is an electrogenic Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporter and that hkNBC is also electrogenic.

Intracellular pH; acid extruder; oocytes; bicarbonate because almost every cellular process is sensitive to changes in intracellular pH (pH\(_{i}\)), the regulation of pH\(_{i}\) is important for normal cell function (9). Steady-state pH\(_{i}\) is determined by the balance between acid extruders (i.e., transporters that move acid out of cells or alkali into cells) and acid loaders. Acid extruders include Na\(^{+}\)/H\(^{+}\) exchangers (the NHEs) (6), various H\(^{+}\) pumps, and the Na\(^{+}\)-driven Cl\(^{-}\)/HCO\(_{3}^{-}\) exchanger, which is present in invertebrate cells (34, 39) as well as cultured fibroblasts (28), cultured mesangial cells (13), and central nervous system neurons (37). Acid loaders include Cl\(^{-}\)/HCO\(_{3}^{-}\) exchangers (the AE\(_{s}\)) (3). In addition, a K\(^{-}\)-HCO\(_{3}^{-}\) cotransporter, which would function as an acid loader, has been reported in squid giant axons (21, 22).

Electrogenic Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporters (10) can function either as acid extruders or acid loaders. In renal proximal tubules, the electrogenic Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporter has a Na\(^{+}\) to HCO\(_{3}^{-}\) stoichiometry of 1:3 and moves HCO\(_{3}^{-}\) out of the cell (4, 12); i.e., the cotransporter functions as an acid loader. In glial cells (8, 19), however, the electrogenic Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporter appears to have a stoichiometry of 1:2 and moves HCO\(_{3}^{-}\) into the cell (i.e., it is an acid extruder). Finally, electroneutral Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporters have been reported in vascular smooth muscle cells (1) and in the heart (18, 27). These transporters would have a stoichiometry of 1:1 and mediate HCO\(_{3}^{-}\) uptake. On the other hand, there is also a report that the cardiac cotransporter is electrogenic (15), presumably with a stoichiometry of 1:2.

Romero and co-workers (32) expression cloned the cDNA encoding an electrogenic Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporter (NBC) in the kidney of the tiger salamander Ambystoma tigrinum. Others subsequently cloned similar cDNAs from mammalian kidneys (14, 33). The open reading frame of the NBC cDNA is ~3.2 kb in length, corresponding to 1,035 amino acids. The amino acid sequences of the NBC are ~30% identical to those of the AE\(_{s}\), and these two families of HCO\(_{3}^{-}\) transporters have very similar hydropathy profiles. Expression in Xenopus oocytes of Ambystoma NBC (aNBC) and rat kidney NBC (rKNBC) confirms that both clones encode electrogenic Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporters that are blocked by the stilbene DIDS (32, 33). These two NBCs have similar apparent K\(_{m}\) values for extracellular HCO\(_{3}^{-}\), ~10 mM (20). Antibodies specific for rKNBC localize the cotransporter to the basolateral membrane of the renal proximal tubules of rat and rabbit (36).

As noted above, unlike the Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporters identified in kidney and glial cells, the cardiac Na\(^{+}\)-HCO\(_{3}^{-}\) cotransporter described by one group is electroneutral (18, 27). Therefore, we decided to attempt to clone the cardiac NBC by screening a human heart cDNA library with rKNBC. We identified a cDNA from human heart (hhNBC) that is identical to the one from human kidney (hkNBC), except that the 85 amino-terminal amino acids in hhNBC are replaced by 41 amino-terminal amino acids in hhNBC. While our paper was being submitted, a paper appeared by Abuladze et al. (2), reporting the cloning of a human NBC that is identical to the one from human kidney (hkNBC), except that the 85 amino-terminal amino acids in hhNBC are replaced by 41 amino-terminal amino acids in hhNBC. We conclude that hhNBC and hkNBC, when expressed in Xenopus oocytes, are electrogenic. This work is the first demonstration that a human NBC is electrogenic.

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METHODS

Cloning hhNBC. The open reading frame of rknBC (GenBank Accession no. AF004017) was cut into three pieces with BstI I, radiolabeled with [α-32P]dCTP by random priming, pooled, and used to screen a human heart χ-ZAPII cDNA library (Stratagene, La Jolla, CA). The titrated plaques (0.64 × 106) were plated and blotted on nitrocellulose filters. Hybridization was performed in 0.5 M sodium phosphate, 7% SDS, 1% BSA at 65°C overnight. The membranes were washed at 50°C in 1× standard sodium citrate (SSC; 150 mM NaCl/15 mM sodium citrate)/0.1% SDS and then autoradiographed. We then performed a PCR with pBluescript vector primers adjacent to the cloning sites to amplify the cDNA inserts from primary positive plaques.1 PCR products were separated on 1.0% agarose gel, transferred via capillary to a Hybond membrane (Clontech, Palo Alto, CA), and separately hybridized with each probe used for the screening library. Positive plaques were plated for a second screening with the same probes. We isolated four positive phage 6 clones and excised the inserts by rescuing the plasmids. Sequencing was performed by the Keck Sequencing Center at Yale University. We analyzed the sequence using DNAsis (Hitachi Software, San Bruno, CA).

The above approach yielded nucleotide sequence data from both the 5′ and the 3′ end of the putative clone. However, we were missing the middle of the putative clone. We performed RT-PCR with human heart poly(A)+ RNA Clontech (catalog no. 6533–1; a pool of whole heart from Caucasians ages 20–78 who had died of trauma) to obtain the missing middle portion. The upstream primer sequence was 5′-CCG GAG AAG GAC CAG CTG AAG-3′, corresponding to a region near the 3′ end of clone 15.1, which contains the 3′ fragment of hhNBC. The downstream primer sequence was 5′-ATC AGA GTA GGG AGG AAA GAG-3′, corresponding to a sequence at the TAG stop codon of clone 4.1, which contains the 3′ fragment of hhNBC. The result of the PCR2 was a DNA fragment that represented the middle and 3′ end of the putative clone, which we sequenced. We ligated this PCR product to clone 15.1 (the 5′ end) at the Pml I site, to obtain the putative full-length clone of hhNBC (GenBank Accession no. AF069510). We confirmed the sequence of this full-length clone near the ligation site.

Cloning hhNBC. The 5′ fragment of hhNBC cDNA was isolated from human pancreas poly(A)+ RNA by RT-PCR with primers designed on the basis of the published hhNBC sequence (14). The upstream primer was 5′-TTG GGA GGC TTA GCA GGG AAG ATG TGG-3′ (−21 to +15) and the downstream primer was 5′-TCT CTT GGT TGG ATG CCG GTT CCG-3′ (+496 to +522). PCR was performed as described above,2 and the final single PCR product was cloned into the pCRII vector (Invitrogen, Carlsbad, CA). To obtain a full-length hhNBC, we ligated the above PCR product to hhNBC at the AflII site. The inserted DNA fragment, as well as the ligation site, were sequenced to confirm that the ligation product is identical to the published hhNBC sequence (GenBank accession no. AF007216).

Northern blots. A human multiple-tissue Northern blot (catalog no. 7760–1) was purchased from Clontech. A 32P-labeled, random-hexamer-primed cDNA probe (GIBCO BRL, Gaithersburg, MD) was made from the unique 5′ region of the hhNBC (7–271 bp of the coding region of hhNBC). Hybridization was performed in the ExpressHyb hybridization buffer (Clontech) at 68°C for 2 h, at a probe concentration of 0.83 × 106 counts·min−1·µl−1. The membrane was washed at 37°C in 2× SSC/0.1% SDS for 40 min and then at 50°C in 0.1× SSC/0.1% SDS for 1.5 h. The membrane was exposed to Kodak X-Omat film (Kodak, Rochester, NY), which was developed 1 day later for the detection of high-intensity signals. For the detection of low-intensity signals, the film was autoradiographed for 7 days.

Membrane isolation and Western blot analysis. Oocyte plasma membranes were isolated as described by Preston et al. (30), with some modification. Groups of four oocytes were washed with the PBS and homogenized in 0.5 ml of fresh hypotonic lysis buffer (7.5 mM potassium phosphate, pH 7.4, 1 mM EDTA, 1 mM PMSF, 1 µg/ml pepstatin A, 1 µg/ml leupeptin). The cellular debris was removed by centrifuging at 810 g for 5 min. For the collection of membranes, the supernatant was then centrifuged at 15,000 g for 30 min at 4°C. The pellets were gently washed with the lysis buffer and were dissolved in 40 µl of a sample-loading buffer containing 2% SDS. The samples were separated on a 7.5% SDS-polyacrylamide gel and transferred to a polyvinylidene fluoride membrane. Blots were preincubated for 1 h in blocking buffer containing 0.2% l-block (Tropix, Bedford, MA) and 0.05% Tween 20 in Tris-buffered saline (TBS; 50 mM Tris, pH 7.4; 150 mM NaCl) and then were incubated with an antibody specific to the carboxy terminus of rknNBC (1:500 dilution) (36). After several washes with TBS containing 0.05% Tween 20, blots were incubated with anti-rabbit IgG conjugated to horseradish peroxidase (HRP; Sigma, St. Louis, MO) for 1 h (2,000 dilution in blocking buffer). Blots were washed and developed by HRP/hydrogen peroxide-catalyzed oxidation of luminol under alkaline conditions (Pierce, Rockford, IL).

pH, and membrane potential measurements in oocytes. The hhNBC insert in the plasmid described above contains 44 bp of the 5′-untranslated region (UTR), as well as the entire coding region of hhNBC. The insert was digested with EcoRI and ligated into pGH19, an oocyte expression vector (40). The resulting construct and the hhNBC construct in pGH19 were linearized with Not I before transcription and then in vitro transcribed with the mMessage mMachine kit (Ambion, Austin, TX) using T7 RNA polymerase. The ratio of cap analog to GTP was decreased by increasing the final GTP concentration to 3 mM, thereby maximizing the synthesis of full-length transcripts. Oocytes of X. laevis were obtained as described by Romero et al. (32). Defolliculated oocytes (stages V and VI) were injected with 50 nl of RNA (0.5 mg/ml) water and incubated in OR3 buffer (500 mM Leibovitz L-15 media with l-glutamine, 5 mM HEPES, pH 7.5) supplemented with 5 U/ml penicillin-streptomycin (33). Injected oocytes were maintained for 3–7 days at 18°C before use.

pH-sensitive microelectrodes were made from borosilicate glass capillaries (2.0-mm outer diameter; Warner Instrument, Hamden, CT) that were pulled on a micropipette puller (model P-97; Sutter, Novato, CA), dried in an oven at 200°C for at least 2 h, and silanized in the vapor of bis(dimethylamino)-dimethyl silane (Fluka, Ronkonkoma, NY) in a closed vessel. The electrode tip was then filled with hydrogen

1 We used 35 cycles of PCR, with denaturation at 94°C for 1 min, annealing and extension at 68°C for 3 min, and 7 min at 68°C for the last extension.

2 In the first 10 PCR cycles, we denatured at 94°C for 30 s, annealed at 65°C for 1 min, and extended at 68°C for 2 min. The second 20 cycles were identical, except that we lengthened the duration of the extension by 20 s in each cycle. After the 30th cycle, we allowed an additional 7 min for extension at 68°C.
RESULTS

Sequence analysis of hhNBC cDNA. By screening a human heart cDNA library and subsequently employing a PCR approach, we cloned a full-length cDNA, hhNBC. The open reading frame of hhNBC encodes a protein of 1,079 amino acids, with a predicted molecular mass of 121 kDa. As shown in Fig. 1A, this clone has a 5’ coding region that is different from that of the previously published human kidney clone, hkNBC (14). The first 255 bp (i.e., 85 amino acids) of the coding region of hhNBC replaces the first 123 bp (41 amino acids) of hkNBC (Fig. 1B). In addition, hhNBC has a phenylalanine at amino acid position 255 instead of the serine reported for hkNBC. We obtained the portion of hhNBC that encodes the F255 by screening a cDNA library. Except for these differences, hhNBC is identical to hkNBC, including the 3’-UTR.

The unique amino terminus of hhNBC consists mainly of charged amino acids, such as aspartate, glutamate, histidine, lysine, and arginine. These charged residues comprise ~50% of the unique 85 amino acids in hhNBC, considerably higher than the ~22% of residues that are charged among the corresponding 41 amino acids of hkNBC. Thus the amino-terminal region of hhNBC is very hydrophilic (Fig. 1C). This region is reminiscent of AE2 and AE3, which also have large, amino-terminal domains consisting of highly charged amino acids. There is 20–30% sequence similarity among the amino-terminal charged domains of hhNBC, AE2, and AE3, but not AE1. Finally, the unique amino terminus of hhNBC contains a second putative consensus protein kinase A (PKA) phosphorylation site at amino acid position 49, in addition to a conserved PKA site at amino acid position 1023.

Tissue distribution of hhNBC mRNA. Using the unique 5’ region (nucleotide positions 7–271 of the coding regions) of hhNBC as a probe, we performed a high-stringency Northern blot analysis in various human tissues. After a 1-day exposure, the probe showed substantial hybridization to an ~9-kb transcript in pancreas but not to any of the other tissues on the blot (Fig. 2A). Because we expected to detect hhNBC mRNA in the heart, having cloned hhNBC from human heart tissues, we exposed the film for 7 days to determine whether the signal could be detected in heart. We found that hhNBC mRNA is strongly expressed in pancreas and weakly expressed in heart and brain (Fig. 2B). hhNBC mRNA is undetectable in the kidney. It is interesting to note that, in Ambystoma (i.e., tiger salamander) tissues, Romero et al. (32) did not detect signals in pancreas, even though they probed with a full-length cDNA.

Abuladze et al. (2) probed a Northern blot, obtained from the same source as ours, with part of the unique 5’ region of hhNBC. They detected strong expression in pancreas and moderate expression in brain, but not heart. This disparity may be due to the probe specificity and/or stringency. We used nearly the entire unique nucleotide (7–271) as a probe, whereas Abuladze et al. used a synthetic oligonucleotide probe corresponding to nucleotides 118–212 (95 bp).

Immunoblot experiments on expression of hhNBC in oocytes. To study the functional properties of hhNBC, we expressed hhNBC in Xenopus oocytes by injecting them with hhNBC cRNA. We first tested whether the open reading frame of hhNBC could be translated into protein with the expected molecular mass. We used a rabbit reticulocyte system (without microsomes) to perform an in vitro translation of hhNBC cRNA and observed a 120-kDa product (not shown), as expected from the deduced amino acid sequence of hhNBC cDNA, which predicts a molecular mass of 121 kDa. We then injected the same cRNA into the oocytes and tested the expression of hhNBC by Western blot analysis, using an antibody against the carboxy terminus of rkNBC (36). Because the deduced amino acid sequences of hhNBC and rkNBC are ~98% identical at their carboxy termini, we expected the antibody against rkNBC to react with the hhNBC protein. Figure 3 shows that the antibody reacted with a 130-kDa protein in oocytes injected with rkNBC cRNA (lane 3). The same antibody also recognized an ~135-kDa band in oocytes injected with hhNBC cRNA (lane 2). The slightly greater molecular mass is expected because the 85 amino acids at the amino terminus of hhNBC replace the 41 amino acids at the amino terminus of rkNBC. In addition to the major band, the antibody detects a...
smaller band (~130 kDa), which may be the result of proteolysis or a less extensive glycosylation. Water-injected control oocytes showed no immunoreactivity (lane 1).

Functional properties of hhNBC expressed in Xenopus oocytes. To study the functional properties of hhNBC, we used microelectrodes to monitor pH$_i$ and membrane potential ($V_m$) in control oocytes as well as in oocytes expressing hhNBC. Figure 4A illustrates the effects of exposing a water-injected oocyte to a solution buffered with 1.5% CO$_2$/10 mM HCO$_3^-$.

In both oocytes, applying CO$_2$/HCO$_3^-$ caused a decrease in pH$_i$. In the case of the control oocyte, pH$_i$ fell rapidly and by a relatively large amount and then recovered very slowly. In the case of the hhNBC oocyte, the pH$_i$ decrease was smaller and slower. Moreover, pH$_i$ recovered rather rapidly. Thus the pH$_i$ profile in the hhNBC oocyte is consistent with an extremely robust expression of the cotransporter, which not only produced a pH$_i$ recovery but also blunted the initial CO$_2$-induced acidification. Applying CO$_2$/HCO$_3^-$ also caused characteristic changes in $V_m$, a slowly developing depolarization in the water-injected oocyte but a very large and rapid hyperpolarization in the hhNBC oocyte. The hyperpolarization wanes in the case of the hhNBC oocyte because, for three reasons, the hhNBC-mediated inward transport of Na$^+$ and HCO$_3^-$ gradually slows down:

1) CO$_2$ diffuses into the cell and produces HCO$_3^-$.
2) hhNBC actively transports HCO$_3^-$ into the cell, and
3) hhNBC transports Na$^+$ into the oocyte. In each case, an hhNBC substrate slowly builds up inside the cell, slowing inward transport. Note, however, that $V_m$ is always more negative in the presence than in the absence of CO$_2$/HCO$_3^-$. Thus, as previously shown for aNBC (32).
and rkNBC (33), the direction of electrogenic Na\(^+\)-HCO\(_3\) cotransport by hhNBC is inward in oocytes.

To test whether the expressed transporter is coupled to Na\(^+\), we next removed Na\(^+\) from the bath in the continued presence of CO\(_2\)/HCO\(_3\). Removing extracellular Na\(^+\) (replacing Na\(^+\) with choline) had little effect on pH\(_i\) in the control oocyte (Fig. 4A) but produced a pH\(_i\) decrease in the hhNBC oocyte. This intracellular acidification is consistent with the “reversal” of HCO\(_3\) transport (i.e., Na\(^+\)-coupled HCO\(_3\) efflux rather than influx). Removing extracellular Na\(^+\) also produced characteristic V\(_m\) changes in the two oocytes. In the control oocyte, Na\(^+\) removal elicited a small hyperpolarization, reflecting a small Na\(^+\) conductance, as previously observed (32, 33). In the hhNBC oocyte, however, Na\(^+\) removal produced a large depolarization (from −80 to −10 mV), consistent with the electrogenic efflux of Na\(^+\) with more than one HCO\(_3\). Returning Na\(^+\) to the outside of the oocytes reversed the pH\(_i\) and V\(_m\) changes.

To test the unlikely possibility that hhNBC encodes a Na\(^+\)-driven Cl\(^-\)/HCO\(_3\) exchanger, we removed Cl\(^-\) from the bath (replacing Cl\(^-\) with gluconate). As shown in Fig. 4C, removing extracellular Cl\(^-\) in the presence of CO\(_2\)/HCO\(_3\) had no significant effect on either the rate of pH\(_i\) recovery or the membrane potential (n = 4). If the pH\(_i\) recovery had been due to a Na\(^+\)-driven Cl\(^-\)/HCO\(_3\) exchanger, then Cl\(^-\) removal should have speeded up the pH\(_i\) recovery, at least transiently. Thus our results indicate that hhNBC does not transport Cl\(^-\), although we cannot rule out the unlikely possibility that hhNBC may require intracellular Cl\(^-\).

Finally, we addressed the question of whether this electrogenic, Na\(^+\)-dependent (and Cl\(^-\)-independent) HCO\(_3\) cotransport process can be blocked by the stilbene derivative DIDS, which is known to inhibit electrogenic Na\(^+\)-HCO\(_3\) cotransporter in other systems (11). Before exposing the oocyte to DIDS, we briefly pulsed the cell with CO\(_2\)/HCO\(_3\) and then returned it to the CO\(_2\)/HCO\(_3\)-free ND96 media (arrow in Fig. 4D). This brief CO\(_2\)/HCO\(_3\) pulse produced a hyperpolarization of nearly 60 mV in this experiment. We then applied 200 µM DIDS in the absence of CO\(_2\)/HCO\(_3\). In the continued presence of DIDS, the second exposure to CO\(_2\)/HCO\(_3\) elicited a hyperpolarization of >20 mV. Three other similar experiments showed that 200 µM DIDS blocked, on average, 70 ± 4% of the voltage change elicited by CO\(_2\)/HCO\(_3\) (P < 0.01). Figure 4D also shows that DIDS failed to block completely the depolarization and acidification elicited by removing extracellular Na\(^+\). In contrast, 200 µM DIDS completely blocks aNBC expressed in oocytes (31, 32). Not shown are experiments in which we found that 500 µM DIDS reduced the hyperpolarization by ~90% (n = 3).

Effect of substituting the amino terminus of hkNBC for the amino terminus of hhNBC. Our functional studies described above indicate that, despite its unique amino terminus, hhNBC is an electrogenic Na\(^+\)-HCO\(_3\) cotransporter, similar to rat kidney NBC or salamander kidney NBC. However, the electrogenericity had yet to be examined for human kidney NBC, which is nearly identical to hhNBC, except for a different amino terminus. To address the functional impact of the charged amino terminus of hhNBC, we therefore constructed hkNBC by performing RT-PCR with primers

Fig. 3. Immunoblot of hhNBC in oocytes. Membrane preparations from oocytes injected with cRNA for hhNBC, rat kidney NBC (rkNBC), and control water were separated by 7.5% SDS-PAGE and immunoblotted with an antibody (MBP-NBC-5) specific to rkNBC (36). In oocytes injected with rkNBC cRNA, the antibody detected a single band of ~130 kDa, whereas in oocytes injected with hhNBC cRNA, the antibody detected a major band at ~135 kDa as well as a slightly smaller minor band.

Fig. 2. Northern blot of human tissue. cDNA probe corresponded to the 5’ coding region (nucleotides 7–271) of hhNBC. Probe recognizes an ~9-kb transcript. A: 1 day of exposure. B: 7 days of exposure. Tissues examined include pancreas (P), kidney (K), skeletal muscle (S), liver (L), lung (Lu), placenta (Pl), brain (B), and heart (H).
specific to the amino terminus of hkNBC and replacing the unique amino terminus of the hhNBC cDNA with the one for hkNBC. We then expressed the hkNBC in oocytes and monitored the pHi and $V_m$ as described above.

Figure 5 shows that applying CO$_2$/HCO$_3^-$ caused an acidification, followed by a pH$_i$ recovery. The application of CO$_2$/HCO$_3^-$ also causes a rapid hyperpolarization that slowly wanes ($n = 5$), as in hhNBC-expressing oocytes. Removing extracellular Na$^+$ (replacing Na$^+$ with choline) produced an intracellular acidification, as well as a rapid depolarization. The pH$_i$ and $V_m$ effects of adding CO$_2$/HCO$_3^-$ and removing Na$^+$ were fully reversible. Thus, as is the case for the other NBCs, hkNBC is electrogenic.

We noticed that the CO$_2$/HCO$_3^-$ pulse generally produced a smaller hyperpolarization in hkNBC-expressing oocytes than hhNBC-expressing oocytes. hhNBC and hkNBC differ in the nucleotide sequence surrounding the initiator methionine. The hhNBC cDNA has a perfect Kozak sequence (AGGATGG), whereas the hkNBC cDNA has an imperfect sequence (AAGATGT). The highly conserved G in position +4 immediately following the ATG codon in hhNBC is critically important for halting the scanning 40S ribosomal subunit and thus to initiate translation (26). Thus hhNBC and hkNBC may be translated with different efficiencies.

**DISCUSSION**

Cloning of hhNBC from human heart. In this study, we report the cloning of an NBC-related cDNA from human heart tissues. Studies on pHi regulation of heart cells have provided conflicting evidence for both an electroneutral Na$^+$-HCO$_3^-$ cotransporter (18, 27) and an electrogenic Na$^+$-HCO$_3^-$ cotransporter (15). The evidence for the electroneutral cotransporter was obtained in guinea pig myocytes and sheep Purkinje fibers, whereas the data supporting the presence of an electrogenic Na$^+$-HCO$_3^-$ cotransporter was obtained in cat myocardium. Thus it is possible that the conflicting conclusions of the two groups reflect a species difference. Another possibility is that both transporters are present in the same tissue.

We cloned hhNBC from heart tissue of humans. hhNBC has a unique amino-terminal domain, which consists mainly of charged or polar amino acid residues, and is definitely electrogenic. Because there are no functional studies of the Na$^+$-HCO$_3^-$ cotransporter in human cardiac tissue, it is possible that hhNBC repre-
Possible physiological function of hhNBC. We found that the unique amino terminus of hhNBC, which encodes 85 amino acids, is strongly expressed in the pancreas, as shown by Northern blot analysis. Indeed, Abuladze et al. (2) reported that cotransporters Na\(^+\)-HCO\(_3\) are electrogenic, despite the differences in their amino termini, our results indicate that different regions of the respective amino termini are not responsible for the electrogenicity of NBC. However, hhNBC appears to have a low sensitivity to DIDS than Ambystoma Na\(^+\)-HCO\(_3\) cotransport (31, 32) or rkNBC (M. F. Romero, unpublished results). This result suggests that the amino terminus, which is believed to be cytoplasmic, may be able to influence the binding of DIDS to the extracellular surface.

Electrogenicity of hhNBC. Burnham et al. (14) have now demonstrated that hhNBC, like the renal NBC from salmon and rat, is electrogenic. We have previously suggested that hhNBC is electrogenic (31, 32). Our results on hhNBC and nkNBC thus the first molecular evidence for the existence of an electrogenic Na\(^+\)-HCO\(_3\) cotransporter from a human source. Moreover, our data demonstrate that more than one variant of an electrogenic Na\(^+\)-HCO\(_3\) cotransporter exists in humans.

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duct cells have a Na⁺-dependent HCO₃⁻ transporter at the basolateral membrane that mediates net HCO₃⁻ uptake into the duct cells (23, 24). This pancreatic transporter differs functionally from hNBC in at least one crucial respect: the renal cotransporter mediates net HCO₃⁻ efflux, whereas the pancreatic cotransporter mediates net HCO₃⁻ influx. The difference between net HCO₃⁻ efflux (kidney) and influx (pancreas) could be dictated simply by the ion and voltage gradients in the respective cell type or by differences in posttranslational modification. However, it is possible that the unique 85 amino acids of hNBC change the stoichiometry of the cotransporter from 1:3 to 1:2.

Another possible role for the unique 85 amino acids at the amino terminus of hNBC is suggested by the observation that these 85 amino acids include a novel consensus phosphorylation site for PKA. Both hhNBC and hNBC have consensus PKA sites near the carboxyl terminus, but only hNBC has such a site near the amino terminus. It is interesting to recall that secretin contains a unique 85 amino acid sequence that is phosphorylated by PKA. It is interesting to recall that secretin interacts with specific membrane receptors on pancreatic duct cells and activates the cAMP/PKA cascade. Thus the presence of a second PKA site in the amino terminus of hNBC is suggested by the observation that these 85 amino acids include a novel consensus phosphorylation site for PKA. Both hhNBC and hNBC have consensus PKA sites near the carboxyl terminus, but only hNBC has such a site near the amino terminus. It is interesting to recall that secretin interacts with specific membrane receptors on pancreatic duct cells and activates the cAMP/PKA cascade. Thus the presence of a second PKA site in the amino terminus of hNBC could be important for activation of HCO₃⁻ transport in the pancreas by secretin.

In summary, an NBC isoform was cloned from human heart tissues (hhNBC). The hhNBC clone, when expressed in Xenopus oocytes, reveals physiological properties similar to the renal electroneutral Na⁺-HCO₃⁻ transporter.

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