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Topical Review

Na^+:HCO_3^- Cotransporters (NBC): Cloning and Characterization

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Abstract. The sodium bicarbonate cotransporter (NBC1) is essential for bicarbonate transport across plasma membranes in epithelial and nonepithelial cells. The direction of the $NaHCO_3$ movement in secretory epithelia is opposite to that in reabsorptive epithelia. In secretory epithelia (such as pancreatic duct cells) NBC is responsible for the transport of bicarbonate from blood to the cell for eventual secretion at the apical membrane. In reabsorptive epithelia (such as kidney proximal tubule cells) NBC is responsible for the reabsorption of bicarbonate from cell to the blood. In nonepithelial cells this transporter is mainly involved with cell pH regulation. Recent molecular cloning experiments have identified the existence of four NBC isoforms (NBC1, 2, 3 and 4) and two NBC-related proteins AE4 and NCBE (Anion Exchanger 4 and Na-dependent Chloride-Bicarbonate Exchanger). All but AE4 are presumed to mediate the cotransport of Na^+ and HCO_3^- under normal conditions and may be functionally altered in certain pathologic states. NBC1 shows a limited tissue expression pattern, is electrogenic and plays an important role in bicarbonate reabsorption in kidney proximal tubule. In addition to the kidney, NBC1 is expressed in pancreatic duct cells, is activated by cystic fibrosis transmembrane conductance regulator (CFTR) and plays an important role in HCO_3^- secretion. NBC2 and NBC3 have a wider tissue distribution than NBC1, are electroneutral, and are involved with cell pH regulation. The characterization of NBC4 is incomplete. The NBC-related protein called NCBE mediates Na-dependent, Cl^-/Bicarbonate Exchange. The purpose of this review is to summarize recent advances on the cloning of NBC isoforms and related proteins and their role and regulation in physiologic and pathologic states.

Key words: Na^+:HCO_3^- cotransporter — Kidney — Pancreas — CFTR — Acid-base regulation

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Abbreviations: NBC, Na^+:HCO_3^- cotransporter; NBCE, Na-dependent Chloride-Bicarbonate Exchanger; NHE, Na^+/H^+ exchanger; AE, Cl^-/HCO_3^- or anion exchanger; CFTR, cystic fibrosis transmembrane conductance regulator; DIDS, disulfonic stilbene derivatives; DOCA, Deoxycorticosterone acetate

Acid-base Transporters — General Overview

Intracellular pH (pHi) in mammalian tissues is regulated through coordinated action of several acid-base transporters, including Na^+/H^+ exchangers (NHEs), Na-independent Cl^-/HCO_3^- or anion exchangers (AEs), Na-dependent Cl^-/HCO_3^- exchangers (NCBEs), and Na^+:HCO_3^- cotransporters (NBCs) (Kopito, 1990; Alpern, 1990; Soleimani & Singh, 1995; Romero & Boron, 1999; Counillon & Pouyssegur, 2000; Soleimani & Burnham, 2000). In nonepithelial cells, this coordinated regulation maintains the cell pH in a narrow physiologic range which is vital to cell function and survival. Figure 1 is a general schematic representation of these acid-base transporters in nonepithelial cells. It is worth mentioning that not all these transporters are expressed in the same cell. Usually, nonepithelial cells express a Na^+/H^+ exchanger (mostly the ubiquitous NHE-1), a Cl^-/HCO_3^- exchanger (the widely distributed AE-2) and a Na^+:HCO_3^- cotransporter or a Na-dependent, Cl^-/HCO_3^- exchanger.

In epithelial cells, the above acid-base transporters show distinct membrane-domain localization, with certain transporters demonstrating exclusive expression in

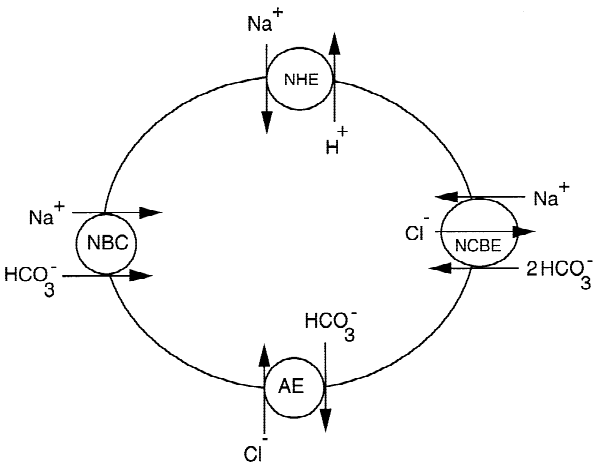

Fig. 1. A schematic representation of acid-base transporters in nonepithelial cells. NBC: Na^+:nHCO_3^- cotransporter; AE: anion exchanger; NHE: Na^+/H^+ exchanger; NCBE: Na-dependent Cl^-/HCO_3^- exchanger.

either the luminal or basolateral membrane. For example, the Na^+/H^+ exchanger NHE-3 is exclusively expressed on the apical membrane, whereas the Na^+:HCO_3^- cotransporter (NBC1) is predominantly expressed on the basolateral membrane of epithelial cells (Soleimani & Singh, 1995; Romero & Boron, 1999; Counillon & Pouyssegur, 2000; Soleimani & Burnham, 2000). This polar distribution of acid-base transporters results in vectorial transport of bicarbonate from lumen to blood or vice versa. In the kidney, the net effect of this coordinated action is reabsorption of bicarbonate from the lumen, whereas in the hepatic bile duct, these transporters cause bicarbonate secretion into the lumen.

Na^+:HCO_3^- Cotransporter

Background

The Na^+:HCO_3^- cotransporter (NBC) was first described in the kidney proximal tubule (Boron & Boulpaep, 1983; Alpern, 1985; Yoshitomi, Burkhardt & Fromter, 1985; Grassl & Aronson, 1986). Functional studies in amphibian and mammalian kidneys indicated the presence of NBC in the basolateral membrane of proximal tubule cells (Boron & Boulpaep, 1983; Alpern, 1985; Yoshitomi et al., 1985; Grassl & Aronson, 1986). NBC was found to be electrogenic, with an stoichiometry of 3 equivalent of bicarbonate per sodium and was shown to transport bicarbonate from cell to the blood (Yoshitomi et al., 1985; Soleimani, Grassl, & Aronson, 1986). These studies demonstrated that basolateral NBC works in tandem with luminal H^+ transporters (NHE-3 and H^+-ATPase) and is essential for bicarbonate reabsorption in the proximal tubule, which is the site for the reclaiming of the majority of filtered bicarbonate (Alpern, 1990; Soleimani & Singh, 1995).

The proximal tubule Na^+:HCO_3^- cotransporter has been studied extensively with respect to inhibitor profile, signal transduction pathways and adaptive regulation in pathophysiologic disorders. These studies demonstrate that NBC is sensitive to inhibition by disulfonic stilbene derivatives (DIDS) (Boron & Boulpaep, 1983; Alpern, 1985; Yoshitomi et al., 1985; Grassl & Aronson, 1986; Soleimani & Burnham, 2000). They further demonstrate that NBC is acutely upregulated by Ca-dependent protein kinase, angiotensin II and cholinergic stimulants and is inhibited by cAMP-dependent protein kinase and parathyroid hormone (Ruiz et al., 1992, 1995, 1996, 1997; Ruiz & Arruda, 1996). NBC is chronically upregulated in metabolic acidosis and potassium depletion and is downregulated in metabolic alkalosis (Akiba, Rocco & Warnock, 1987; Alpern, 1990; Soleimani et al., 1990; Soleimani et al., 1991).

Although NBC was first identified in the kidney proximal tubule, subsequent studies demonstrated its presence in numerous types of cells, including brain, liver, colon, cornea, heart and lung (Astion, Obaid & Orkand, 1989; Fitz, Perisco & Scharschmidt, 1989; Gleeson, Smith, & Boyer, 1989; Rajendran, Oesterlin, & Binder, 1991; Jentsch et al., 1985; Lagadic-Gossmann, Buckler, & Vaughn-Jones, 1992; Camilion et al., 1996; Lubman, Chao, & Crandall, 1995) suggesting that this pathway plays an important role in mediating HCO_3^- transport in both epithelial as well as nonepithelial cells. Functional data support the presence of more than one NBC isoform as judged by direction and stoichiometry of the transporter. In kidney proximal tubule, NBC activity leads to cell acidification, whereas in some other tissues (such as heart) its function leads to cell alkalinization. Further, NBC has a stoichiometry of 3 equivalents of HCO_3^- per Na^+ ion in the kidney but shows a stoichiometry of 2 or 1 HCO_3^- per Na^+ in other tissues (Soleimani et al., 1987; Astion et al., 1989; Camilion et al., 1996).

In addition to the Na^+:HCO_3^- cotransporter, a Na-dependent Cl^-/HCO_3^- exchanger is also expressed in several tissues and is shown to be involved in cell pH regulation. The Na-dependent Cl^-/HCO_3^- exchanger is an electroneutral transporter and mediates transport of Na^+ and HCO_3^- into the cell in exchange for cell Cl^- (Alpern, 1990; Romero & Boron, 1999; Soleimani & Burnham, 2000). Based on functional and molecular data showing transport of HCO_3^- and Na^+ and sensitivity to inhibition by disulfonic stilbenes (Alpern, 1990; Romero & Boron, 1999; Soleimani & Burnham, 2000) and structural homology to NBCs (*see* the section on cloning), it is clear that the Na-dependent Cl^-/HCO_3^- exchanger is related to the NBC family.

While much information has been gathered on molecular properties and gene regulation of NHE and AE isoforms (Kopito, 1990; Soleimani & Singh, 1995; Counillon & Pouyssegur, 2000), molecular characteristics of Na-coupled HCO_3^- transporters (gene structure and regulation) have not been well delineated. This has been due to a lack of information on the genes encoding these transporters. Recent molecular cloning experiments have identified the presence of several NBC isoforms. These isoforms display distinct tissue-distribution patterns and are differentially regulated in certain pathophysiologic states. Characterization of NBC isoforms still is in its early stage. Specifically, regulatory mechanisms by signal transduction pathways, substrate specificity, inhibitory profile and adaptive regulatory mechanisms in pathophysiologic states for each isoform remain to be examined. While functional studies over the last two decades have characterized NBC activity in various tissues, one has to be cautious with attributing these properties to a specific NBC isoform, as more than one NBC isoform is likely expressed in the same tissue or membrane domain. An exception may be the kidney proximal tubule cells where functional studies nicely correlate with NBC1 properties, indicating that this protein is the major Na-dependent, HCO_3^- cotransporter in this nephron segment (Romero & Boron, 1999; Soleimani & Burnham, 2000). In the following sections, we will discuss cloning and characterization of NBC isoforms and their possible roles in pathophysiologic disorders. We will further discuss the characteristics of proximal tubule NBC, as a prototype of NBCs, with respect to electrogenicity and stoichiometry, substrate- and inhibitor specificity, and functional and molecular regulation in acute and chronic states.

Electrogenicity and Stoichiometry

Kidney

The electrochemical gradients of Na^+ and HCO_3^- across the basolateral membrane of the kidney proximal tubule predict that there should be a net flux of HCO_3^- from blood to proximal tubule cell if NBC carries one Na^+ for one HCO_3^-. However, in all studies to date, NBC has been found to be electrogenic and associated with a net flux of negative charge across the basolateral membrane (Boron & Boulpaep, 1983; Alpern, 1985; Grassl & Aronson, 1986; Soleimani et al., 1987; Soleimani & Aronson, 1989). In studies in amphibian proximal tubule, the transport of Na-coupled HCO_3^- transport was associated with a net movement of negative charge across the basolateral membrane (Boron & Boulpaep, 1983). Similarly, in mammalian proximal tubule cells (Alpern, 1985), NBC was found to be electrogenic. In the presence of CO_2/HCO_3^- buffer, alterations in membrane potential altered NBC activity in basolateral membrane (BLM) vesicles (Grassl & Aronson, 1986). These observations indicate that the stoichiometry of the cotransport process must involve more than 1 HCO_3^- per Na^+. Knowledge of the precise stoichiometry is important for predicting the direction of net transport under physiologic and pathophysiologic conditions. The greater the HCO_3^- per Na^+ stoichiometry, the more effectively the inside-negative membrane potential of the cell can drive the net exit of HCO_3^- against the Na^+ and HCO_3^- gradients. Indeed, based on established measurements (Burckhardt, Sato & Fromter, 1984; Yoshitomi et al., 1985), the membrane potential would not be sufficient to drive net HCO_3^- efflux across the basolateral membrane of the proximal tubule cell under physiologic conditions if the stoichiometry of cotransport were only 2 HCO_3^-:Na^+. Studies in membrane vesicles and perfused tubules demonstrated that NBC has an apparent stoichiometry of 3 HCO_3^- per Na^+ ion in mammalian kidney (Soleimani et al., 1987; Yoshitomi et al., 1985). With such a stoichiometry, the inside-negative membrane potential, normally on the order of −60 mV (Yoshitomi et al., 1985), is sufficient to drive HCO_3^- exit against the inward concentration gradients of HCO_3^- and Na^+ that are present across the basolateral membrane of the intact proximal tubule cell.

It is worth mentioning that the cotransport of Na^+ with 3 HCO_3^-, the cotransport of Na^+ with 2 HCO_3^- and 1 OH^- and/or cotransport of Na^+ with 1 HCO_3^- and 1 CO_3^{2-} are thermodynamically equivalent processes and can not be distinguished. Studies in membrane vesicles indicated that HCO_3^- exit across the basolateral membrane of the kidney proximal tubule occurs via a cotransport of $1Na^+$:$1CO_3^{2-}$:$1HCO_3^-$ on separate distinct sites (Soleimani & Aronson, 1989), consistent with a stoichiometry of 3 equivalents of base per Na (Soleimani et al., 1987).

Nonrenal Tissues

As was indicated, NBC is expressed in several mammalian cell types including epithelial as well as nonepithelial cells. A major difference with respect to the functional mode of the NBC in kidney and other tissues is its direction of transport. In kidney proximal tubule cells this transporter mediates the exit of HCO_3^- from the cell to the blood (Boron & Boulpaep, 1983; Yoshitomi et al., 1985; Alpern, 1985; Grassl & Aronson, 1986) whereas in other epithelial cells (and certain nonepithelial cells) such as liver (Fitz et al., 1989; Gleeson et al., 1989) and heart (Lagadic-Gossmann et al., 1992; Camilion et al., 1996), this transporter mediates the entry of HCO_3^- from blood to the cell. As such, NBC function in the kidney proximal tubule leads to cell acidification. However, in heart or liver, NBC function leads to cell alkalinization. Further, the kidney NBC has a stoichiometry of 3 base

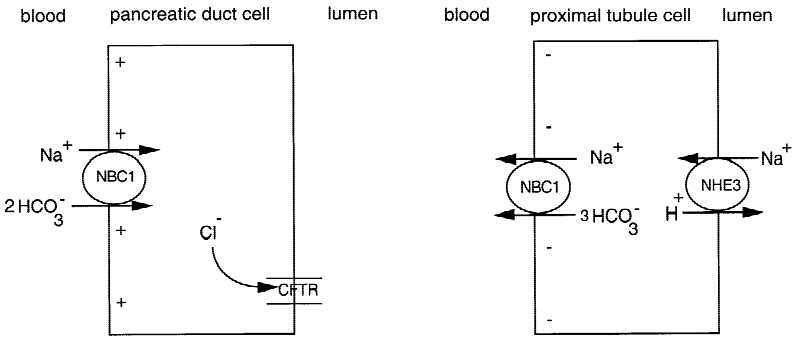

Fig. 2. Schematic diagrams illustrating the types and direction of acid-base transporters in kidney proximal tubule cells and pancreatic duct cells. In kidney proximal tubule (*right panel*) the Na^+:HCO_3^- cotransporter (NBC1) works in efflux mode and has a stoichiometry of 3 HCO_3^- per Na^+. In the pancreas (*left panel*), the Na^+:HCO_3^- cotransporter (NBC1) works in the influx mode and presumably has a stoichiometry of 2 HCO_3^- per Na^+. The direction of NBC movement (efflux *vs.* influx) is determined by the basolateral membrane potential. An inside negative membrane potential (hyperpolarization) causes the NBC to function in the efflux mode, whereas an inside positive membrane potential (depolarization) causes the NBC to function in the influx mode. "−" sign indicates hyperpolarized membrane potential whereas "+" denotes depolarized membrane potential.

equivalents per 1 Na (Soleimani et al., 1987), whereas heart NBC, for example, is either electroneutral (Lagadic-Gossmann et al., 1992) or has a stoichiometry of 2 bases per Na (Camilion et al., 1996), perhaps dependent on myocardial cell type.

The difference in the direction of NBC movement in kidney *vs.* other tissues has raised two possibilities: 1. The difference in the direction is due to varying membrane potential or cell ionic compositions in kidney cells (*vs.* other tissues), or 2. The difference in the direction suggests the presence of distinct NBC isoform in the kidney (*vs.* other tissues). Recent studies provide evidence in support of both possibilities. Molecular studies illustrate that the proximal tubule NBC (Burnham et al., 1997; Romero et al., 1997; Burnham et al., 1998; Romero et al., 1998; Abuladze et al., 1998a) is distinct from the cardiac NBC (see the section on NBC cloning), indicating that the opposite functional modes of cotransport (influx in the cardiac cells and efflux in the kidney cells) are likely due to the presence of two different isoforms in these tissues. Comparison of the studies in kidney and pancreas, however, indicate that the same NBC isoform can work in opposite directions in two different tissues. For example, NBC1 is expressed in both kidney proximal tubule (Burnham et al., 1998; Romero et al., 1998; Abuladze et al., 1998a) and pancreatic duct cells (Abuladze, et al., 1998b; Marino et al., 1999; Shumaker et al., 1999), however, it works in the influx mode in the pancreatic duct cells (Ishiguro et al., 1996; Shumaker et al., 1999) but operates in the efflux mode in the kidney proximal tubule (Alpern, 1990; Romero & Boron, 1999; Soleimani & Burnham, 2000). The influx mode of NBC1 in the pancreatic duct cells is due to a depolarized membrane potential that results from CFTR activation with subsequent Cl^- secretion (Shumaker et al., 1999). The schematic diagram in Fig. 2 illustrates the modes and directions of operation of the NBC in kidney proximal tubule and pancreatic duct cells.

Cloning and Distribution of NBC Isoforms

Clearing the Confusion: Simple and Universal Terminology

The current nomenclature describing the NBC/AE family has resulted in an extremely confusing situation. While all investigators agree to the term NBC (for sodium bicarbonate cotransporter), the nomenclature for isoforms, variants, and orthologs from different species has been more arbitrary. The term "isoform" is usually restricted to the product of a unique gene rather than to the result of a variation (most likely) resulting from alternate splicing of a single gene. As it stands, the same isoform or variant has been given two or sometimes three different names by different laboratories. In one case (NBCn1-A), a human cDNA has been given a name that implies that it is a splice variant of a rat gene.

One of the first examples of the confusion concerns NBC1, first cloned from human kidney (GenBank Accession no. AF007216) and amphibian kidney (Burnham et al., 1997; Romero et al., 1997). A variant of NBC1, which was subsequently cloned from pancreas (GenBank Accession no. AF011390), was first thought to be a new isoform (and not a variant) (Abuladze et al., 1998b). However, pancreas NBC1 (pNBC1) is in fact the result of differential splicing of the same gene (SLC4A4) that is transcribed to produce kidney NBC1 (Abuladze et al., 2000). Another splice variant of NBC1, hhNBC1 (for human heart NBC1, accession no. AF069510) encodes an amino-acid sequence that is identical to the pancreatic variant but differs in the 5′-noncoding region (Choi et al., 1999). It is worth noting that isoforms other than NBC1 (*see* below) appear likely to be more physiologically relevant (under normal conditions) in the heart.

A similarly confusing situation also involves NBC2, an isoform first described by Ishibashi, Sasaki & Ma-

rumo (1998) as a cDNA clone isolated from a human retina library. An NBC cDNA subsequently isolated from human skeletal muscle that has been designated as muscle NBC3, or just "NBC3" (accession no. AF047033) is clearly a variant of NBC2 (Pushkin et al., 1999). A rat ortholog of this transporter was cloned by another group (Choi et al., 2000) and named NBCn1 (GenBank accession no. AF070475), indicating the electroneutrality of this transporter. The rat NBCn1 protein sequences (there are three splice variants) have more than 92% identity to the human skeletal muscle "NBC3" which in turn is 98% identical over 4.1 kb (at the nucleotide level) to human retina NBC2 (Ishibashi et al., 1998), which was cloned first.

In GenBank and elsewhere, the term "SLC4A" for "SoLute Carrier 4A" has been used for the family of bicarbonate transporters which includes the anion exchangers (AEs) as well as the NBCs. This classification may not resolve the confusion with the nomenclature, as many investigators are more familiar with the term "NBC" than "SCL4A". Indeed, some of the confusion has carried over into the SLC4A-naming convention, as SCL4A5 and SLC4A6 appear to have been removed from the list of approved names because of redundancy with SLC4A7 and SLC4A8 respectively. It is clear that "SLC4A6" was originally applied to retinal NBC2, but then "SLC4A7" was applied to a splice variant of NBC2 designated "muscle NBC3" (Pushkin et al., 1999a) suggesting that it was a separate isoform. At the same time, regulation of mRNA expression (and a partial sequence) of a genuine third isoform (also called NBC3), was described in another publication (Amlal, Burnham & Soleimani, 1999). "SLC4A5" was originally assigned to pNBC1, despite strong structural indications that SLC4A5 might be a splice variant of kNBC1 (kidney form). The wide-spread confusion on this issue illustrates the point that, in biology, names make a difference. They are the basis of taxonomy. Although it is possible to "do" biology using overlapping names, it is nearly impossible to talk about it.

In contrast to the NBCs, the nomenclature for the NHEs (Na^+/H^+ exchanger) and AEs (anion exchanger) is clear and straightforward: each isoform is designated with a numeric suffix (1, 2, 3, etc.) (Kopito, 1990; Soleimani & Singh, 1995; Counillon & Pouyssegur, 2000). The designation of the numerical suffix reflects the chronological order of cloning; the first NHE is designated NHE1 and the most recent one is designated as NHE5. Similarly, the first AE to be cloned is designated AE1 and the last one is named AE4 (Kopito, 1990; Tsuganezawa et al., 2001). We therefore suggest that all NBCs be designated with numerical assignments added as a suffix to the term NBC. The numerical suffix (1, 2, etc.) should reflect the chronological order of cloning for that isoform. Based on this classification, NBC isoforms would be designated NBC1, NBC2, etc.

Using the above nomenclature, the four human NBC isoforms that have been cloned to date would be referred to as NBC1, NBC2, NBC3 and NBC4. In addition, several 5′- or 3′-terminal variants for some isoforms have been cloned. With respect to naming of orthologs, assignment of strict structural correlates may be impossible where invertebrate, bacterial, or plant sources are concerned. However, where human genes are concerned, confusion is not inevitable, and is to the detriment of the biology.

NBC1. NBC1, the first mammalian sequence to be reported was cloned from human kidney (GenBank accession no. AF007216) using a molecular probe that was identified by searching for sequence similarities (to the anion exchangers) in the expressed sequence tag database of GenBank (Burnham et al., 1997). The amphibian (*Ambystoma tigrinum*) kidney NBC1 (GenBank Accession no. AF001958) was cloned by expression in *Xenopus* oocytes (Romero et al., 1997). Both human and ambystoma open reading frames are of similar molecular weight (1035 amino acids), but differ in message size. The human kidney NBC1 cDNA was 7.6 kb in length, which is consistent with the size of the human mRNA as it appears on a Northern blot. However, the amphibian kidney mRNA is much shorter, at approximately 4.2 kb. The difference results from an extensive 3-prime noncoding region in the human NBC-1 kidney cDNA. The amino acid sequences of the two species are 80% identical.

Northern blots probed with full-length human kidney NBC1 gave strong hybridization signals in human pancreas lanes and weak signals in (whole) human brain lanes, suggesting that NBC1 is expressed in these two tissues (Burnham et al., 1997). The pancreas NBC1 (GenBank accession no. AF011390) (Abuladze et al., 1998b) is identical to the human kidney NBC1 except for a unique N-terminus including 85 amino acids of open reading frame, which replace the first 41 amino acids of the human kidney sequence. When the unique 5-prime ends of the two variants were used to probe multiple-tissue Northern blots, the kidney variant hybridized only with kidney mRNA, whereas the pancreas variant gave strong signals with prostate, colon, stomach, thyroid, brain, and spinal chord, as well as pancreas mRNA (Abuladze et al., 1998b). The entire SLC4A4 (NBC1) gene is some 450 kilobases in length and has 26 exons (Abuladze et al., 2000). The junction at which the kidney-specific variant diverges from the more widely expressed variant from pancreas has been determined to be an intron-exon boundary (Abuladze et al., 2000), and an alternative promoter in intron 3 has been shown to drive transcription of the renal variant.

More recently, a third splice variant, which encodes an open reading frame identical to the pancreas variant of NBC1 (but differs in the 5-prime noncoding region), has been cloned from a human heart cDNA library (Choi et al., 1999). The hybridization signal of heart-NBC1 mRNA on Northern blots is vanishingly faint compared to pancreas, suggesting that other NBC isoforms may be responsible for the majority of both the electroneutral (Lagadic-Gossmann et al., 1992) as well as the electrogenic (Camilion et al., 1996) Na^+:HCO_3^- cotransporter, which has been described functionally in the heart.

Other than human, amphibian, rat (Burnham et al., 1998; Romero et al., 1998), and mouse, *Oryctolagus cuniculus* (AF119816) and *Bos taurus* (AF308160) NBC1 isoforms have recently been cloned. The latter two were cloned from corneal endothelium, the rabbit being a kNBC1 variant, and the bovine being a pNBC1 variant. Remarkably, the two types of variants (kidney and pancreas) are preserved across species, suggesting an (as yet) unknown functional distinction. Both splice variants are expressed in human eye, and mutations in the SCL4A gene result in multiple ocular abnormalities (Igarashi et al., 1999). A sequence described as the "rat pancreas variant" (GenBank accession number AF107265) is identical to the human pancreas variant at the nucleotide level, raising the possibility that the annotation may be in error. The tissue-expression pattern of NBC1 in rat and mouse is similar. However, in rat, the expression of NBC1 in the kidney is limited to the proximal tubule under normal conditions (Abuladze et al., 1998a; Burnham et al., 1998; Schmitt et al., 1999), whereas in mouse, expression of NBC1 in the kidney involves proximal tubule as well as medullary thick ascending limb (Wang et al., 2001). An NBC1 with a novel C-terminus has also been cloned from rat brain (Bevensee et al., 2000).

NBC2. NBC2, which is now designated SLC4A7, is a unique isoform with 59% (amino acid) identity with NBC1. NBC2 was first identified by searching an EST database for sequences similar to AE1, and then isolated from a human retina cDNA library (Ishibashi et al., 1998). Its first reported GenBank accession no. is AB012130, and it is widely expressed in many tissues (including testis, spleen, colon, small intestine, ovary, thymus, prostate, skeletal muscle, heart, kidney, stomach, trachea, and bone marrow). It appears to be faintly expressed in pancreas and liver. Two variants of NBC2 appear to be expressed in a tissue-dependent manner, one of ~8 kb which predominates in human retina, and one of ~5 kb which predominates in human testis (Ishibashi et al., 1998).

Since the cloning of retinal NBC2 (Ishibashi et al., 1998), two splice variants of this isoform have been cloned. One variant was cloned from human skeletal muscle (GenBank accession no. AF047033) (Pushkin et al., 1999a). This cotransporter has a 7.7 kilobase transcript, and encodes a 1214-residue polypeptide; it was originally described in skeletal muscle and heart (Pushkin et al., 1999a). Subsequent studies have shown a wider distribution pattern for this variant and demonstrated that it is expressed in kidney, salivary gland and other tissues (Pushkin et al., 1999b; Luo et al., 2001). This splice variant was originally designated as "muscle NBC3" rather than as an NBC2 variant. The reason for confusion regarding its terminology seems to have originated from the fact that the protein sequence of this transporter has a 124-amino acid stretch that is different from the retinal NBC2. In addition, there are differences at the amino and carboxy termini that might suggest that the two are in fact unique isoforms. However, a close analysis of the alignment of "muscle NBC3" with retinal NBC2 indicates that retinal NBC2 and "muscle NBC3" share more than 2.8 kb of identical nucleotide sequence, raising the possibility that "muscle NBC3" is actually a variant of retinal NBC2 produced by alternate splicing of the NBC2 gene. Further, both cDNAs localize to the same arm of the same chromosome. Taken together, these results indicate that muscle NBC3 is likely to be a variant of NBC2, and is not a distinct isoform. Another entry into GenBank (NBC2b, accession no. AF089726) is a variant of retinal NBC2. We suggest that muscle NBC3 be designated as NBC2c, and retinal NBC2 be designated as NBC2a. So, the three GenBank accession numbers (AB012130, AF089726, and AF047033) refer to NBC2a, NBC2b, and NBC2c, respectively. Although this distorts the chronological order in which the clones were first published, it would be even more confusing to refer to the third isoform as "NBC2c" since it has already been released via GenBank as "NBC2b".

The confusion is no less profound among the rat orthologs of NBC2. An mRNA, which is clearly transcribed from the rat ortholog of SLC4A7 and is a variant of NBC2 (with GenBank accession no. AF070475), was cloned from rat smooth muscle, and is actually referred to in the literature as NBCn1 (Choi et al., 2000). This ortholog has a 7.5 kb transcript and encodes a 1218-amino acid polypeptide. Similar to human NBC2, it has a wide tissue expression, is ~92% identical to human NBC2 (*see* above) and has ~50–55% identity to NBC1. Three variants of NBC2 have been identified in rat and are referred to as NBCn1-B, NBCn1-C, and NBCn1-D. The question of what happened to "NBCn1-A" (as a name) has a curious answer. According to the OMIM (online Mendelian inheritance in man) account, "NBCn1-A" is the name given to the human skeletal muscle isoform, NBC2c. However, as noted above, this isoform is referred to as "NBC3" in the primary publication describing its cloning. It is hoped that "NBCn1-A" is a name destined for obscurity. The relationships among the various rat and human splice variants is obscured by the two naming conventions, and it would be helpful to all investigators in the field if the rat

NBC2 orthologs would be given names which are consistent with the human sequences, yet which distinguish them as rat sequences.

NBC3. Studies in NT-2 cells identified a third human NBC isoform (referred to as NBC3, GenBank accession no. AF107099) (Amlal et al., 1999). This isoform has been assigned the gene name "SLC4A8". It appears to have three transcripts on Northern blots, which are expressed in a tissue-dependent manner (Amlal et al., 1999). A large, ~9 kb transcript is expressed in brain, spinal chord, and adrenal gland. An intermediate transcript of ~4.5 kb is widely expressed in brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, stomach, thyroid, spinal chord, lymph node, trachea, adrenal gland, and bone marrow. A small transcript (~3 kb) appears in neuronal tissue. NBC3 encodes a 1044-amino-acid polypeptide (Amlal et al., 1999). Two variants of NBC-3 have been released by GenBank (accession numbers AB018282 and AF069512). The first was identified from randomly sequenced cDNA clones selected from a human brain library (Nagase et al. 1998), and appears to be a partial coding sequence and transcript. The second contains an alternate 5-prime end, but is identical in other respects. Tissue distribution studies of the latter variant show expression in brain and several other tissues (Grichtchenko et al., 2001). Overall, NBC3 has an amino-acid sequence that is 56% identical with NBC1 and 76% identical with NBC2. The mouse NBC3 ortholog has been cloned and shows high expression in brain (Wang et al., in press). Interestingly, in the kidney, NBC3 expression is limited to the inner medullary collecting duct cells (Wang et al., 2001).

NBC4. Recently, a new member of the Na^+:HCO_3^- cotransporter family was cloned from human heart (GenBank accession numbers AF207661) (Pushkin et al., 2000a). This isoform is designated as NBC4, has a 6 kb transcript and encodes a 1074-residue polypeptide. NBC4 has the highest expression levels in liver, testis and spleen and is moderately expressed in heart. Two C-terminal variants of NBC4 have been cloned (Pushkin et al., 2000b). The nucleotide sequence of NBC4 actually contains 1667 nucleotides from another cDNA, Dynactin I, on its 5′ end. This error is likely due to the fact that the dynactin gene is immediately adjacent to the NBC4 gene.

Very recently two other isoforms with high structural homology to the members of NBC family have been cloned. One, which was originally referred to as NBC5 (FASEB annual meeting 2000), shows ~50% homology to NBC1 (GenBank Accession no. AB038263). It is only expressed in the kidney cortical collecting duct cells (Tsuganezawa et al., 2001). Subsequent functional studies indicate that this transporter is actually a Cl^-/HCO_3^- exchanger and does not transport Na (Tsuganezawa et al., 2001). As a result, this transporter has been renamed AE4 (rather than NBC5), and seems to have a structure intermediate between the NBCs and the other AE's (Tsuganezawa et al., 2001).

The latest member of the NBC/AE family was recently cloned from an insulin-secreting cell cDNA library using human kidney NBC1 as a probe in low-stringency hybridizations (Wang et al., 2000). Functional studies indicate that this transporter mediates Na-dependent Cl^-/HCO_3^- exchange, and accordingly it has been named "NCBE" for Na-dependent, Chloride/Bicarbonate Exchanger (GenBank Accession no. AB033759). It is expressed in brain, ileum and kidney (Wang et al., 2000). Its sequence is most closely related to NBC2 and NBC3.

Table 1 summarizes the cloning of NBC and related proteins. Table 2 summarizes the tissue distribution and subcellular localization of NBC isoforms and related proteins in epithelial tissues. An unrooted phenotypic dendrogram illustrating the relationships of the bicarbonate transporter/exchanger family is shown in Fig. 3. The anion-exchanger (AE1-3) part of the family appears to have branched off from a common ancestor nearly simultaneously, whereas NBC1 and NBC4 are derived from a form that was intermediate between the AEs and NBC2 and NBC3.

Functional Expression and Membrane Localization of NBC Isoforms

NBC1. Studies in mammalian cells expressing NBC1 demonstrated a DIDS-sensitive, Na-dependent, HCO_3^- cotransport (Burnham et al., 1997; Amlal, Burnham & Soleimani, 1999). Expression studies in oocytes similarly indicate that NBC1 is a Na^+:HCO_3^- cotransporter (Romero et al., 1997). Both pancreatic and kidney variants of NBC1 were shown to be electrogenic (Romero et al., 1997; Abuladze et al., 1998b) and carrying a net negative charge, indicating the transport of more than 1 HCO_3^- per Na^+. The Na-binding site of kidney NBC1 shows affinity for Li, although at a much lower level (Amlal et al., 1998b). These results are in agreement with functional studies in kidney membrane vesicles and perfused tubules (Boron & Boulpaep, 1983; Alpern, 1985; Grassl & Aronson, 1986; Soleimani and Aronson, 1989). Immunocytochemical studies have localized NBC1 to the basolateral membranes of kidney proximal tubule and pancreatic ducts (Schmitt et al., 1999; Marino et al., 1999).

As was indicated, NBC1 is electrogenic and carries a net negative charge, consistent with the transport of more than one HCO_3^- per Na^+ (Romero & Boron, 1999; Soleimani & Burnham, 2000). Expression studies in oocytes indicate that NBC1 has a stoichiometry of 2 HCO_3^- per Na^+ (Sciortino & Romero, 1999). However, expression studies in renal cells indicate a stoichiometry of 3

Table 1. NBC isoforms, their splice variants and alternative names

Isoform	Variants	Alternative Names	Accession Numbers	References
NBC1 (SLC4A4, 4q21)	kNBC1		NM_003759, AF007216 AF157492	J. Biol. Chem. 272 (31), 19111–19114 (1997)
	pNBC1		AF069510, AF011390,	J. Biol. Chem. 273
	hNBC1	hhNBC	AF053754, AF310248	(28), 17689–17695 (1998) Am. J. Physiol. 276 (3), C576–C584 (1999)
NBC2 (SLC4A7, 3p22)	retina NBC2 NBC2b	SBC2 NBC3, mNBC3, NBCn1, BT	NM_003615, AB012130 AF047033 AF089726, AF053755	Biochem. Biophys. Res. Commun. (1998) J. Biol. Chem. 274 (23), 16569–16575 (1999) Genomics 57 (2), 321–322 (1999)
NBC3 (SLC4A8, Chr. 12)		NDCBE	NM_004858, AF069512, AB018282 AF107099	DNA Res. 5 (5), 277-286 (1998) Am. J. Physiol. 276 (6), F903–F913 (1999)
NBC4 (2p13)	NBC4a NBC4b		AF207661, NM_021196 XM_002746	Biochim. Biophys. Acta 1493 (1–2) 215–218 (2000) IUBMB Life. 50(1):13–9 (2000)
NCBE (SLC4A10, 2q23-q24)			NM_022058 AB040457	J. Biol. Chem. 275 (45), 35486–35490 (2000)

Table 2. Tissue distribution and membrane localization of NBC isoforms and their splice variants

NBC isoform	Variants	Tissue distribution	Membrane localization
NBC1	kNBC1	predominantly kidney	basolateral
	pNBC1	pancreas, liver, GI tract	basolateral
NBC2	retina NBC2	retina, other tissues	likely basolateral
	mNBC3	muscle, other tissues	apical or basolateral
NBC3		brain, kidney medulla, others	likely basolateral
NBC4	NBC4a	wide-spread	unknown
	NBC4b	wide-spread	unknown
NCBE		limited tissue expression	likely basolateral
AE4		kidney	apical

HCO_3^- per Na^+ (Gross et al., 2000). When the same cotransporter (NBC1) was expressed in pancreatic duct cells, it showed a stoichiometry of 2 HCO_3^- per Na^+ (Gross et al., 2000). Taken together, these results indicate that the stoichiometry of NBC1 is tissue-dependent and can vary between 2 or 3 base equivalents depending on the tissue. These results also reconcile the conflicting functional reports on the varying stoichiometry of NBC in brain and kidney (Yoshitomi et al., 1985; Soleimani et al., 1987; Soleimani & Burnham, 2000).

NBC2. Two studies have examined the functional properties of NBC2. In the first, the human skeletal-muscle NBC was expressed in oocytes and examined. As was indicated above, this cotransporter (GenBank accession no. AF047033) is a variant of NBC2. In oocytes expressing this transporter, a Na-dependent HCO_3^- cotransport activity was observed using the pH-sensitive dye BCECF (Pushkin et al., 1999). This activity was not coupled to chloride, indicating that it is distinct from the Na-dependent Cl^-/HCO_3^- exchanger. The NBC activity

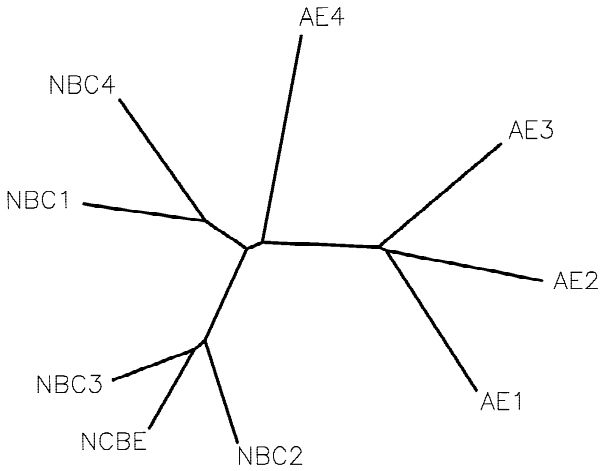

Fig. 3. A phenotypic dendrogram illustrating the evolutionary relationships among the AE/NBC superfamily. Branch lengths are proportional to evolutionary distance calculated using a "blossum" matrix. NBC: Na^+:nHCO_3^- cotransporter; AE: anion exchanger.

was resistant to inhibition by DIDS but was inhibited by amiloride and its analogues (Pushkin et al., 1999a). In oocytes expressing the rat homologue of NBC2 (also referred to as NBCn1), a Na-dependent HCO_3^- cotransport was observed that was resistant to amiloride but partially sensitive to DIDS (Choi et al., 2000). Both studies indicated that this cotransporter is electroneutral (Pushkin et al., 1999a; Choi et al., 2000). Antibodies against the skeletal-muscle NBC labelled a protein in the rabbit kidney which showed colocalization with H^+ ATPase in the cortical collecting ducts; it localized to the apical domain of alpha intercalated cells and basolateral domain of beta intercalated cells (Pushkin et al., 1999b). Based on its apical localization in alpha intercalated cells it was suggested that the skeletal muscle NBC contributes to net bicarbonate reabsorption in the collecting duct (Pushkin et al., 1999b). Antibodies against the rat NBC2 (NBCn1) labelled a protein on the basolateral membrane of kidney thick ascending limb and collecting ducts (Vorum et al., 2000).

NBC3. Tissue expression studies have demonstrated that this cotransporter is highly expressed in brain and kidney inner medulla (Amlal et al., 1999). Three studies have examined the functional properties of this transporter. The first series of studies were performed in cultured inner medullary collecting duct (mIMCD-3) cells. Sublethal acidosis downregulated NBC1 expression by 90% but increased NBC activity by >5-folds in mIMCD-3 cells (Amlal et al., 1999). This was due to the upregulation of NBC3 expression by >7-fold. Depleting the cells of chloride did not affect the Na-dependent bicarbonate cotransport, indicating that NBC3 does not function in Na-dependent Cl^-/HCO_3^- exchange mode (Amlal et al., 1999). Two new studies have examined the functional expression of NBC3 in oocytes. In one study, oocytes expressing NBC3 demonstrated a Na-dependent, Cl^-/HCO_3^- exchange (Grichtchenko et al., 2001), whereas in the other, they showed a Na-dependent HCO_3^- cotransport (Wang et al., 2001). The reason for this disparity is not clear at the present. Additional studies are needed to determine whether the coupling of intracellular chloride to extracellular Na^+ and HCO_3^- is dependent on the level of NBC3 expression.

NBC4. NBC4 was the latest isoform to be cloned (Pushkin et al., 2000). Currently, there are no data available regarding its functional properties or its membrane localization.

NBC5 (or AE4). NBC5 (or AE4) is actually a Cl^-/HCO_3^- exchanger and does not function in Na-dependent HCO_3^- cotransport mode (Tsuganezawa et al., 2001). It was cloned from rabbit kidney cortical collecting ducts by RT-PCR using degenerate primers based on the cDNA sequence of AE1 and AE3. Its sequence shows the highest homology to NBC1. Expression studies in oocytes and cultured cells indicated that it mediates Cl^-/HCO_3^- exchange and does not transport Na (Tsuganezawa et al., 2001). Because of this functional property it was named AE4. It is only expressed on the apical domain of beta-intercalated cells in kidney cortical collecting ducts (Tsuganezawa et al., 2001) and is thought to be responsible for bicarbonate secretion into the kidney lumen.

NCBE. NCBE functions as a Na-dependent, Cl^-/Bicarbonate Exchange (Wang et al., 2000). It is expressed in brain, kidney and ileum (Wang et al., 2000). Its membrane localization has not been studied but, based on its expression and function, it is likely expressed on the basolateral domain of epithelial cells. A Na-dependent, Cl^-/base exchanger has been cloned from Drosophila and is termed Na-Dependent, Anion Exchanger (NDAE) (Romero et al., 2000).

Role and Regulation in Pathophysiologic States

Cystic Fibrosis

Cystic fibrosis (CF) is the most common genetically-transmitted disorder in the United States (Rosenstein & Zeitlin, 1998). An autosomal recessive disease, cystic fibrosis results from mutational inactivation of a cAMP-sensitive Cl^- channel (cystic fibrosis transmembrane conductance regulator, CFTR) with resultant impairments in the respiratory, pancreatic, hepatobiliary, and genitourinary systems (Rosenstein & Zeitlin, 1998). In the pancreas, the expression of CFTR is mainly limited to the apical membrane domain of the duct cells (Case & Argent, 1993). These cells constitute less than 10% of the exocrine pancreas and are responsible for all of bicarbonate secretion from pancreas. The pancreatic insufficiency in CF patients is mainly due to dysfunction of

acinar cells which constitute more than 90% of the exocrine pancreas and are responsible for the secretion of digestive enzymes. Several studies have shown that the major initial defect in cystic fibrosis is the impairment of secretin-stimulated ductal Cl^- and HCO_3^- secretion (Case & Argent, 1993; Rosenstein & Zeitlin, 1998). Based on experimental and histopathologic evidence, it was suggested that the reduction in secretin-stimulated HCO_3^- secretion from pancreatic duct epithelial cells alters intraductal pH and, as a result, precipitates proteins secreted from acinar cells (Scheele et al., 1996). This cascade of events results in protein plugs and disrupts vesicular trafficking in the acinar cell's apical domain (Scheele et al., 1996). Taken together, these alterations lead to pancreatic fibrosis and insufficiency in 80% of patients with CF (Scheele et al., 1996; Rosenstein & Zeitlin, 1998). Attempts toward understanding or correcting this HCO_3^- secretion defect have been hampered by a lack of knowledge regarding the cellular and molecular mechanisms mediating HCO_3^- transport in pancreatic ductal epithelial cells. The accepted model of pancreatic ductal HCO_3^- secretion suggests that (1) intracellular HCO_3^- accumulates due to basolateral diffusion of CO_2 and subsequent action of carbonic anhydrase, (2) G protein-coupled receptors activate cAMP-sensitive cystic fibrosis transmembrane regulator (CFTR), and (3) resultant increases in luminal Cl^- drives an apical Cl^-/HCO_3^- exchanger (Case & Argent, 1993). However, this model is inconsistent with recent data demonstrating the Na^+-dependence of ductal HCO_3^- uptake at the basolateral membranes (Ishiguro et al., 1996). A mathematical modeling of pancreatic duct cells indicates the important role of basolateral Na-dependent HCO_3^- transport in pancreatic duct cells (Sohma et al., 2000).

A recent study demonstrated that HCO_3^- enters duct cells (from blood) via the basolateral electrogenically-driven Na^+:HCO_3^- cotransporter NBC1 (Shumaker et al., 1999). It was further illustrated that cAMP, which mediates the stimulatory effect of secretin on HCO_3^- secretion, potentiated Na^+:HCO_3^- cotransport only in cells expressing functional CFTR (Shumaker et al., 1999). This stimulatory effect of cAMP was blocked under voltage-clamped conditions, indicating that the stimulation of Na^+:HCO_3^- cotransport by cAMP is due to generation of a favorable electrical potential as a result of membrane depolarization by Cl^--secreting CFTR (Shumaker et al., 1999). Based on these studies, it was proposed that the driving force for HCO_3^- entry into pancreatic duct cells in the agonist-stimulated state is activation of the apical CFTR in response to intracellular cAMP (Shumaker et al., 1999). The observed depolarization of cell membrane potential (which results from Cl^- exit at the luminal membrane via CFTR) stimulates the entry of HCO_3^- via the basolateral electrogenically-driven Na^+:HCO_3^- cotransporter NBC1. HCO_3^- is then secreted into the duct lumen predominantly via Cl^-/HCO_3^- exchanger(s). It was further proposed that the defect in HCO_3^- secretion in response to secretin (which acts via cAMP) in patients with cystic fibrosis is partly due to lack of entry of HCO_3^- at the basolateral membrane (due to a lack of depolarization of the cell membrane and therefore absence of a driving force for electrogenic Na^+:HCO_3^- cotransport) (Shumaker et al., 1999). Accordingly, the following HCO_3^- transport model has been proposed in the pancreatic duct cells (Fig. 4). According to this model, secretin increases intracellular cAMP which then results in the activation of CFTR and secretion of Cl^-, leading to depolarization of both luminal and basolateral membranes. The depolarization of the basolateral membrane increases the driving force for NBC and as a result, enhances HCO_3^- entry into the duct cells. HCO_3^- will then be secreted at the apical membrane (mostly via an apical Cl^-/HCO_3^- exchanger and a bicarbonate conductive pathway that is poorly defined at the present). These findings have important ramifications regarding our understanding of the physiologic role of CFTR in these cells, and provide important insight into the pancreatic pathophysiology of cystic fibrosis.

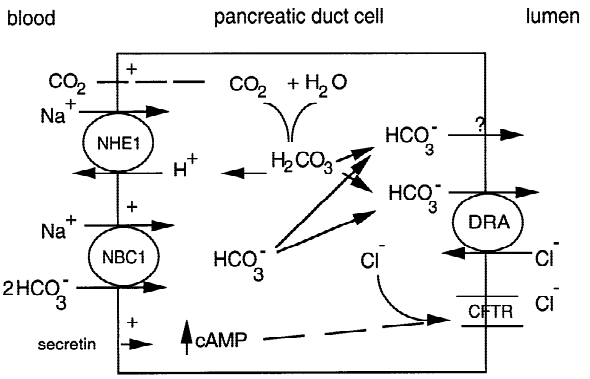

Fig. 4. The proposed model of HCO_3^- secretion in the pancreatic duct cells. Activation of CFTR by cAMP depolarizes the basolateral membrane potential which in turn stimulates the electrogenic Na^+:$2HCO_3^-$ cotransporter (NBC1). The activity of basolateral Na^+/H^+ exchanger additionally generates intracellular HCO_3^- under the action of cytosolic carbonic anhydrase and the CO_2, which diffuses from the blood. The HCO_3^- that is thus entered or generated is secreted at the apical membrane predominantly via an apical Cl^-/HCO_3^- exchanger (DRA: *D*own *R*egulated in *A*denoma) and possibly a bicarbonate conductive pathway. "+" sign denotes depolarized membrane potential.

Proximal Renal Tubular Acidosis

Decreased reabsorption of bicarbonate in kidney proximal tubule results in metabolic acidosis secondary to renal bicarbonate wasting (Alpern et al., 1986). This condition is called proximal tubular acidosis and is dis-

tinct from distal renal tubular acidosis, which is caused by defective bicarbonate reabsorption in the distal tubule (Alpern et al., 1986). The molecular basis of proximal renal tubular acidosis remains unclear at the present. As bicarbonate reabsorption in the proximal tubule is mediated via apical NHE3 and H^+-ATPase and basolateral NBC (Alpern, 1990; Soleimani & Singh, 1995; Romero & Boron, 1999; Soleimani & Burnham, 2000), proximal renal tubular acidosis should ultimately result from the impairment of the activity of one these transporters. Mice deficient in NHE3 do not develop renal tubular acidosis (Schultheis et al., 1998), indicating that NHE3 dysfunction is not the cause of proximal RTA. Based on the important role of NBC1 in HCO_3^- reabsorption in kidney proximal tubule, it was postulated that dysregulation of NBC1 may play an important role in the pathogenesis of proximal renal tubular acidosis (Soleimani & Burnham, 2000).

Recently, mutations in the gene encoding the renal Na^+:HCO_3^- cotransporter, NBC1, were identified in two patients with proximal renal tubular acidosis (Igarashi et al., 1999; Shiohara et al., 2000). The serum bicarbonate concentration was less 10 mEq/l in both patients, indicating severe metabolic acidosis. In addition to RTA, the patients had glaucoma, band keratopathy, corneal calcification and short stature acidosis (Igarashi et al., 1999; Shiohara et al., 2000). In one patient, a homozygous G to A transition at nucleotide 1,678 in kidney NBC1 gene was identified. Expression studies with the mutant cDNAs in cultured mammalian cells indicated decreased NBC activity (Igarashi et al., 1999). These results demonstrate that severe metabolic acidosis was caused by the impairment of bicarbonate reabsorption in the kidney proximal tubules. The metabolic acidosis and short stature improved as a result of alkali therapy indicating that growth retardation was due to severe acidosis (Shiohara et al., 2000). In addition to proximal renal tubular acidosis and band keratapathy, the blood chemical analysis indicated increased amylase concentration (Igarashi et al., 1999), suggesting possible evidence of pancreatitits. It is intriguing to speculate that decreased NBC activity in pancreatic duct cells may lead to decreased ductal bicarbonate secretion and as a result cause pancreatitis (in a manner that has been postulated for cystic fibrosis).

Potassium Depletion

Functional studies indicate that potassium depletion increases bicarbonate reabsorption in the kidney proximal tubule (Rector, Bloomes & Saldin, 1965; Kunau et al., 1968; Capasso et al., 1987a,b). This is due to increased activities of apical Na^+/H^+ exchanger (NHE3) and basolateral Na^+:HCO_3^- cotransporter (Soleimani et al., 1990). To better understand the molecular mechanism of NBC upregulation, the expression of NBC1 was studied in the kidneys of potassium depleted animals. These studies indicated enhanced NBC1 mRNA expression and activity in rat proximal tubules as early as 72 hrs after potassium deprivation (Amlal, Habo & Soleimani, 2000). The results support the notion that potassium deprivation increases HCO_3^- reabsorption in proximal tubule by enhancing NBC1 expression and activity. Interestingly, potassium deprivation induced the expression of NBC1 in medullary thick ascending limb and inner medullary collecting ducts (Amlal et al., 2000), suggesting enhanced HCO_3^- reabsorption in several nephron segments. These results further demonstrate that the upregulation of NBC1 is an early event and precedes the onset of hypokalemia, indicating that the signal responsible for enhanced NBC1 expression is likely activated via intracellular potassium depletion rather than hypokalemia.

Glucocorticoid Excess

The effect of glucocorticoids on NBC1 was recently studied (Ali et al., 2000). Rats were injected subcutaneusly with either dexamethasone (100 μg day) or DOCA (30 mg/kg), potent glucocorticoid or mineralocorticoid analogs, respectively. Animals were sacrificed after 5 days and NBC1 mRNA expression and activity were measured in proximal tubule suspensions. Northern hybridizations indicated that treatment with dexamethasone, but not DOCA, enhanced NBC1 mRNA expression and activity in proximal tubule (Ali et al., 2000). Based on these studies, it is suggested that glucocorticoids but not mineralocorticoids enhance NBC1 expression and activity. Coupled to increased apical NHE3 activity (Freiberg, Kinsella & Sackton, 1982), this in turn could result in increased HCO_3^- reabsorption in proximal tubule with subsequent metabolic alkalosis in pathophysiologic states associated with increased glucocorticoid production such as Cushing synrome (Hulter et al., 1980; Hulter, Sigala & Sebastian, 1981).

In conclusion, NBCs are expressed in various tissues and are essential to acid-base and electrolyte homeostasis. NBC1 is electrogenic and plays an important role in the reabsorption and secretion of HCO_3^- in the kidney and pancreas, respectively. NBC2 and NBC3 are electroneutral, have a wide tissue distribution, and are predominantly responsible for bicarbonate uptake and cell pH regulation. NBC3 is highly expressed in the brain and kidney inner medulla and may play an important role in pH regulation in these two tissues. More studies are needed to examine the characteristics of NBC isoforms and related transporters in pathophysiologic states. Further, future studies in genetically-engineered mice deficient in NBCs should answer questions regarding the role of these transporters in electrolyte and acid-base homeostasis in kidney, pancreas and other organs.

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