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The Renal Sodium Bicarbonate Cotransporter NBCe2: Is It a Major Contributor to Sodium and pH Homeostasis?

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Abstract

The sodium bicarbonate cotransporter (NBCe2, aka NBC4) was originally isolated from the human testis and heart (Pushkin et al. IUBMB Life 50:13–19, 2000). Subsequently, NBCe2 was found in diverse locations where it plays a role in regulating sodium and bicarbonate transport, influencing intra-cellular, extracellular, interstitial, and ultimately plasma pH (Boron et al. J Exp Biol. 212:1697–1706, 2009; Parker and Boron, Physiol Rev. 93:803–959, 2013; Romero et al. Mol Asp Med. 34:159–182, 2013). NBCe2 is located in human and rodent renal-collecting duct and proximal tubule. While much is known about the two electrogenic sodium bicarbonate cotransporters, NBCe1 and NBCe2, in the regulation of sodium homeostasis and pH balance in the rodent kidney, little is known about their roles in human renal physiology. NBCe2 is located in the proximal tubule Golgi apparatus under basal conditions and then disperses throughout the cell, but particularly into the apical membrane microvilli, during various maneuvers that increase intracellular sodium. This review will summarize our current understanding of the distribution and function of NBCe2 in the human kidney and how genetic variants of its gene, *SLC4A5*, contribute to salt sensitivity of blood pressure.

Keywords

Renal sodium; Sodium bicarbonate cotransporter; pH homeostasis; NBCe2; Salt sensitivity of blood pressure

Renal Bicarbonate Transport and NBCe2 *(SLC4A5)*, NBCe1 *(SLC4A4)*, and the Cl^-/HCO_3^- Exchanger *(SLC26A6)*

Various transporters are involved with bicarbonate transport since reabsorbing bicarbonate filtered by the kidney is necessary to maintain acid-base and pH balance in the body.

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Compliance with Ethical Standards

Conflict of Interest Drs. Felder, Jose, Xu, and Gildea declare no conflicts of interest relevant to this manuscript.

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Approximately 4320 meq/day of HCO_3^-(24 meq/L × 180 L/day) are filtered by the glomeruli. Much (~90 %) of this bicarbonate is reabsorbed by the renal proximal tubule (RPT) and the remainder in the thick ascending limb and collecting duct. The reabsorption process is thought to occur via the combination of H^+ ions secreted into the tubular lumen by the sodium hydrogen exchanger (NHE3) and the vacuolar H^+ATPase [1], and reabsorption of the filtered bicarbonate by a series of events initiated by carbonic anhydrase type 4 (CA IV) (Fig. 1). CA IV dissociates carbonic acid (H_2CO_3) into H_2O and CO_2, which is membrane permeable, diffuses into the cell. Bicarbonate can also be reabsorbed from or secreted into the tubule lumen, the latter occurring if there is an excess generated in the renal tubule, through members of the solute carrier protein family *(SLC4)*. Therefore, we will briefly review this transporter family and what is known about its location in the kidney.

There are 10 members of the bicarbonate transporter family (encoded by the *SLC4* gene family), many of which are found in the kidney [2••, 3••]. This review is focused on the electrogenic members of the *SLC4* family, extensively studied by Boron and Boulpaep, and discovered in the renal proximal tubule (RPT) [4], as well as NBCe2 *(SLC4A5)* originally found in the liver, testis, and spleen by Pushkin [5], which mediate cotransport of 2–3 bicarbonate ions along with each sodium ion. NBCe2 is found predominantly at the apical side of the proximal tubule cell (vide infra), while NBCe1, originally found in the salamander and rodent [4, 6], is found exclusively at the basolateral membrane [3••, 7•, 8]. Although there are six published splice variants of NBCe2, only two (NBCe2-A and NBCe2-C) are expressed with all the transmembrane domains intact and thus may be the only functional members of the NBCe2 variant family [9]. NBCe2-A contains a unique 16 amino acid insert almost at the end of the carboxy terminal tail between transmembrane segments 11 and 12 when compared with NBCe2-C (Fig. 2). NBCe2-C is the only NBCe2 variant to have electrogenic activity, and thus, the 16 amino acid insert probably prevents NBCe2-A from expressing electrogenic activity. In order to visualize both isoforms by immunoblotting or immunohistochemistry, an amino terminal tail-directed antibody should be selected.

NBCe1 is transcribed under the control of two distinct promoters making four possible messenger RNAs. However, only three isoforms have been identified as follows: NBCe1-A, NBCe1-B, andNBCe1-C [9]. Of these three transcripts, only NBCe1-A is expressed in the kidney where it is located in the basolateral membrane of the S1 and S2 segments of the proximal tubule [8]. By contrast, NBCe2 is found in the luminal membrane of all segments of the RPT (vide infra).

Recently, we demonstrated that under basal conditions, the human RPT expresses low levels of NBCe2 which can be enhanced by increasing the intracellular sodium concentration, either by increasing the extracellular concentration of sodium or adding monensin, an ionophore, into the incubating media. Presumably, this results in an increase in sodium bicarbonate cotransport [10•]. The increase in NBCe2 activity in RPT cells (RPTC) is transient in those carrying wild-type *SLC4A5* and persistent in those carrying rs7571842 *SLC4A5* [11••]. Others have determined that approximately 75 % of bicarbonate secretion into the intestinal lumen in the sea bass is due to the activity of carbonic anhydrase, while another 10–20 % is 4,4’-diisothiocyanatostilbene-2,2’-disulfonate sensitive. NBCe2 and

NBCe1 are inhibited by 4,4'-diisothiocyanatostilbene-2,2'-disulfonate [2••, 3••]. This suggests that a sodium bicarbonate cotransporter, such as NBCe2, may be involved with this activity [12]. Thus, there is some precedent for a sodium bicarbonate cotransport beyond the well-accepted carbonic anhydrase pathway in the regulation of bicarbonate transport [1, 2••, 3••, 4–6, 7•, 9].

Human *SLC4* proteins come from 10 different genes that encode sodium-dependent transporters and sodium- independent exchangers, including bicarbonate [1, 2••, 3••, 4–6, 7•, 8, 9, 13]. They can be ubiquitously or discretely expressed with specific functions. In the renal proximal tubule, it appears that bicarbonate and chloride secretion into the tubule lumen via the apical membrane is also mediated by *SLC26A6* encoding the putative anion transporter 1 (PAT1) (aka CFEX) [14]. When studied in RPTCs from the spontaneously hypertensive rat (SHR) and its normotensive control, the Wistar-Kyoto rat (WKY), PAT1 activity was found to be increased in the SHR [15–17]. However, there are other *SLC26*–encoded proteins in the renal proximal tubule that may contribute to bicarbonate transport [14–20]. The Cl^-/HCO_3^- exchanger activity in the kidney appears to be the sum of the activities of *SLC26A4, SLC26A6*, and *SLC26A9* in WKY and SHR RPTCs; all these transporters are overexpressed in the SHR [15]. Simão et al. [15] make a compelling argument that these transporters may be an adaptive process to the sustained increase in sodium and bicarbonate transport in the RPTCs in the SHR. However, the increased transport could also be a result of gain-of-function single nucleotide polymorphisms (SNPs) in *SLC4A5* [11••] (vide infra).

NBCe1 and NBCe2 may regulate renal sodium and bicarbonate transport to varying degrees depending on the salt balance of the individual. The activities of other sodium-dependent transporters and sodium-independent exchangers in the *SLC4* and *SLC26* families affect the activities of NBCe1 and NBCe2. These transporters and exchangers, in turn, affect the activity of proteins involved in sodium and hydrogen transport, such as NHE3, Na^+/K^+−ATPase, and the H^+−ATPase in the renal proximal tubule. Since a full review of all the sodium, bicarbonate, and hydrogen transporters is beyond the scope of this review, we suggest the following review articles [1, 2••, 3••, 7•, 9, 18–21].

The Role of the Kidneys in the Regulation of Blood Pressure

Seminal studies on blood pressure regulation were performed by Guyton et al. [22] who hypothesized that ultimately the kidney contributes to the most critical regulation of intra-arterial pressure aided by interactions with the nervous, cardiovascular, and endocrine systems, among others. The gastrointestinal tract also contributes to the regulation of blood pressure [23–26]. Within the kidney, the angiotensin type 1 receptor (AT_1R) is considered one of the dominant regulators of blood pressure homeostasis [27]. However, the D_1−like dopamine receptors (D_1 receptor (D_1R) and D_5 receptor (D_5R)) and angiotensin type 2 receptor (AT_2R) exert important counter regulatory roles when excess salt and volume need to be excreted [28]. There is no evidence that NBCe2 and NBCe1 are regulated directly by anti-natriuretic (AT_1R) or natriuretic renal receptors (D_1R, D_5R, and AT_2R) since adding their agonists in RPTCs were ineffective in changing the expression ofNBCe1 orNBCe2

(unpublished data from our laboratory). We speculate that intracellular sodium may be the primary stimulus in the regulation of NBCe1 and NBCe2.

NBCe2 Localization in the Kidney

NBCe2 protein is minimally expressed in the human and rodent RPT under basal conditions; this may have limited the detection of its protein expression by immunohistochemistry and its functional activity, even though its mRNA was detectable [29••]. Well-characterized antibodies against NBCe2 have been lacking. In addition, the commercially available antibodies were ambiguously labeled due to changes in nomenclature for NBCe2 (aka NBC4) which further hindered progress in this area of research. Newer NBCe2 antibodies have made it recently possible to determine the location of NBCe2 in the human kidney [10•]. We validated a commercial NBCe2 antibody by preadsorption of NBCe2 immunoreactivity with the immunizing peptide. We then used this antibody to study NBCe2 expression in empty vector- and NBCe2-shRNA-treated cells, by western blot and immunofluorescence microscopy of RPTCs and HEK293 cells expressing an epitope-tagged NBCe2 lentiviral expression construct. NBCe2 immunofluorescence was alsofound in the cortical collecting duct [10•], but not in the distal convoluted tubule in fresh and frozen renal tissue sections; the latter finding in agreement with a previous report [30].

NBCe2 Subcellular Location and Translocation

Understanding the subcellular localization of NBCe2 is important in determining whether or not NBCe2 contributes to the transport of bicarbonate from the tubular (luminal) fluid into the RPTC. We studied NBCe2 expression in connecting tubule and cortical collecting duct (identified by L1-CAM) and RPT (identified by CD13 and *Lotus tetragonolobus* agglutinin) in fresh human renal slices [10•]. NBCe2 staining in the renal cortical collecting duct is consistent with earlier reports of its expression in principal cells [30]. We found that an increase in intracellular sodium caused by increasing the NaCl concentration in the incubation medium from 120 to 170 mmol/L or exposure to the ionophore, monensin (1–10 μmol/L), was the stimulus that increased NBCe2 expression and activity, i.e., increase bicarbonate transport across the luminal membrane [10•]. Under basal conditions, in the RPTC, NBCe2 is concentrated in the Golgi bodies with some diffuse staining throughout the cell. Increasing intracellular sodium causes the recruitment of NBCe2 to intracellular punctate structures subjacent to the apical membrane of the RPT. Electron microscopy demonstrated migration of NBCe2 from the sub-apical compartment to the microvilli following the increase in intracellular sodium. Total internal reflection fluorescence microscopy demonstrated vesicle-like structures at the apical membrane in polarized RPTCs. This location is similar to that shown for NHE3 in RPTs of rats after the induction of hypertension where NHE3-mediated sodium transport is still functional, albeit at a lower level, even after its movement in the microvilli or in the inter-microvilliary cleft. [31] We also confirmed apical RPT localization of NBCe2 by western blot studies of apical membranes isolated by two different methods: CD-13 immunoprecipitation and magnesium precipitation [10•].

NBCe1 Versus NBCe2

In the kidney, NBCe1 (particularly NBCe1-A) has been well characterized to provide electrogenic transport of sodium and bicarbonate across the basolateral membrane of the RPT in rodents [1, 2••, 3••, 9, 32] and humans [33]. NBCe1, encoded by *SLC4A4* [1, 2••, 3••, 9], is located in the human RPT [10•, 30, 33], medullary thick ascending limb (mTAL), and collecting duct [30]. NBCe1, by transporting bicarbonate from inside the RPT across the basolateral membrane, promotes H^+ ion secretion into the tubular lumen which can then combine with bicarbonate that was exchanged with chloride via PAT1. The resultant carbonic acid is acted upon by CA IV to continue the process of bicarbonate reabsorption. The renal tubular reabsorption of bicarbonate helps to maintain normal plasma bicarbonate and pH [1, 2••, 3••, 4, 13, 18, 19]. Germline deletion of *SLC4A4* (NBCe1) [33] or *SLC4A5* (NBCe2) [34] in mice causes metabolic acidosis and hypertension in the case of *SLC4A5*. The latter occurs because of an increase in sodium reabsorption in the distal nephron [35].

The electrogenic sodium bicarbonate cotransporter activities of NBCe1 and NBCe2 are considered indistinguishable [2••, 3••]. In humans, the relationship of these two transporters is better understood, in that RPTC NBCe1 appears to be located in the basolateral membrane [36], whereas NBCe2 is located in the apical membrane [10•]. Our immunofluorescence studies were performed in various model systems to ensure the validity of our interpretation of the results. We measured NBCe2 protein in the apical membranes and NBCe1 in the basolateral membranes of RPT of flash-frozen human kidney, primary cultures of RPTCs exfoliated into human urine, primary cultures of RPTCs isolated from surgical-discard fresh human kidneys, and immortalized human RPTCs from cell lines isolated from seven different individuals. We localized NBCe2 expression to the RPT subapical membrane in flash-frozen human kidney tissue, as well as in fresh renal cortical tissue. Higher expression of NBCe2 and lower expression of NBCe1 were found when intracellular sodium was increased (vide supra). We also demonstrated that bicarbonate-dependent pH recovery in RPTCs was due, in part, to NBCe2 at the apical membrane [10•]. These data support our hypothesis that NBCe2 expression is increased and recruited to the RPTC apical membrane microvilli by an increase in intracellular sodium.

Dysregulation of NBCe2 Activity

Essential hypertension is likely caused by genetic variants in key blood pressure-regulating pathways, instigated or exacerbated by environmental factors. The nephron segments responsible for the bulk of sodium retention in human polygenic/essential hypertension are the renal proximal tubule (RPT) and the medullary thick ascending limb of Henle (mTAL) [37–40]. However, renal distal tubular mechanisms also contribute to the increased sodium retention in hypertension [39, 40], especially in monogenic forms of hypertension [39–41]. NBCe2 is located in these nephron segments [10•, 30]. The gene *SLC4A5*, which encodes NBCe2 [2••, 3••, 9, 19], has been significantly associated with high blood pressure and/or salt sensitivity [42••, 43••, 44••, 45••, 46, 47]. The increased activity ofNBCe2 [44••, 45••, 46, 47] at the luminal membrane [43••], and decreased activity of NBCe1, at the basolateral membrane [48], in hypertension do not conflict with the increase in RPT sodium transport in the genetic hypertension [37–40]. The increased sodium reabsorption in the renal proximal

tubule of young SHRs is a consequence of the high activity of the main mechanisms of sodium transport in this nephron segment, NHE3, and Na^+/K^+-ATPase [49–51]; *SLC26A6* activity is also increased in the SHR [15–17]. The increased activity of these exchangers and pump [37–40, 49–53], with the increased activity of NBCe2 in the RPT [43••] and the decreased activity of NBCe1 [48] result in a decrease in overall HCO_3^- reabsorption. Low plasma HCO_3^- and high anion gap are associated with hypertension [54–57]. Mice with germline deletion of *SLC4A5* on SV129/C57 background were reported to be acidotic with elevated blood pressure that was thought to be due to increased distal tubule bicarbonate transport via other bicarbonate sodium transporters, e.g., *SLC26A4* and *SLC4A7* [34, 35]. Increasing bicarbonate consumption in wild-type SV129/C57 mice elevated their blood pressure to the levels seen in *SLC4A5* knockout mice [34]. Mice with germline deletion of *SLC4A5* on mostly C57BL/6 background have normal blood pressure on a normal diet but an acid diet caused hypertension that was due to increased epithelial sodium channel-mediated sodium reabsorption [35, 58].

Human essential hypertension is also caused by increased renal reabsorption of electrolytes, including bicarbonate and sodium [59, 60]. Salt sensitivity has been estimated to be present in 51 % of hypertensive and 26 % of normotensive subjects [60]. Salt sensitivity of blood pressure, even in the absence of hypertension, is similar to hypertension in that they both lead to significant increases in morbidity and mortality due to stroke, blindness, heart attack, and renal failure [61]. We, and others, examined the relationship between SNPs in NBCe2 and salt sensitivity. At the University of Virginia (UVA), we examined the genetic associations with blood pressure in 185 subjects of European ancestry ages 18–70 years and body mass index (BMI) of 18–30 [43••]. In a collaborative study, the genetic associations with blood pressure traits were performed on specimens from the HyperPATH Cohort with subjects with mild hypertension studied from four international centers (Brigham and Women's Hospital, University of Utah Medical Center, Vanderbilt University, and Hospital Broussais (Paris, France)) [43••, 62]. We tested the hypothesis that SNPs in *SLC4A5* are associated with salt sensitivity (≥7-mmHg increase in mean arterial pressure during a randomized transition between high- and low-sodium diets) in 185 whites consuming an isocaloric constant diet starting with either 7 days of low (10 mmol Na^+/day) or 7 days of high sodium (300 mmol Na^+/day) intake and then switching to the other diet. Three variants were associated with salt sensitivity, two in *SLC4A5* ($P<0.001$) and one in GRK4 ($P=0.020$). Of these, two SNPs in *SLC4A5* (rs7571842 and rs10177833) demonstrated highly significant results and large effect sizes, using logistic regression. These two SNPs had P values of 1.0×10^{-4} and 3.1×10^{-4} with odds ratios of 0.221 and 0.221 in unadjusted regression models, respectively, with the G allele at both sites conferring protection. The association of these SNPs with salt sensitivity was replicated in the HyperPATH Cohort at Harvard with a meta-analysis demonstrating significant associations of both SNPs with salt sensitivity (rs7571842 ($P=1.2\times10^{-5}$); rs1017783 ($P=1.1\times10^{-4}$)) [43••]. Our results [43••] are consistent with the association ofrs1017783 and increased blood pressure in African-Americans, Mexican-Americans, Euro-Americans, and Taiwanese [42••, 44••, 45••, 46, 47, 63••, 64]. Another *SLC4A5* SNP (rs 10022637) ($P=2.07\times10^{-6}$) was found to be associated with salt sensitivity in a large cohort of Han Chinese; *SLC4A5* rs1017783 was not genotyped [65].

Some investigators believe that despite the strong association between the *SLC4A5* locus and salt sensitivity in the absence of hypertension, NBCe2 may not contribute to the phenotypes of salt sensitivity or hypertension since it has only a minor role to play (if at all) in renal sodium and bicarbonate transport, under conditions of "normal" sodium intake. However, there are two compelling reasons to support the notion that *SLC4A5* contributes, at least in part, to the sodium retention in hypertension. Increased sodium transport is involved in genetic hypertension [37–41, 43••, 59, 66••]. Although *SLC4A5* may not be a major contributor to sodium balance, relative to other sodium transporters, it may be an important player under conditions of high salt intake. Thus, even a decrease of only 0.1 % in sodium excretion over a period of time can lead to hypertension. For illustrative purposes, an average individual excretes 1 % of filtered sodium (~250 mmol/day). A reduction in sodium excretion of only 0.1 % leads to sodium retention of 25 mmol/day or 125 mmol in 5 days provided that there is no corresponding natriuresis. A short-term (5 days) change in sodium diet in normotensive and hypertensive human subjects from low to high and vice versa can also lead to a directional change in plasma sodium of about 3 mmol [67, 68]. One-month reduction of sodium intake from ~ 170 to 100 mmol/day has also been reported to be associated with a 0.4-mmol decrease in plasma sodium [68]. Fortunately, extrarenal regulatory mechanisms [69] participate in the maintenance of sodium homeostasis and pressure-natriuresis mechanism, in addition to the increased production and action of natriuretic hormones/factors and decreased production and action of anti-natriuretic hormones/factors help to eliminate most of the ingested sodium [25]. However, we have demonstrated that *SLC4A5* mRNA, as well as its protein prod-uctNBCe2, is increased by sodium intake [10•]. Individuals with polymorphisms in *SLC4A5* would have a further increase in NBCe2 expression and therefore an increase in sodium and bicarbonate cotransport in their RPT. Thus, we have demonstrated a functional link between *SLC4A5* polymorphisms and renal sodium transport that could make contributions to salt sensitivity [11••]. As previously mentioned, studies from various investigators including ourselves have demonstrated a genetic link between NBCe2 polymorphisms and hypertension [11••, 42••, 43••, 44••, 45••, 46, 47, 63••, 64, 65]. However, these polymorphisms are not in the coding region for *SLC4A5* (NBCe2). We have further clarified the mechanism by which *SLC4A5* rs10177833 may lead to an increase in expression and activity of NBCe2. HNF4A is a transcriptional regulator present in the RPT that plays a key regulatory role in a large number of pathways [70–72]. We found that *SLC4A5* rs10177833 causes an increase in HNF4A binding to the *SLA4A5* gene resulting in an increase in NBCe2 mRNA, NBCe2 protein expression, and increased NBCe2-mediated bicarbonate and sodium transport under conditions of elevated intracellular sodium [11••] (Fig. 3).

Summary and Conclusion

Ion transporters and exchangers mediate the balance of influx and outflux of ions through the cell membrane in all tissues. The normal balance of pH and sodium is critical to the maintenance of life in the short-term and health in the long-term. Since pH is so critical to immediate cell health, nature seems to have endowed cells throughout the body with a rich variety of bicarbonate cotransporters and hydrogen pump and channel. We have studied NBCe2 and NBCe1 regulation of renal bicarbonate transport and how they work in concert

to maintain sodium balance. A consequence of a hyperactive NBCe2, as a result of SNPs in the *SLC4A5* gene, may be that in a subset of salt-sensitive individuals, NBCe2 polymorphisms, e.g., *SLC4A5* rs1017783, lead to an increase in renal sodium bicarbonate reabsorption, which apparently is only partially compensated by a partial reduction in NBCe1 and increase in PAT1 activities. We speculate that blocking the increased synthesis and/or activity of NBCe2 may be a novel approach to mitigate the increased renal sodium reabsorption in some salt-sensitive individuals.

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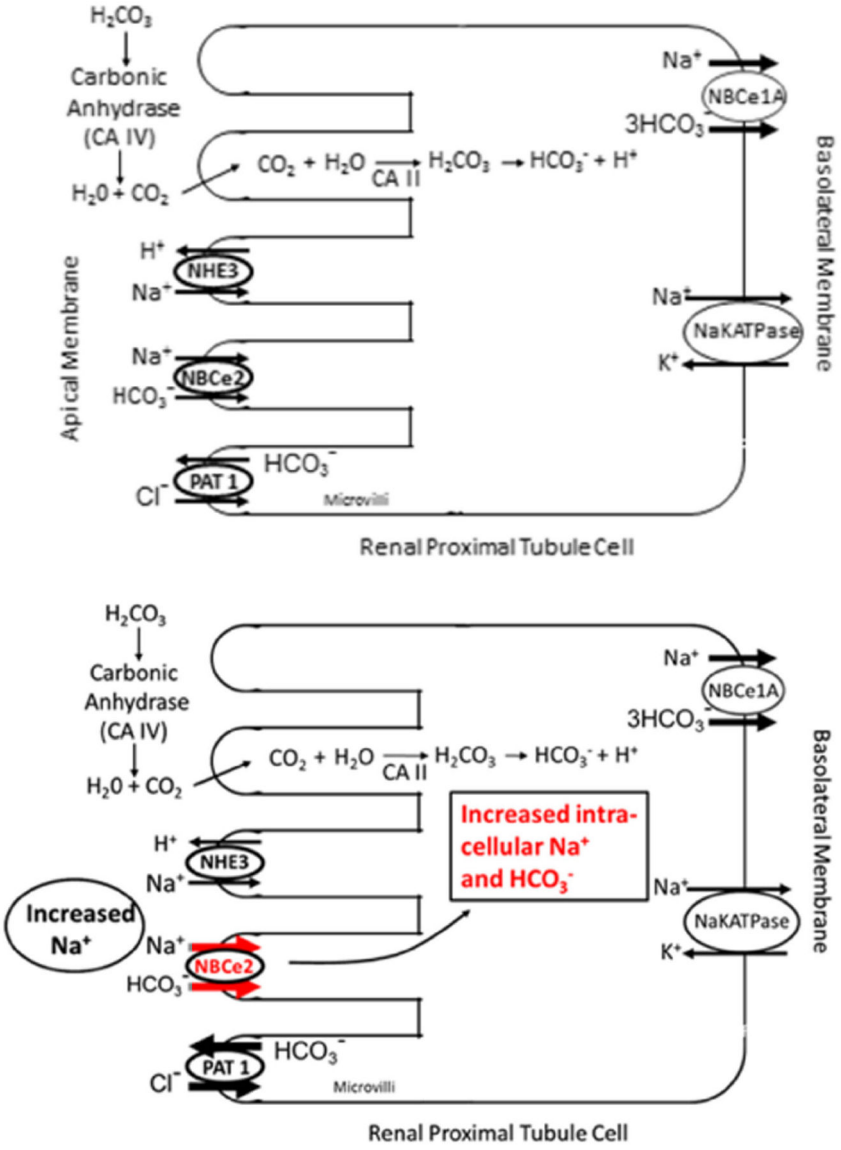

Fig. 1.

Model of a human renal proximal tubule cell (RPTC). *Top figure*—under basal conditions the principal avenue for bicarbonate (HCO_3^-) uptake from the lumen into cytosol is via the breakdown of carbonic acid (H_2CO_3) to water and carbon dioxide (CO_2) by luminal carbonic anhydrase type IV (CA IV), followed by the diffusion of CO_2 into the cytosol via the membranes of the microvilli. CA II then converts water and CO_2 back into carbonic acid where it spontaneously breaks down into bicarbonate and hydrogen ion, with the latter secreted into the lumen via the sodium hydrogen exchanger type 3 (NHE3). Two to three bicarbonate ions can then be reabsorbed into the blood stream along with each sodium ion via NBCe1-A at the basolateral membrane. *Bottom figure*—increasing intracellular sodium concentration caused by high extracellular sodium concentration or monensin increases NBCe2 mRNA and protein expressions and activity that become persistent in the presence of single nucleotide polymorphisms of NBCe2, while only marginally attenuating the protein expression and activity of NBCe1-A in RPTCs. This results in a net increase in

sodium transport into the basolateral space. PAT1 activity increases because of an increase in intracellular bicarbonate. NHE3 activity also increases because the increase in luminal NBCe2 activity increases intracellular H^+ following the conversion of intracellularly transported HCO_3^- to H_2CO_3 and its dissociation to H^+ and HCO_3^- resulting in a further increase in sodium reabsorption

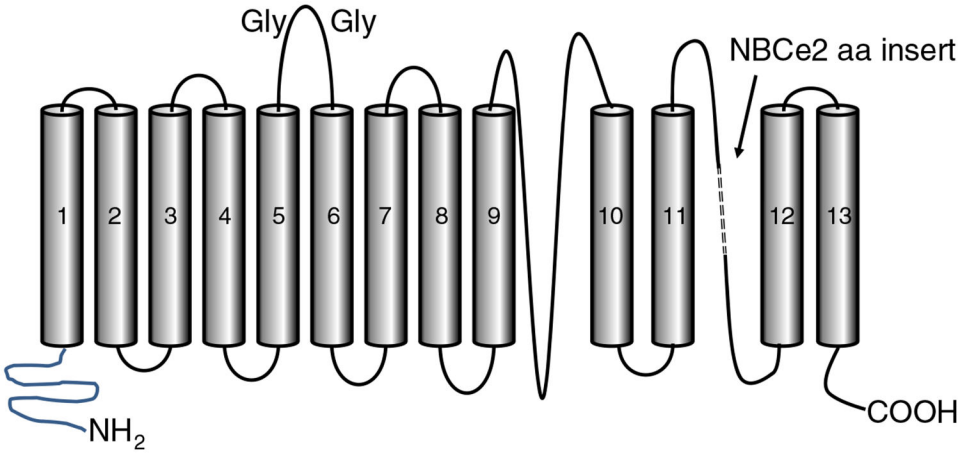

Fig. 2.

Generic model of NBCe2 showing its three domain structure, carboxy and amino terminal tails, and glycosylation *(Gly)* sites along the extracellular domain between transmembrane domains 5 and 6. NBCe2 exists in two isoforms (NBCe2-A and NBCe2-C). NBCe2-A differs from NBCe2-C mainly by the presence of an 18 amino acid *(aa)* insert in the connector between transmembrane domains 11 and 12 (adapted from reference [9])

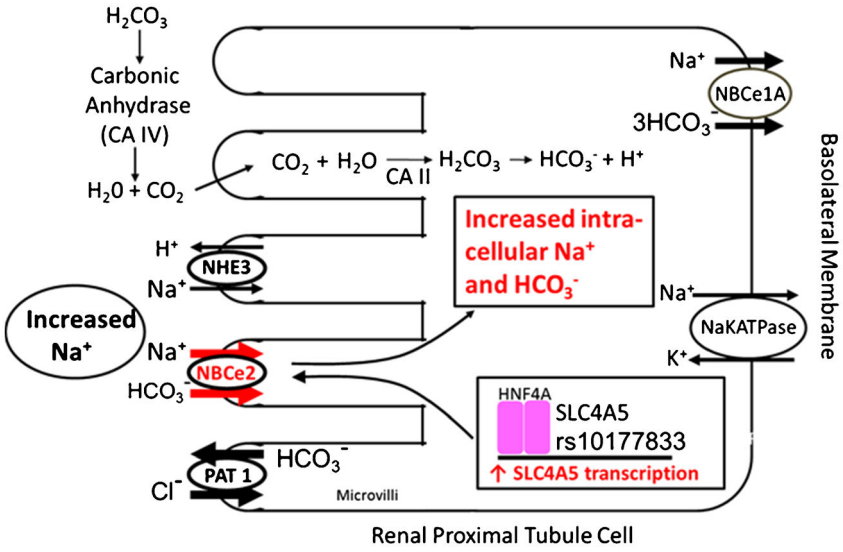

Fig. 3.

The mechanism causing the increase in sodium transport in RPTCs carrying the *SLC4A5* rs10177833 is shown on the same model depicted in Fig. 1. These RPTCs have an increase in HNF4A binding to the *SLC4A5* gene resulting in an increase in NBCe2 mRNA, as well as an increase in NBCe2 protein and activity under high salt conditions. The resulting increase in intracellular sodium and bicarbonate is associated with an increase in PAT1 activity and slight reduction in NBCe1 activity