Molecular Cloning of a New anion exchanger Selectively Expressed in Rat Kidney.

Kenichi Ishibashi
Department of Pharmacology, Jichi Medical School.

Summary

A family of HCO3 transporter (solute carrier family 4A, SLC4A) have been identified that include three anion exchangers (AE) 1-3 (SLC4A1-3) and four Na/HCO3 cotransporters (NBC) 1-4 (SLC4A6-9). In the kidney, NBC1 functions as HCO3 absorption at proximal tubule and AE1-3 function as HCO3 absorption at collecting duct. However, the molecular identity of HCO3 transporter at apical membrane of beta intercalated cells to secrete HCO3 at cortical collecting duct (CCD) is currently unknown.

Here, we report the cloning of a new gene of NBC subfamily (NBC5) from rat kidney by homology cloning. EST-data base was screened with NBC sequences. Three new NBC sequences were identified. They are tentatively named NBC4, NBC5,and NBC6. NBC4 was ~60% identical with NBC2 and widely expressed and later deposited in GeneBank as KIAA0739 by Kazusa DNA research EST project. NBC5 had lowest identity with NBC1 in three and was selectively expressed in the kidney. NBC6 was ~70% identity and predominantly expressed in the brain. To obtain the full length of NBC6, rat and human brain cDNA libraries were screened with NBC6 EST clone. However, the full length was not obtained.

On the other hand, the full length of NBC5 was obtained by screening a rat kidney cDNA library. NBC5 is composed of 954 amino acids with 44% identity with NBC1 and 33% identity with AE1. NBC5 has putative 12 transmembrane domains.

In Northern blot, NBC5 was selectively expressed in kidney (3.3kb) and absent in other tissues (heart, brain, spleen, lung, liver, muscle, testis). In RT-PCR of nephron segments, NBC5 was expressed at Cortical Collecting Duct (CCD) and virtually absent in glomeruli, proximal tubules, thick ascending limb of Henle and inner medullary collecting ducts.

Using carboxy-terminus 15 amino acid peptide sequence of NBC5, a polyclonal antibody was produced in rabbit. Immunohistochemistry of rat kidney revealed that NBC5 was expressed at the apical membrane of some restricted cells in CCD.

NBC5 was subcloned in expression vector under the control of CMV promotor. In the transfection of NBC5 in human embryonic kidney (HEK) cells, Cl removal alkalinized the cell but Na removal did not change cell pH. In mock transfected cells did not change cell pH to the either procedure. This pH change was not inhibited by removal of ambient HCO3 nor 0.1 mM SITS. The result indicated the the function of NBC5 is Cl/OH exchange and it is Na independent.

To gain the insight into the physiological roles of NBC5, three kind of rat models were tested for the expression of NBC5. Acidosis and alkalosis models were produced by adding 0.1 M NH4Cl or NaHCO3 to the drinking water for 7 days, respectively. The dehydrated rats were produced by depriving water for 2 days. In each animal model, the expression of NBC5 mRNA was increased by 1.5-3 times higher than control. The result indicated some role of NBC5 in acid-base regulation and volume regulation. As NBC5 was stimulated in both acidosis and alkalosis, the functional regulation of its activity is more likely than the number of transporters.

In summary, we have identified a new anion exchanger of CCD. NBC5 may be a bona fide HCO3 transporter at apical membrane of beta intercalated cells. Its lower homology with other NBCs may indicate that NBC5 may comprise the third subfamily in HCO3 transporter superfamily (SLC4A). Further analysis of other members of SLC4A will reveal the new mechanism of cellular pH regulation in our body.