Life Sciences 93 (2013) 435–440

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Identification of SLC26A transporters involved in the Cl^-/HCO_3^- exchange in proximal tubular cells from WKY and SHR

Sónia Simão, Pedro Gomes, Maria João Pinho, Patrício Soares-da-Silva *

Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

ARTICLE INFO

Article history:
Received 27 November 2012
Accepted 29 July 2013

Keywords:
slc26a family
Cl^-/HCO_3^- exchanger
Kidney
Proximal tubular epithelial cells
SHR

ABSTRACT

Aims: slc26a proteins are responsible for a large number of functions either in normal physiology or in human disease. We have previously reported that proximal tubular epithelial (PTE) cells immortalized from spontaneously hypertensive rats (SHRs) were endowed with increased Cl^-/HCO_3^- exchanger activity and slc26a6 protein expression compared with PTE cells immortalized from normotensive Wistar Kyoto (WKY) rats. The aim of the present study was to identify slc26a members responsible for the Cl^-/HCO_3^- exchange in WKY and SHR PTE cells.
Main methods: Cl^-/HCO_3^- exchanger activity was assessed as the initial rate of pH_i recovery after removal of HCO_3^- or after removal of Cl^-. The presence of slc26a genes was evaluated by means of reverse transcriptase-PCR (RT-PCR) in WKY and SHR PTE cell lines and in the kidney of WKY and SHR. Transcript abundance was measured by quantitative real-time polymerase chain reaction (PCR).
Key findings: We detected slc26a4, slc26a6, slc26a7 and slc26a9 transcripts in the rat kidney of WKY and SHR. In WKY and SHR PTE cell lines we detected slc26a4, slc26a6 and slc26a9 transcripts, which were, respectively, 12-, 4- and 15-fold upregulated in SHR cells. Gene silencing with small interfering RNAs (siRNAs) targeting slc26a4, slc26a6 and slc26a9 reduced Cl^-/HCO_3^- exchanger activity in both cell lines.
Significance: These results indicate that Cl^-/HCO_3^- exchanger activity is mediated by, at least in part, slc26a4, slc26a6 and slc26a9 in cultured WKY and SHR cells. The overexpression of these slc26a members in SHR cells may correspond to an adaptive process to cope with the sustained increase in proximal tubular sodium reabsorption.

© 2013 Elsevier Inc. All rights reserved.

Introduction

Essential hypertension is a complex disease with several underlying causes. One reason for the development and maintenance of this form of hypertension is the defective capacity of the kidneys to excrete water and salt in appropriate relation to the intake. Regulation of renal NaCl reabsorption is critical in the control of extracellular volume and blood pressure (Zeng et al., 2004). The majority of the filtered NaCl is reabsorbed by the proximal tubule (PT) cells (Aronson and Giebisch, 1997) and the main transporters responsible for this reabsorption are the apical Na^+/H^+ exchanger subtype 3 (NHE3) and the Cl^-/HCO_3^- exchanger, which are essential for intracellular pH and cell volume regulation (Petrovic et al., 2003).

The solute carrier 26 (SLC26) proteins are a family of anion exchangers responsible for a large number of functions in normal physiology and in human diseases (Mount and Romero, 2004; Ohana et al., 2009; Soleimani and Xu, 2006). Several rare recessive human pathologies, in particular diastrophic dysplasia, congenital chloride diarrhea and Pendred syndrome, are associated with mutations in SLC26A2, SLC26A3 and SLC26A4, respectively. The SLC26 family comprises 10 different genes responsible for the transport/exchange of a great variety of monovalent and divalent ions, including chloride, iodide, sulphate and bicarbonate (Mount and Romero, 2004; Ohana et al., 2009; Soleimani and Xu, 2006). Some SLC26 members show very specific tissue distribution while others are widely expressed. SLC26 family members are expressed in many epithelia and play a central role in anion secretion and absorption. Our group recently demonstrated the presence of an apical Cl^-/HCO_3^- exchanger, the slc26a6 protein, in proximal tubular epithelial (PTE) cell lines from Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) (Simão et al., 2008). An increased activity of the Cl^-/HCO_3^- exchanger in PTE cells from SHR when compared with WKY cells was also reported (Simão et al., 2008).

Recent studies in slc26a6-null mice suggest that this protein mediates only a part of apical proximal tubule Cl^- transport (Wang et al., 2005), indicating that other transporters may also be involved in this process. The purpose of this study was to identify the presence of slc26a members in the kidney and in immortalized PTE cells of WKY and SHR and to determine their relative contribution to the Cl^-/HCO_3^- exchange in both cell lines.

Abbreviations: BCECF-AM, acetoxymethyl ester of 2′, 7′-bis (carboxyethyl)-5(6)-carboxyfluorescein; EDTA, ethylenediamine tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NHE, Na^+/H^+ exchanger; PCR, polymerase chain reaction; PT, proximal tubule; PTE, proximal tubular epithelial; RT-PCR, reverse transcriptase-PCR; siRNA, small interfering RNA; SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto rat.

* Corresponding author. Tel.: +351 22 5513642; fax: +351 22 5513643.
E-mail address: pss@med.up.pt (P. Soares-da-Silva).

0024-3205/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2013.07.026

Materials and methods

Cell culture

Immortalized renal PTE cells were obtained from primary cultures from S1 segments of proximal tubules of 4- to 8-week-old WKY and SHR (Woost et al., 1996). These cell lines formed polarized monolayers with apical microvilli, tight junctional complexes and convolutions of the basolateral plasma membrane. WKY and SHR cell lines express a proximal tubular phenotype and are morphologically and functionally similar to primary cultures (Woost et al., 1996). Cells were maintained in a humidified atmosphere of 5% CO_2–95% air at 37 °C. WKY and SHR PTE cells were grown in Dulbecco's modified Eagle's medium nutrient mixture F-12 Ham (Sigma Chemical Company, St. Louis, MO) supplemented with 100 U/ml penicillin G (Sigma), 0.25 μg/ml amphotericin B (Sigma), 100 μg/ml streptomycin (Sigma), 4 μg/ml dexamethasone (Sigma), 5 μg/ml transferrin (Sigma), 5 μg/ml insulin (Sigma), 5 ng/ml selenium (Sigma), 10 ng/ml epidermal growth factor (Sigma), 5% fetal bovine serum (Sigma) and 25 mM N-2-hydroxyethylpiperazine-N′-2-ethanosulfonic acid (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); Sigma). For subculturing, the cells were dissociated with 0.10% trypsin–ethylenediamine tetraacetic acid (EDTA) (Sigma), split 1:8 and cultured in Costar plates with 21-cm^2 growth areas (Costar, Badhoevedorp, The Netherlands). For intracellular pH (pH_i) measurement experiments, cells were grown in 10-mm diameter collagen-coated glass coverslips or in 96-well plates (Costar) and for polymerase chain reaction (PCR) cells were grown in 6-well plates (Costar). The cell medium was changed every 2 days, and the cells reached confluence after 3–5 days of incubation. For approximately 2 h prior to each experiment, the cells were maintained in fetal bovine serum-free medium. Experiments were performed 4 days after the initial seeding; each cm^2 contained about 50 μg of cell protein.

pH_i measurements

In pH_i measurement experiments, WKY and SHR PTE cells were grown in 10-mm diameter collagen-coated glass coverslips or in 96-well plates. pH_i was measured as previously described (Pedrosa et al., 2004b). Briefly, 4 days after seeding glass coverslips were incubated with 10 μM of acetoxymethyl ester of 2′, 7′-bis (carboxyethyl)-5(6)-carboxyfluorescein (BCECF-AM) at 37 °C for 30 min. Coverslips were then washed with pre-warmed modified Krebs buffer before initiation of the fluorescence recordings. The Krebs medium had the following composition (in mM): 115 NaCl, 25 $NaHCO_3$, 5.4 KCl, 2.8 $CaCl_2$, 1.2 $MgSO_4$, 0.3 NaH_2PO_4, 0.3 KH_2PO_4, 10 HEPES, and 5 glucose, at pH 7.4 (adjusted with Tris base). Coverslips were mounted diagonally in an acrylic fluorometric cuvette that was inserted in a PerkinElmer cuvette holder (model LS 50) and subsequently placed in the sample compartment of a FluoroMax-2 spectrofluorometer (Jobin Yvon-SPEX, Edison, NJ). The cuvette volume of 3.0 ml was constantly stirred and perfused at 5.0 ml/min with modified Krebs buffer pre-warmed to 37 °C. Under these conditions, the cuvette medium was replaced within 150 s. After a stabilization period of 5 min, fluorescence was measured every 5 s alternating between 440- and 490-nm excitation (1-nm slit size) and 525-nm emission (3-nm slit size). In the experiments performed in WKY and SHR cells cultured in 96-well plates, pHi measurements were performed after the cells were loaded with 10 μM BCECF-AM at 37 °C for 30 min. Cells were placed in the sample compartment of a dual-scanning microplate spectrofluorometer (Spectramax Gemini XS, Molecular Devices, Sunnyvale, CA), and fluorescence was measured every 17 s alternating between 440- and 490-nm excitation and 535-nm emission with a cutoff filter of 530 nm. The ratio of intracellular BCECF fluorescence at 490 nm and 440 nm was converted to pH_i by comparison with values from an intracellular calibration curve using nigericin 10 μM in a high-K^+ solution (in mM:15 NaCl, 130 KCl, 0.3 KH_2PO_4, 0.3 NaH_2PO_4, 5 glucose, 1.2 $MgSO_4$, 2.8 $CaCl_2$ and 10 HEPES) with pH ranging from 6.6 to 7.8.

Cl^-/HCO_3^- exchanger activity

Cl^-/HCO_3^- exchanger activity can be assessed by different approaches, i.e., Cl^-/HCO_3^- exchanger activity can be defined as the initial rate of pH_i recovery after removal of HCO_3^- or after addition of Cl^-. The Na^+-independent HCO_3^- transport system activity was assayed as the initial rate of pH_i recovery after an alkaline load (CO_2/HCO_3 removal), in the absence of Na^+, as previously described (Pedrosa et al., 2004b). Briefly, cells were loaded in serum-free medium with 10 μM BCECF-AM, for 30 min at 37 °C in 5% CO_2–95% air atmosphere. The cells were washed free of dye and loaded with Krebs–Henseleit solution (25 mM $NaHCO_3$). Then, extracellular solution was replaced by a Krebs–Henseleit $NaHCO_3$-free solution. In the $NaHCO_3$-free bathing solution, $NaHCO_3$ was replaced by an equimolar concentration of choline. Cells were placed in the sample compartment of a FluoroMax-2 spectrofluorometer or in a dual-scanning microplate Spectramax Gemini spectrofluorometer and fluorescence was monitored. Additionally, the Cl^--dependent transport system activity was assayed as the initial rate of intracellular alkalinization when PTE cells were incubated alternately with Ringer's buffer (in mM: 5 glucose, 5 potassium gluconate, 1 $CaCO_3$, 1 $MgSO_4$, 2.5 NaH_2PO_4, 25 $NaHCO_3$, 10 Hepes, pH 7.4) containing either 140 mM NaCl or 140 mM sodium gluconate. Cells were placed in the sample compartment of a FluoroMax-2 spectrofluorometer and fluorescence was monitored as described above.

RNA extraction

Total RNA was isolated from kidney cortexes of 12-week-old WKY and SHR and from WKY and SHR PTE cell monolayers using TRIZOL (Invitrogen) according to manufacturer's instructions. The RNA obtained was dissolved in diethylpyrocarbonate (DEPC)-treated water and quantified by spectrophotometry at 260 nm. The total RNA extracted was treated with DNase (Ambion, USA), to eliminate potential genomic DNA contamination.

Reverse transcriptase-PCR (RT-PCR)

Approximately 250 ng of total RNA was reverse transcribed to cDNA with SuperScript III First Strand Synthesis SuperMix for RT-PCR (Invitrogen) using 50 ng/μl random hexamers as primers at 50 °C, according to the manufacturer's instructions. The cDNA was amplified by PCR using one set of rat specific primers for slc26a4 (forward: 5′-GTG GGG TCC GTT GTT C-3′ and reverse: 5′-CCG TTG TAG TTT TTG GTT GAG-3′), slc26a6 (forward: 5′-CGG GAG GCA ACA CGC AGA T-3′ and reverse: 5′-GGT GGC TGA GGA ACG GAA GAC-3′), slc26a7 (forward: 5′-ATC ATT GCT GCC TCA TTT GCT-3′ and reverse: 5′-TTT GCC CCC GTG CTG T-3′) and slc26a9 (forward: 5′-GCA AAA ACC TCC CCC ACA CCA-3′ and reverse: 5′-GAC TTCC CTC CAG CCC CAT CC-3′). PCR was performed with Platinum TaqPCRx DNA Polymerase (Invitrogene) with 1× enhancer. Amplification conditions were as follows: hot start of 2 min at 95 °C; 30 cycles of denaturing (95 °C for 30 s), annealing (54 °C for 30 s) and extension (68 °C for 1 min); and a final extension of 7 min at 68 °C. The PCR products were separated by electrophoresis in a 1.5% agarose gel and visualized under UV light in the presence of ethidium bromide. The obtained PCR products were purified and sequenced in both directions, by GATC Biotech AG (Konstanz, Germany), using specific primers described above. Nucleotide homology searching was performed against nonredundant and dbEST using basic local alignment tool (BLAST) via online connection to the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA). Multiple nucleotide sequence comparisons were done with CLUSTALW

Download English Version:

https://daneshyari.com/en/article/2551531

Download Persian Version:

https://daneshyari.com/article/2551531

Daneshyari.com