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Apical and basolateral 4F2hc and the amino acid exchange of L-DOPA in renal LLC-PK_1 cells

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Summary. The present study aimed to examine the presence and define the role of 4F2hc, a glycoprotein associated with the LAT2 amino acid transporter, in L-DOPA handling by LLC-PK_1 cells. For this purpose we have measured the activity of the apical and basolateral inward and outward transport of [^{14}C] L-DOPA in cell monolayers and examined the influence of 4F2hc antisense oligonucleotides on [^{14}C] L-DOPA handling. The basal-to-apical transepithelial flux of [^{14}C] L-DOPA progressively increased with incubation time and was similar to the apical-to-basal transepithelial flux. The spontaneous and the L-DOPA-stimulated apical fractional outflow of [^{14}C] L-DOPA were identical to that through the basal cell side. The L-DOPA-induced fractional outflow of [^{14}C] L-DOPA through the apical or basal cell side was accompanied by marked decreases in intracellular levels of [^{14}C] L-DOPA. In cells treated with an antisense oligonucleotide complementary to 4F2hc mRNA for 72 h, [^{14}C] L-DOPA inward transport and 4F2hc expression were markedly reduced. Treatment with the 4F2hc antisense oligonucleotide markedly decreased the spontaneous fractional outflow of [^{14}C] L-DOPA through the apical or the basal cell side. It is likely that the Na^+-independent and pH-sensitive uptake of L-DOPA include the hetero amino acid exchanger LAT2/4F2hc, which facilitates the *trans*-stimulation of L-DOPA and its outward transfer at both the apical and basal cell sides.

Keywords: LAT2 – 4F2hc – L-DOPA – LLC-PK_1 cells – Amino acid exchanger

Introduction

Recent studies from our laboratory have shown that LLC-PK_1 cells transport quite efficiently [^{14}C]-L-DOPA and [^{14}C]-L-leucine through the apical cell side and several findings demonstrate that this uptake process is a facilitated mechanism (Soares-da-Silva and Serrao, 2004). Though most of [^{14}C]-L-DOPA was entering the cells in a Na^+-independent manner, a minor component of [^{14}C]-L-DOPA uptake (~25%) was found to require extracellular Na^+, which contrasts with the Na^+-independent L-leucine apical transfer. This is in line with previous observations showing that L-DOPA uptake in human and rat kidney slices is a Na^+-dependent and ouabain-sensitive process (Soares-da-Silva and Fernandes, 1992; Soares-da-Silva et al., 1993a, b). Apart from this, [^{14}C]-L-DOPA and [^{14}C]-L-leucine uptake in LLC-PK_1 cells were both sensitive to inhibition by BCH, but not to MeAIB, and sensitive to inhibition by neutral, but not acidic and basic amino acids. In addition, [^{14}C]-L-DOPA and [^{14}C]-L-leucine uptake in LLC-PK_1 cells shows *trans*-stimulation by unlabelled and L-DOPA and L-leucine. Taken together, these findings agree with the view that [^{14}C]-L-DOPA may be transported by systems B^0 (Na^+-dependent) and L (Na^+-independent), whereas [^{14}C]-L-leucine may be transported through system L only. However, [^{14}C]-L-DOPA and [^{14}C]-L-leucine appear to be handled through system L in a very different manner, as [^{14}C]-L-DOPA was transported by a low affinity and pH-sensitive process whereas [^{14}C]-L-leucine was transported by a high affinity and pH-insensitive transport, which may correspond to LAT2 and LAT1 transporters, respectively (Soares-da-Silva and Serrao, 2004). LAT1 and LAT2 are two isoforms of system L, for leucine preferring, that convey the Na^+-independent transport of large branched and aromatic neutral amino acids. LAT1 is expressed in non-epithelial cells such as brain, spleen, thymus, testis, skin, liver, placenta, skeletal muscle, and stomach (Kanai and Endou, 2001; Prasad et al., 1999) and has a high affinity for the amino acid substrates. The second isoform of system L, LAT2, is highly expressed in polarized epithelia (Segawa et al., 1999), suggesting an important role in transepithelial amino acid transport, but has an affinity for the amino acid substrates lower than that for LAT1 (Segawa et al.,

1999; Wagner et al., 2001). Another difference between LAT1 and LAT2 is concerned with their sensitivity to extracellular pH for amino acid uptake (Prasad et al., 1999).

Recently, our group has demonstrated for the first time that overexpression of LAT2 in the kidney of the spontaneous hypertensive rat (SHR) is organ specific and precedes the onset of hypertension, this being accompanied by an enhanced ability to take up L-DOPA (Pinho et al., 2003). It was, therefore, suggested that overexpression of renal LAT2 might constitute the basis for the enhanced renal production of dopamine in the SHR in an attempt to overcome the deficient dopamine-mediated natriuresis generally observed in this genetic model of hypertension (Pinho et al., 2003). This adaptive mechanism may be limited to renal tissues, because at the intestinal level where defective transduction of the D_1 receptor signal also occurs (Lucas-Teixeira et al., 2000), it is accompanied by increases in neither dopamine tissue levels (Lucas-Teixeira et al., 2000) nor intestinal LAT2 expression (Pinho et al., 2003). In the SHR and in some forms of human hypertension, despite a normal dopamine receptor density, there is defective transduction of the D_1 receptor signal in renal proximal tubules (Hussain and Lokhandwala, 1998; Jose et al., 2002a, b). This coupling defect is genetic (precedes the onset of hypertension and co-segregates with the hypertensive phenotype), is receptor specific (not shared by other humoral agents), and is organ and nephron-segment selective (occurs in proximal tubules but not in cortical collecting ducts or the brain striatum). A consequence of the defective dopamine receptor/adenylyl cyclase coupling in the SHR and hypertensive subjects is a decreased ability to promote natriuresis (Hussain and Lokhandwala, 1998; Jose et al., 2002a, b). However, dopamine production and excretion in SHR is normal or even increased when compared with that in WKY (Racz et al., 1985; Sanada et al., 1995; Yoshimura et al., 1990). This would suggest that in SHR the increased ability to form dopamine at the kidney level might correspond to an attempt to overcome the deficient dopamine-mediated natriuresis (Jose et al., 2002b), as has been reported in aged Fischer 344 rats (Vieira-Coelho et al., 1999).

One aspect that is still debatable concerns the localization of LAT2 on the cell membranes of transporting epithelia. According to Rossier et al (Rossier et al., 1999), LAT2 is expected to play a role in transepithelial amino acid transport in particular in the basolateral extrusion step, where it co-localizes with 4F2hc at the basolateral membrane of kidney proximal tubules and small intestine epithelia. To be functional LAT2 has been shown to require the association with the single transmembrane domain 4F2hc protein through an intermolecular disulfide bond (Palacin and Kanai, 2004; Verrey, 2003). As mentioned below, LLC-PK_1 cells transport [^{14}C]-L-DOPA through the apical cell side and several findings suggest that this uptake process is promoted through LAT2; LAT2 siRNA reduced by 85% [^{14}C] L-DOPA accumulation, a time- and concentration-dependent effect (Soares-da-Silva et al., 2004). The present work was aimed to evaluate the presence and define the role of 4F2hc, a glycoprotein associated with the LAT2, in L-DOPA handling by LLC-PK_1 cells. For this purpose we have measured the activity of the apical and basolateral inward and outward transport of [^{14}C] L-DOPA in cell monolayers and examined the influence of 4F2hc antisense oligonucleotides on [^{14}C] L-DOPA handling. It is reported that the Na^+-independent uptake of L-DOPA may include the hetero amino acid exchanger LAT2/4F2hc, which facilitates the *trans*-stimulation of L-DOPA outward transfer. The LAT2/4F2hc hetero amino acid exchanger appears to be of equal importance at the apical and basolateral cell membranes.

Methods

Cell culture

LLC-PK_1 cells (ATCC CRL 1392; passages 198–206) were maintained in a humidified atmosphere of 5% CO_2-95% air at 37°C and grown in Medium 199 (Sigma Chemical Company, St. Louis, Mo, USA) supplemented with 100 U ml^{-1} penicillin G, 0.25 μg ml^{-1} amphotericin B, 100 μg ml^{-1} streptomycin (Sigma), 3% fetal bovine serum (Sigma) and 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanosulfonic acid (HEPES; Sigma). For subculturing, the cells were dissociated with 0.05% trypsin-EDTA, split 1:4 and subcultured in Costar flasks with 75- or 162-cm^2 growth areas (Costar, Badhoevedorp, The Netherlands). For uptake studies, the cells were seeded in collagen treated 24 well plastic culture clusters (internal diameter 16 mm, Costar) at a density of 40,000 cells per well or onto collagen treated 0.2 μm polycarbonate filter supports (internal diameter 12 mm Transwell, Costar) at a density 13,000 cells per well (2.0×10^4 cells cm^2). The cell medium was changed every 2 days, and the cells reached confluence after 3–5 days of incubation. For 24 hours prior to each experiment, the cell medium was free of foetal bovine serum. Experiments were generally performed 2–3 days after cells reached confluence and 6–8 days after the initial seeding and each cm^2 contained about 80 μg of cell protein.

Transport of [^{14}C] L-DOPA

In experiments performed in the presence of different concentrations of sodium, sodium chloride was replaced by an equimolar concentration of choline chloride.

On the day of the experiment, the growth medium was aspirated and the cell monolayers were preincubated for 15 min in Hanks' medium at 37°C. The Hanks' medium had the following composition (mM): NaCl 137, KCl 5, $MgSO_4$ 0.8, Na_2HPO_4 0.33, KH_2PO_4 0.44, $CaCl_2$ 0.25, $MgCl_2$ 1.0, Tris HCl 0.15 and sodium butyrate 1.0, pH = 7.4. The incubation medium also contained benserazide (30 μM) and tolcapone (1 μM) in order to inhibit the enzymes aromatic L-amino acid decarboxylase and catechol-*O*-methyltransferase, respectively. In experiments designed to study the

transepithelial flux of [^{14}C] L-DOPA, cells were incubated with 2.5 μM [^{14}C] L-DOPA applied from the basal cell border or the apical cell border and uptake (accumulation in the cell monolayer) and flux (transfer to opposite chamber) were measured over a 24 min period. An aliquot of the medium (25 and 100 μl from the apical or basal compartment, respectively) was collected at the indicated time points, and the aliquot was replaced with an equal volume of Hanks' medium. The data at 3, 6, 9, 12 and 24 min represent cumulative values. The monolayers were continuously agitated during transport measurement. In some experiments, unlabelled L-DOPA (1 mM) was applied from the chamber opposite to that containing [^{14}C] L-DOPA. [^{3}H]-Sorbitol (0.2 μM) was used to estimate paracellular fluxes and extracellular trapping of L-DOPA during L-DOPA uptake studies. Paracellular fluxes were estimated dividing concentration of [^{3}H]-sorbitol in the apical chamber by the concentration of [^{3}H]-sorbitol in the basal chamber. Extracellular trapping was calculated dividing the amount of [^{3}H]-sorbitol in the cell monolayer by the amount of [^{3}H]-sorbitol in the basal chamber. At the end of the transport experiment, the medium was immediately aspirated and the filter was washed three times with ice-cold Hanks' medium. Subsequently, the cells were solubilized by 0.1% v/v Triton X-100 (dissolved in 5 mM Tris.HCl, pH 7.4) and radioactivity was measured by liquid scintillation counting.

Fractional outflow of intracellular [^{14}C] L-DOPA was evaluated in cells loaded with 0.25 μM [^{14}C] L-DOPA for 6 min and then the corresponding efflux monitored over 24 min, in the absence and the presence of different amino acids. During preincubation and incubation, the cells were continuously shaken and maintained at 37°C. Fractional outflow was calculated using the expression

$$[^{14}\text{C}]\text{L-DOPA}^{\text{fluid}}/([^{14}\text{C}]\text{L-DOPA}^{\text{fluid}} + [^{14}\text{C}]\text{L-DOPA}^{\text{cell}})$$

where [^{14}C] L-DOPA$^{\text{fluid}}$ indicates the amount of [^{14}C] L-DOPA (in pmol/mg protein) which reached the fluid bathing the apical cell side and [^{14}C] L-DOPA$^{\text{cell}}$ (in pmol/mg protein) indicates the amount of [^{14}C] L-DOPA accumulated in the cell monolayer. At the end of the transport experiment, the medium was immediately aspirated, the cell monolayer rapidly washed with cold Hanks' medium and added with 250 μL of 0.1% v/v Triton X-100 (dissolved in 5 mM Tris.HCl, pH 7.4). Radioactivity was measured by liquid scintillation counting.

44F2hc immunobloting

LLC-PK$_1$ cells cultured to 90% of confluence were washed twice with PBS and total cell protein extracted for 4F2hc detection. Briefly, to obtain total cell extract, cells were lysed by brief sonication (15 s) in lysis buffer with protease inhibitors (150 mM NaCl, 50 mM Tris.HCl pH 7.4, 5 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 100 μg ml^{-1} PMSF aprotinin and leupeptin 2 μg ml^{-1} each) and incubated on ice for 1 hour. After centrifugation (14 000 r.p.m., 30 min, 4°C), the supernatant was mixed in 6× sample buffer (0.35 M Tris-HCl, 4% SDS, 30% glycerol, 9.3% DTT, pH 6.8, 0.01% bromphenol blue) and boiled for 5 min. The proteins were subjected to SDS-PAGE (10% SDS-polyacrylamide gel) and electrotransfered onto nitrocellulose membranes. The transblot sheets were blocked with 5% of non-fat dry milk in Tris·HCl 25 mM pH 7.5, NaCl 150 mM and 0.1% Tween 20, overnight at 4°C. Then, the membranes were incubated with rabbit anti-4F2hc polyclonal antibody (1:500; Santa Cruz Biotechnology, CA, USA) and subsequently incubated with a peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Chemiluminescence of immunocomplexes was detected using an ECL kit (Amersham Life, Arlington Heights, IL). Protein concentration was determined using a protein assay kit (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as standard.

44F2hc oligonucleotides and antisense experiments

Antisense oligonucleotides have been used for the selective suppression of mRNA expression in mammalian cells (Bröer et al., 1997). LLC-PK$_1$ cells were treated with an antisense oligonucleotide (1 μM) complementary to rat 4F2hc mRNA for 72 h. In the antisense experiments a phosphorothioate oligonucleotide with the sequence 5′-GGG AGC CTA AAT CCG GAT-3′ was used, corresponding to nt 717–734 of the published 4F2hc sequence (Kanai et al., 1998). The sequence of the nonsense oligonucleotide was 5′-TAG GGC TAA ATC CGA GGG-3′.

Cell viability

Cells were preincubated for 30 min at 37°C and then incubated in the absence or the presence of L-DOPA and test compounds for further 6 min. Subsequently the cells were incubated at 37°C for 2 min with trypan blue (0.2% w/v) in phosphate buffer. Incubation was stopped by rinsing the cells twice with Hanks' medium and the cells were examined using a Leica microscope. Under these conditions, more than 95% of the cells excluded the dye.

Data analysis

Arithmetic means are given with S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for multiple comparisons. A P value less than 0.05 was assumed to denote a significant difference.

Drugs

Benserazide, L-DOPA and trypan blue were purchased from Sigma Chemical Company, St. Louis, Mo, USA. Tolcapone was kindly donated by late Professor Mosé Da Prada (Hoffman La Roche, Basle, Switzerland). [^{14}C] L-DOPA, specific activity 51 mCi/mmol, was purchased from Amersham Pharmacia Biotech (Little Chalfont, UK).

Results

In experiments designed to study the transepithelial flux of [^{14}C] L-DOPA, cells were incubated with 2.5 μM [^{14}C] L-DOPA applied from the apical cell border or the basal cell border and flux (transfer to the opposite chamber) was measured over a 24 min period. As shown in Fig. 1, the

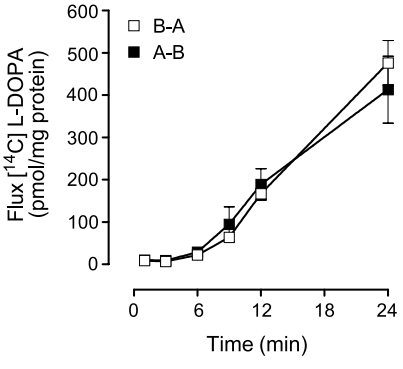

Fig. 1. Basal-to-apical (B-A) and apical-to-basal (A-B) flux of [^{14}C] L-DOPA in LLC-PK$_1$ cells. Cells were incubated with 2.5 μM [^{14}C] L-DOPA applied from the basal cell border or the apical cell border and flux (transfer to opposite chamber) measured over a 24 min period. Each symbol or column represents the mean ± SEM, n = 6. Significantly different from corresponding values for apical-to-basal (*P < 0.05)

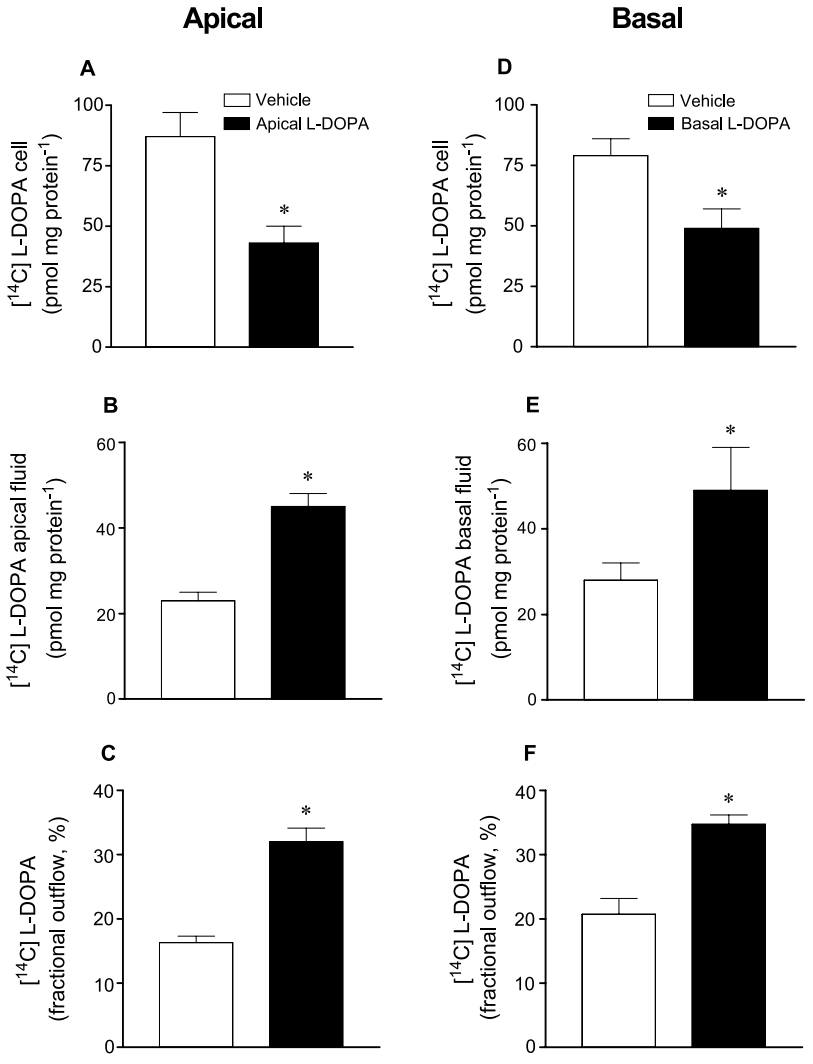

Fig. 2. Spontaneous and L-DOPA-stimulated outflow of [^{14}C] L-DOPA and absolute levels of [^{14}C] L-DOPA in LLC-PK$_1$ cells and the incubation fluid. Cells were incubated for 6 min in the presence of 2.5 μM [^{14}C] L-DOPA and then incubated in the absence (vehicle) and the presence of unlabelled L-DOPA (1 mM) for 9 min. Columns represent the mean of 4 experiments per group; vertical lines show S.E.M. Significantly different from corresponding control value (*$P < 0.05$) using the Student's "t" test

transepithelial flux of [^{14}C] L-DOPA progressively increased with incubation time. The basal-to-apical transepithelial flux of [^{14}C] L-DOPA was similar to the apical-to-basal transepithelial flux (Fig. 1).

In experiments designed to study the efflux of L-DOPA through the apical and basal cell sides, cells cultured in polycarbonate filters were loaded for 6 min with 2.5 μM [^{14}C] L-DOPA applied from both cell sides. For the measurement of apical and basal [^{14}C] L-DOPA efflux, the cells cultured in polycarbonate filters were incubated for 12 min with Hanks' medium in the absence and the presence of unlabelled L-DOPA (1 mM). As shown in Fig. 2, the spontaneous apical fractional outflow of [^{14}C] L-DOPA (Fig. 2C) was identical to that through the basal cell side (Fig. 2F). The addition of unlabelled L-DOPA from the apical cell side increased 2-fold the apical efflux of [^{14}C] L-DOPA (Fig. 2B). Similarly, the addition of unlabelled L-DOPA from the basal cell side increased 2-fold the basal efflux of [^{14}C] L-DOPA (Fig. 2E). The L-DOPA-induced fractional outflow of [^{14}C] L-DOPA through the apical or basal cell side (Fig. 2C and F) was accompanied by marked decreases in intracellular levels of [^{14}C] L-DOPA (Fig. 2A and D).

To evaluate the involvement of 4F2hc on the inward transfer of [^{14}C] L-DOPA, LLC-PK$_1$ cells were treated for 72 h with an antisense oligonucleotide (AS4F2hc, 1 μM) complexed with lipofectin (3%) and then incubated for 6 min with [^{14}C] L-DOPA (0.25 μM). The accumulation of [^{14}C] L-DOPA in LLC-PK$_1$ cells treated with the 4F2hc antisense oligonucleotide (63.5 ± 4.1 pmol mg protein^{-1}) was significantly ($P < 0.05$) lower than in cells exposed to the 4F2hc nonsense oligonucleotide (79.4 ± 6.1 pmol mg protein^{-1}). In another set of experiments, cells cultured in polycarbonate filters were treated for 72 h with the 4F2hc antisense (AS4F2hc, 1 μM) and nonsense (NS4F2hc, 1 μM) oligonucleotides and then incubated for 6 min with

[^{14}C] L-DOPA (2.5 μM). Thereafter, for the measurement of apical and basal [^{14}C] L-DOPA efflux, the cells cultured in polycarbonate filters were incubated for 12 min in Hanks' medium. As shown in Fig. 3, the treatment of LLC-PK$_1$ cells with the 4F2hc antisense oligonucleotide (AS4F2hc, 1 μM) resulted in a marked decrease in spontaneous fractional outflow of [^{14}C] L-DOPA through the apical (Fig. 3B) or the basal (Fig. 3C) cell side. This was accompanied by significant increase in intracellular [^{14}C] L-DOPA (Fig. 3A). Immunoblotting studies also showed that this cell line endogenously expresses 4F2hc and treatment with the 4F2hc antisense oligonucleotide markedly decreases the expression of 4F2hc (Fig. 4).

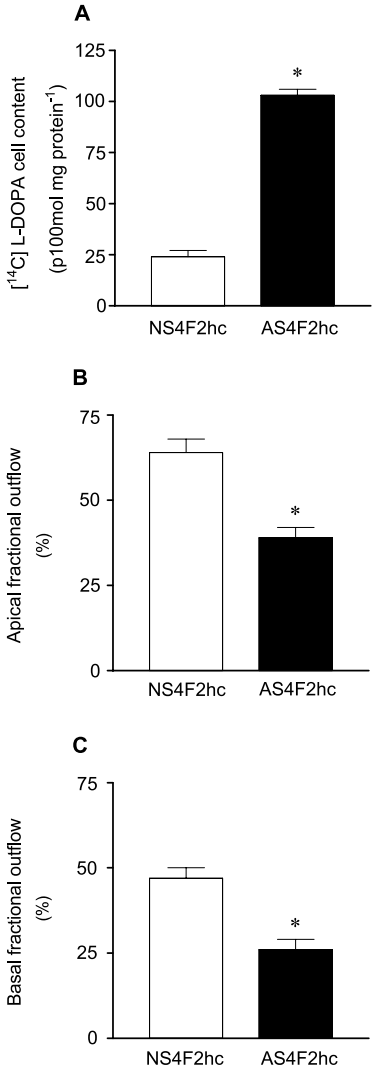

Fig. 3. (A) [^{14}C] L-DOPA cell content and spontaneous (B) apical and (C) basal fractional outflow of [^{14}C] L-DOPA in LLC-PK$_1$ cells treated with non-sense (NS4F2hc, 1 μM) and anti-sense (AS4F2hc, 1 μM) oligonucleotide against 4F2hc. Cells were loaded for 6 min with 2.5 μM [^{14}C] L-DOPA and then incubated for 12 min. Columns represent the mean of 4 experiments per group; vertical lines show S.E.M. Significantly different from corresponding control value ($^*P < 0.05$) using the Student's "t" test

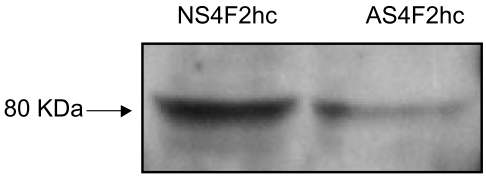

Fig. 4. 4F2hc protein in LLC-PK$_1$ cells using an antibody raised against the rat 4F2hc. The lane contains of 20 μg protein. Immunoblots were repeated 4 times

Discussion

The data gathered here shows that basal-to-apical and apical-to-basal transepithelial fluxes of [^{14}C] L-DOPA in polarised LLC-PK$_1$ cell monolayers are of identical magnitude, and intracellular [^{14}C] L-DOPA is transported quite efficiently through the apical and basal cell membranes. The results of L-DOPA efflux studies in LLC-PK$_1$ cells are quite valuable to define the nature of transporters involved in the handling of the substrate. Measurements of [^{14}C] L-DOPA efflux in the presence of unlabelled L-DOPA did show a consistent efflux, being considerably greater than that in the absence of unlabelled L-DOPA. This suggests that the Na^+-independent outward transfer of L-DOPA may include the hetero amino acid exchanger LAT2/4F2hc, which facilitates the *trans*-stimulation of L-DOPA. This is also in line with evidence recently reported that treatment of LLC-PK1 cells with LAT2 siRNA reduced by 85% [^{14}C] L-DOPA accumulation, in a time- and concentration-dependent manner (Soares-da-Silva et al., 2004). The results present here suggest that the LAT2/4F2hc hetero amino acid exchanger might be of equal importance at the apical and basolateral cell membranes for the outward transfer of intracellular [^{14}C] L-DOPA.

The mRNA corresponding to LAT2 examined by Northern blot analysis was strongly expressed in kidney and small intestine (Pineda et al., 1999; Rossier et al., 1999). *In situ* hybridisation studies specifically localized the renal expression of LAT2 mRNA to the epithelial cells of proximal tubules (Pineda et al., 1999). A similar pattern of expression has been shown on immunolocalization of 4F2hc protein on kidney cortex (Quackenbush et al., 1986; Rossier et al., 1999), suggesting an association between LAT2 and 4F2hc. In fact, as described for other transporters (Mastroberardino et al., 1998), 4F2hc was found to bring LAT2 to the oocyte plasma membrane (Pineda et al., 1999). Subsequently, it was demonstrated

that LAT2 forms a disulfide bond heterodimeric complex with 4F2hc (Rossier et al., 1999), as has been found to occur with other amino acid transporters and their associated glycoproteins (Verrey et al., 2000). The finding that Hg^{2+} completely blocked the stimulated outward transfer of [^{14}C] L-DOPA (Soares-da-Silva and Serrao, 2004), which was prevented by β-mercaptoethanol, strongly suggests that in LLC-PK$_1$ cells LAT2 might be associated with the single transmembrane domain 4F2hc protein through an intermolecular disulfide bond. The involvement of 4F2hc on [^{14}C] L-DOPA transport in LLC-PK$_1$ cells is further supported by the finding that an antisense oligonucleotide of 4F2hc significantly reduced both the inward and outward transfer of [^{14}C] L-DOPA. The degree of inhibition of [^{14}C] L-DOPA outward and inward transfer in cells treated with the 4F2hc antisense oligonucleotide paralleled the decrease in 4F2hc expression. The degree of inhibition (20% decrease) in L-DOPA accumulation by the 4F2hc antisense oligonucleotide is similar to that observed on the inward transport of L-DOPA in C6-BU-1 glioma cells treated with a 4F2hc oligonucleotide antisense (Bröer et al., 1997).

The finding that L-DOPA-induced increases in the fractional outflow of [^{14}C] L-DOPA were identical at both cell sides suggests that LAT2 in LLC-PK$_1$ cells may be present in both the apical and basolateral membranes. The identical decrease in [^{14}C] L-DOPA efflux through the apical and basal cell sides by the antisense oligonucleotide of 4F2hc also suggests that the LAT2/4F2hc hetero amino acid exchanger is equally distributed at the apical and basolateral cell membranes. This is a potential point of conflict since 4F2hc has been suggested to have a basolateral location to facilitate absorption (Verrey, 2003; Verrey et al., 2000). Though in the kidney 4F2hc has been shown to have a basolateral location (Quackenbush et al., 1986; Rossier et al., 1999), as the present data suggest for LLC-PK$_1$ cells, LAT2/4F2hc may also show an apical location in cultured renal epithelial cells. Similar findings were observed in opossum and rat kidney cells cultured in polycarbonate filters (Gomes and Soares-da-Silva, 2002; Pinho et al., 2004). Therefore, it is likely that the distribution of LAT2/4F2hc at the apical cell membrane may correspond to a general characteristic of cultured cell lines. This, obviously, does not limit the usefulness of cultured cell lines in transport studies, but is a matter to be considered when comparisons with original tissues are performed.

In conclusion, it is suggested that the Na^+-independent uptake of L-DOPA may include the hetero amino acid exchanger LAT2/4F2hc, which facilitates the *trans*-stimulation of L-DOPA and its outward transfer. The LAT2/4F2hc hetero amino acid exchanger appears to be of equal importance at the apical and basolateral cell membranes.

Acknowledgements

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