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Regulation of L-leucine transport in rat kidney by dexamethasone and triiodothyronine

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Summary. We have investigated the transport mechanisms involved in the stimulation of renal tubular reabsorption of large amino acids by glucocorticoids *in vivo* through the examination of activity and expression of specific transport systems L and y^+L for L-leucine in membrane preparations of rat kidneys. Kidneys were removed from adult female Wistar rats treated with dexamethasone or triiodothyronine, and the fractions of brush-border and basolateral membranes were isolated by density gradient centrifugation. Functional analysis of L-leucine uptake using rapid filtration technique revealed induction of a sodium-dependent, arginine-inhibitable system y^+L transport component in the basolateral membrane in the dexamethasone-treated group. A minor sodium-independent, BCH-inhibitable, system L transport component was unaffected by glucocorticoids. L-leucine uptake remained unaffected in the triiodothyronine-treated group. Expression of both subunits of the system y^+L transporter was increased in dexamethasone-treated rat kidneys: Western blot analysis showed a significant (46%) increase of 4F2hc protein abundance in the basolateral membrane fraction and competitive RT-PCR revealed an almost 4-times induced expression of y^+LAT1 mRNA. Our results indicate that system y^+L in rat kidney is regulated by glucocorticoids. We suggest that enhancement of both 4F2 heavy chain and y^+LAT1 light chain is necessary for induction of this transport system in the kidney.

Keywords: Amino acid transport – Kidney – Glucocorticoid hormones – Thyroid hormones – Leucine

Abbreviations: BBMV – brush-border membrane vesicles; BLMV – basolateral membrane vesicles; DEX – dexamethasone; T3 – triiodothyronine; BCH – 2-amino-2-carboxybicyclo[2,2,1]heptane-2-carboxylic acid; MeAIB – alpha-(N-methylamino)isobutyric acid

Introduction

The reabsorption of large neutral amino acids such as L-leucine in the kidney is driven by transport systems localized in the apical and basolateral membrane of tubular epithelium. Functional analysis of transport kinetics for these amino acids revealed the participation of sodium-dependent transport systems B°, $B^{\circ,+}$, and of sodium-independent transport system $b^{\circ,+}$ in the apical membrane (Christensen, 1990), and also of sodium-dependent transport systems y^+L (Devés et al., 1992) and ASC (Stevens et al., 1984) as well as sodium-independent transport system L in the basolateral membrane (Christensen, 1990). In the last decade the primary structures of many of these transporters have been elucidated (Palacin et al., 1998; Devés and Boyd, 2000). Whereas system ASC and B° are monomeric transporters with 10 transmembrane domains (Kekuda et al., 1996; Utsunomiya-Tate et al., 1996), the others, except the unidentified system $B^{\circ,+}$, belong to a group of heterodimeric transporters that is currently a focus of intensive research (Verrey et al., 1999). These transporters consist of a type II membrane glycoprotein (the heavy chain) linked by a disulifde bond to an extremely hydrophobic protein (the light chain) belonging to the amino acid/polyamine/choline (APC) superfamily (Mastroberardino et al., 1998; Verrey et al., 1999). Two different heavy chains are known: NBAT (or rBAT), in the kidney mainly expressed in the apical membrane of S3 segment, and 4F2hc, first known as a surface antigen in lymphocytes and localized in the basolateral membrane of epithelial cells (Quackenbush et al., 1987; Bertran et al., 1992; Wells and Hediger, 1992; Kanai et al., 1992; Wells et al., 1992). It has been shown that rBAT and the light chain $b^{\circ,+}AT1$ together induce system $b^{\circ,+}$

activity (Chairoungdua et al., 1999; Pfeiffer et al., 1999b). System L transport is produced by LAT1 or LAT2 associated to 4F2hc (Segawa et al., 1999; Pineda et al., 1999), whereas the y^+LAT1 and y^+LAT2 proteins induce system y^+L activity in conjunction with 4F2hc (Torrents et al., 1998; Pfeiffer et al., 1999a).

Despite these new molecular insights into membrane transport, very little information is available on the regulation of amino acid transport in the kidney. In previous *in vivo* experiments, stimulatory influences of dexamethasone (DEX) and triiodothyronine (T3) on renal tubular uptake of neutral amino acids L-leucine and L-glutamine could be demonstrated (Fleck et al., 1997). Micropuncture experiments revealed the main site of glucocorticoid action on L-leucine transport to be the proximal straight tubule of superficial nephrons of rat kidney (Schwertfeger et al., 2000). The *Xenopus laevis* LAT-type light chains ASUR4 and IU12 are reported to be induced by adrenocorticoids and thyroid hormones respectively in epithelial cells (Spindler et al., 1997; Liang et al., 1997), highlighting the potential for regulation of the heterodimeric amino acid transporters by endocrine factors.

In this study we have isolated apical and basolateral membranes of rat kidney epithelium after *in vivo* treatment with glucocorticoid or thyroid hormones to investigate the transport systems involved in stimulation of L-leucine uptake. We demonstrate the participation of both system y^+L and system L in basolateral transport of L-leucine. DEX enhanced the basolateral uptake of L-leucine by inducing system y^+L, but not system L activity. Using Western blot analysis and competitive RT-PCR, we show glucocorticoid-induced increases of 4F2hc protein and y^+LAT1 mRNA abundance respectively. We suggest that the activity of heterodimeric transport system y^+LAT1 depends on both the heavy chain abundance and the relative expression of the different light chains.

Methods

Chemicals

Unless otherwise specifically stated, chemicals were obtained from Sigma Chemicals (Poole, Dorset, UK). D-[^{3}H]glucose (2.84 GBq mg^{-1}) and L-[^{3}H]leucine (2.36 GBq mg^{-1}) were obtained from NEN DuPont (Hertfordshire, UK).

Animals and hormone treatments

Adult female Wistar rats (Han: Wist) of our Institute's own out-bred stock were used in the study. Rats weighing 200–250 g were fed a standard diet (Altromin 1316, Altromin, Lage, Germany) and tap water *ad libitum* and were housed under standardized conditions in plastic cages, light-dark cycle 12/12 hours, temperature 22 ± 2°C, humidity 50 ± 10%. Dexamethasone (Fortecortin®, Merck, Darmstadt, Germany) was administered intraperitoneally (i.p.) in a dose of 60 μg/100 g b.wt. once daily for three days. Triiodothyronine (Berlin-Chemie, Berlin, Germany): 20 μg/100 g b.wt. were given i.p. for 3 days, once daily. Both substances were dissolved in 0.9% NaCl to give an volume of 1 ml/100 g b.wt. Controls received saline only. Rats were killed under ether anaesthesia, the kidneys were removed, quick frozen in liquid nitrogen and stored at −80°C for future analysis.

Renal membrane vesicle preparation

Renal brush-border membrane vesicles (BBMV) and basolateral membrane vesicles (BLMV) were prepared from isolated rat kidneys by the modified method of Sacktor et al. (1981). All membrane isolation procedures were carried out at 4°C or on ice. The minced kidneys were placed in a buffer containing 0.25 M sucrose, 0.1 mM phenylmethanesulfonyl fluoride (PMSF), and 10 mM Tris-Cl (Tris[hydroxymethyl]aminomethane chloride), pH 7.6, and were then homogenized sequentially by a motorized Teflon-glass homogeniser and then a Polytron homogeniser. After the homogenate was centrifuged at 2,500 × g for 15 min, the supernatant was centrifuged at 24,000 × g for 20 min. The upper fluffy membrane layer of the pellet was resuspended in 0.25 M sucrose buffer and rehomogenized. Percoll was added to a final concentration of 8.5%, and the membrane suspension was centrifuged at 35,000 × g for 35 min. Percoll density-gradient fractions containing BBMV and BLMV were removed and diluted with a buffer containing 0.4 M sucrose, 5 mM $MgCl_2$, 10 mM *N*-2-hydroxyethylpiperazine-*N*′2-ethanesulfonic acid (HEPES), and 5 mM Tris-Cl (pH 7.4), then centrifuged at 34,000 × g for 30 min. For both fractions, the loose fluffy pellet was resuspended in 0.4 M sucrose buffer, centrifuged at 34,000 × g for 30 min and the final pellet suspended in 0.4 M sucrose buffer. All centrifugation steps were performed in a Centrikon Ultracentrifuge (Kontron AG, Zuerich, Switzerland) using a fixed angle rotor (TFT 50.38) with the exception of the Percoll gradient centrifugation step performed in an Optima L-90 ultracentrifuge (Beckman, U.S.A.) using an SW 28 swing-out rotor.

The protein content was assayed using the bicinchoninic acid method (Smith et al., 1985). The BLMV and BBMV fractions showed high-enrichment of specific marker properties: (i) the BLMV fraction was enriched 9 [±1.1]-times in ouabain-sensitive Na^+-K^+-ATPase activity (Scharschmidt et al., 1979) with respect to the original kidney homogenate, whereas BBMV enrichment was <3-times; (ii) the BBMV fraction showed substantial sodium-dependent (SGLT-mediated) glucose uptake which was not detected to any significant extent in BLMV (Fig. 1). D-[^{3}H]glucose uptake was measured in the presence or absence of sodium ions, using choline as replacement (Mueckler, 1992).

Radiotracer uptake experiments

Uptake experiments were performed at room temperature. The uptake reaction was initiated by the addition of 30 μl (BBMV) or 20 μl (BLMV) sodium or choline chloride transport buffer to the same volume of vesicle preparation (20 to 60 μg protein). The transport buffer consisted of 0.2 M NaCl or choline chloride, 5 mM $MgCl_2$, 0.2 mM $CaCl_2$, 10 mM HEPES, and 5 mM Tris-Cl (pH 7.5)

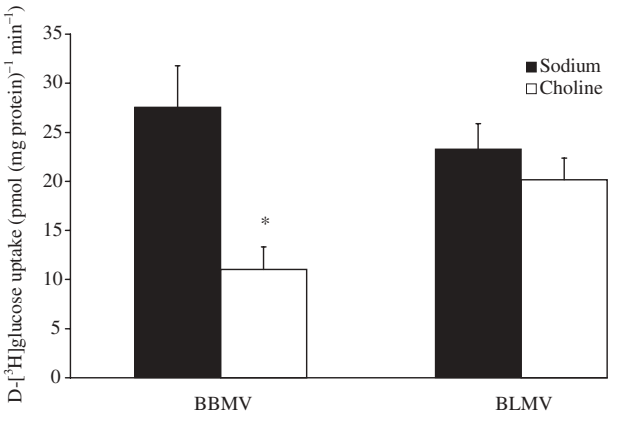

Fig. 1. Uptake of D-glucose into brush-border and basolateral membrane preparations. D-glucose uptake into BBMV and BLMV of kidneys from controls over 1 min period in a medium containing D-[^{3}H]glucose (150 kBq ml^{-1}) and unlabelled D-glucose to 50 μM final concentration in the presence of Na^+ or choline. The data represent mean ± S.E.M. Experiments were performed in triplicate on four separate rat kidney preparations. *Significant difference between values in Na^+ and choline ($P \leq 0.05$)

containing the radiotracers (D-[^{3}H]glucose; L-[^{3}H]leucine) and unlabelled D-glucose or L-leucine to adjust the concentration to 10 μM. The reaction was terminated after a timed period by the addition of 1 ml ice-cold stopping buffer (0.2 mM KCl, 5 mM $MgCl_2$, 0.2 mM $CaCl_2$, 10 mM HEPES, 5 mM Tris-Cl; pH 7.5). Samples were immediately applied to prewetted 0.45 μm pore size cellulose nitrate membrane filters (Whatman, Maidstone, UK) on a rapid filtration unit (Hoefer Scientific, San Francisco, U.S.A.) and washed with 5 × 2.5 ml ice-cold stopping buffer. Washed filters were placed in vials to which 5 ml scintillation fluid was added before radioactivity was counted using a Beckman LS-6000IC liquid scintillation spectrometer (Beckman, High Wycombe, UK). For transport inhibition studies, BLMV were incubated with 20 μl radiotracer solution containing the compound of interest (e.g. Triton X-100, 2-amino-2-carboxybicyclo[2,2,1]heptane-2-carboxylic acid (BCH), amino acids) for 30 seconds before termination and scintillation counting, as detailed above.

Western blot analysis

BBMV and BLMV preparations (20 μg protein) were resolved through a 7.5% SDS-polyacryamide-gel and electrophoretically transferred onto cellulose nitrate membranes. Membranes were blocked overnight with 5% powdered milk before 1 hour incubation with either goat anti-rat 4F2hc polyclonal antibody at 1:500 dilution (Santa Cruz Biotechnology) or rabbit anti-rat NBAT/rBAT polyclonal antibody at 1:1000, followed by a 1 hour incubation with horseradish-peroxidase-labelled anti-goat (dilution 1:1000) or anti-rabbit (1:500) secondary antibodies (both from Scottish Antibody Production Unit, Carluke, UK), respectively. 4F2hc and NBAT were detected by enhanced chemiluminescence (ECL: Amersham). The anti NBAT/rBAT antibody was raised in rabbits (Sigma-Genosys, UK) against a synthetic peptide (Pro-Arg-Ser-Phe-Lys-Asp-Ser-Asp-Lys-Asp-Gly-Asn-Gln-Asp; residues 125–138 of rat NBAT/rBAT sequence, see Wang and Tate, 1995) and purified by affinity chromatography using the peptide antigen.

Competitive RT-PCR

Determination of y^+LAT1-mRNA was performed by competitive RT-PCR (Siebert and Larrick, 1992; Foley et al., 1993). Total RNA was isolated from kidney by means of an RNA Kit II (InViTek, Berlin, Germany). Reverse transcription reactions were performed using an RT-Kit (Promega, Madison WI, U.S.A.). The primers for PCR were targeted to sites in the y^+LAT1 cDNA producing fragments of 470 bp with forward primer 5′-GCG ACG CGG TTG CTG TGA C-3′ and reverse primer 5′-GAT GCC GAT GCC GAT GAG GGA GTT-3′.

The preparation of a homologous competitor RNA used as internal standard for y^+LAT1 during RT-PCR was prepared according to Müller et al. (1998). After reverse transcription of native RNA, cDNA was amplified with composite primers (forward: 5′-TAA TAC GAC TCA CTA TAG GGC GAC GCG GTT GCT GTG AC-3′; reverse: 5′-GCG ATG CCG ATG CCG ATG AGG GAG TTG ACG ATG GGG AAG AAC AGG CTG-3′) generating a homologous dsDNA containing a T7-promoter sequence at the 5′-end and a deletion of 54 bp near the 3′-end. These DNA-fragments were selectively adsorbed on DEAE-membranes (Schleicher and Schuell, Germany) after gel electrophoresis, eluted with 1 M NaCl and 50 mM arginine and used as template for transcription with T7-RNA polymerase (Promega, Madison, WI, U.S.A.). The RNA of 416 bp length was isolated and quantified spectrophotometrically at 260 nm. This competitor RNA was added in a dilution series to RT reactions containing aliquots of the native RNA. PCR was then performed under standardized conditions (40 cycles consisting of 30 s 94°C, 30 s 57°C, 35 s 72°C). Reagents were obtained from Gibco Life Technologies (Eggenstein, Germany). RT-PCR products were separated by agarose gel electrophoresis (1.5%) and visualized with ethidium bromide. The reaction which showed equivalent amounts of DNA generated from native y^+LAT1 mRNA and the competitor RNA was estimated visually and the abundance of the y^+LAT1 mRNA was calculated.

Data analysis

Data are presented as means ± S.E.M. Statistical differences were assessed by analysis of variance (ANOVA) and *post hoc* multiple t-test with Bonferroni correction; differences were taken to be significant when $P \leq 0.05$. Individual uptake experiments and enzyme assays were carried out in triplicate and duplicate, respectively, unless otherwise specified. Curve and line fittings as well as densitometric analysis of Western blots were performed using commercial software (SPSS Science, Chicago, U.S.A.).

Results

Initial time course experiments were carried out to identify the linear phase of the tracer uptake into renal membrane vesicles prepared from control rats. L-leucine uptakes (at 10 μM concentration) in the presence of sodium or choline by rat kidney BBMV and BLMV are shown in Fig. 2. The uptake of L-[^{3}H]leucine was maximum after times between 30 and 60 s in BBMV, and 60 and 120 s in BLMV, respectively. Equilibrium uptake of 10 μM L-[^{3}H]leucine into both BBMV and BLMV was approximately 15 pmol/mg protein, corresponding to an estimated intravesicular volume of 1.5 μl/mg protein. Subsequently,

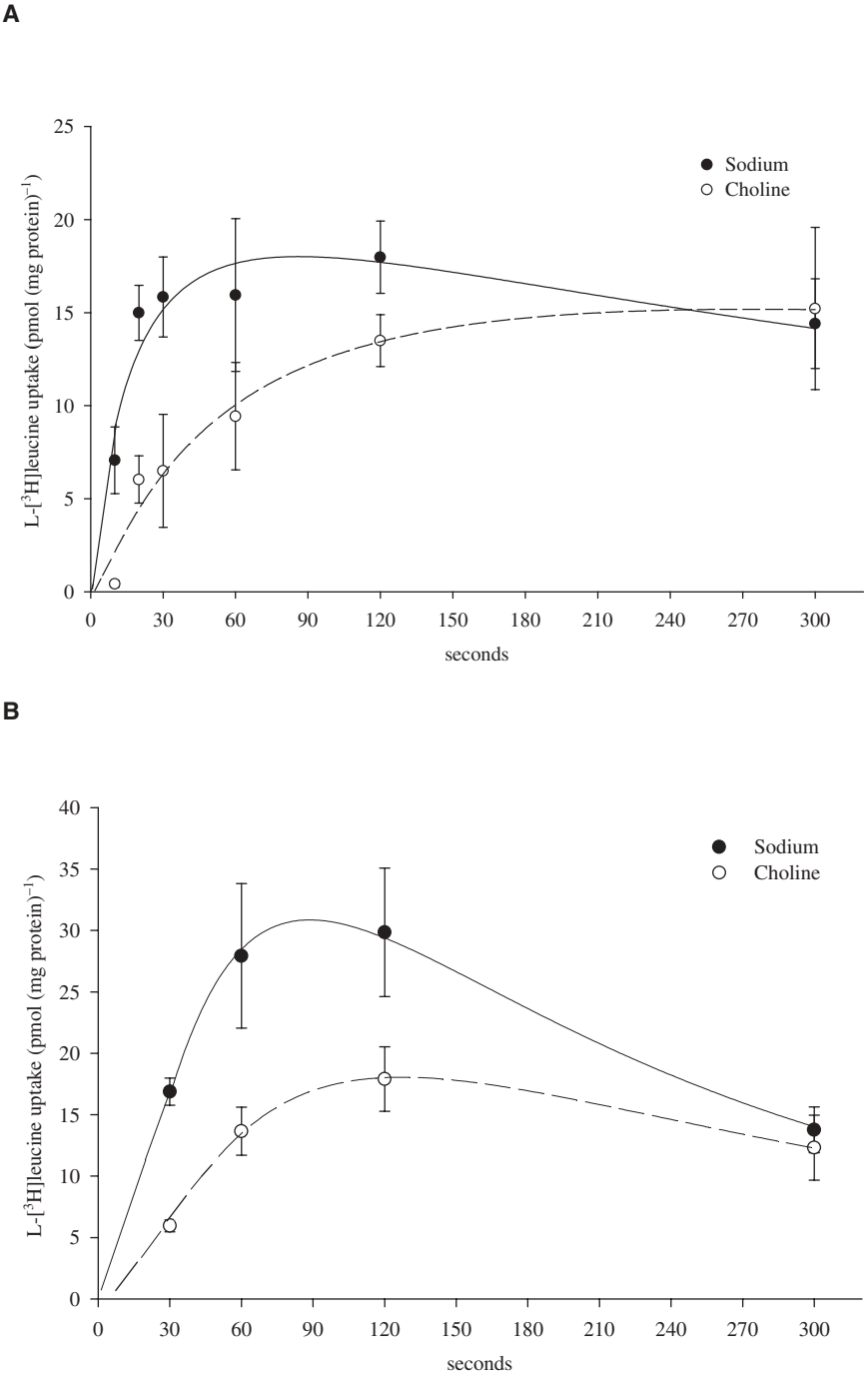

Fig. 2. Time course of L-leucine uptake into brush-border membrane and basolateral membrane vesicles. Time-dependent L-leucine uptake into BBMV (**A**) and BLMV (**B**) of control rat kidneys in a medium containing L-[^{3}H]leucine (130 kBq ml^{-1}) and unlabelled L-leucine to 10 μM final concentration in the presence of Na^+ or choline. The data represent mean ± S.E.M. Experiments were performed on four separate rat kidney preparations. Curves were fitted using non-linear regression with regression coefficients of R = 0.92 and R = 0.95 (**A**), and R = 0.99 and R = 0.99 (**B**), for Na^+ and choline, respectively

uptakes were measured over a time period in which the reaction was linear: in BBMV initial rate uptakes were measured over a 20 s period, in BLMV initial uptakes were measured over a 30 s period. The sodium-dependent uptake of L-leucine exhibited in both membrane preparations an overshoot, which is characteristic for transport driven by a Na^+ electrochemical gradient.

In further experiments the influences of DEX and T3 on sodium-dependent and sodium-independent (choline) 10 μM L-[^{3}H]leucine uptake into BBMV and BLMV were compared with controls. No differences in leucine uptake were found in preparations of BBMV (data not shown). However, 10 μM L-[^{3}H]leucine uptake in BLMV was increased significantly from 16.9 ± 1.1 pmol/mg protein * min in

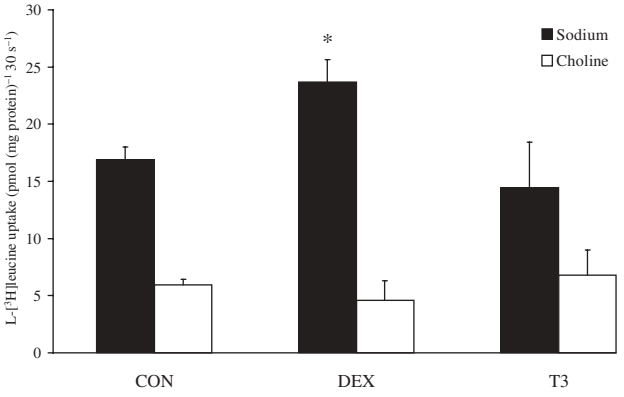

Fig. 3. Uptake of L-leucine into basolateral membrane preparations from rats treated with NaCl, dexamethasone or triiodothyronine. Uptake of L-[^{3}H]leucine (10μM final concentration) into BLMV of kidneys from rats treated with NaCl (*CON*), dexamethasone (*DEX*) or triiodothyronine (*T3*) was measured over 30s period in the presence of Na^+ or choline. The data represent mean ± S.E.M. Experiments were performed in triplicate on four separate rat kidney preparations. *Significantly different from respective control values ($P \leq 0.05$)

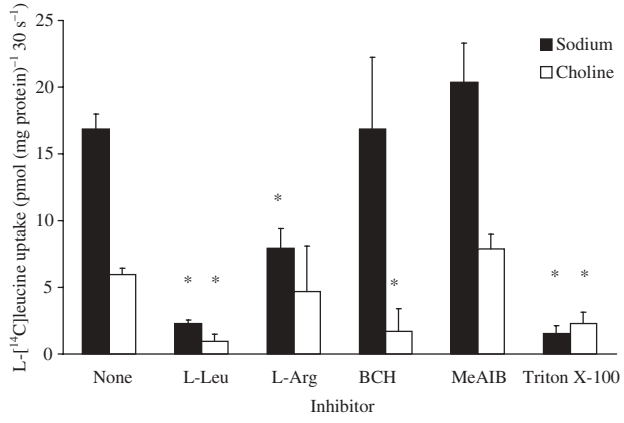

Fig. 4. Characteristics of L-leucine uptake into basolateral membrane preparations from control rats. L-leucine uptake into BLMV of control rat kidneys over 30s period in a medium containing L-[^{3}H]leucine (100kBq ml^{-1}) and unlabelled L-leucine to 10μM final concentration in the presence of Na^+ or choline, and in the absence (*None*) or presence of 5mM L-leucine (*Leu*), 5mM L-arginine (*Arg*), 5mM BCH, 5mM MeAIB or 1% Triton X-100. The data represent mean ± S.E.M. Experiments were performed in triplicate on four separate rat kidney preparations. *Significantly different from respective control values ($P \leq 0.05$)

controls to 23.7 ± 1.9 pmol/mg protein * min in preparations of kidneys from DEX-treated rats (Fig. 3). This increase was largely in the sodium-dependent component of leucine uptake. Leucine uptake in the T3 group was unchanged.

The characterization of transport systems involved in L-leucine uptake on basolateral membrane was performed measuring L-leucine uptake in presence of different amino acids. The data for control rat kidneys are shown in Fig. 4. Both Na^+ dependent and Na^+ independent components of L-leucine uptake were saturable, being markedly inhibited by 5mM L-Leu. The Na^+ dependent component of L-leucine uptake was also substantially inhibited by 5mM L-Arg. The system L specific substrate BCH inhibited Na^+ independent L-leucine uptake, whereas the system A specific substrate alpha-(N-methylamino)isobutyric acid (MeAIB) was without effect on any component of L-leucine uptake. Disruption of BLMV by use of the detergent Triton X-100 abolished the saturable component of L-[^{3}H]leucine uptake, as expected for a process involving tracer uptake into a closed intravesicular space.

DEX only induces the Na^+ dependent, arginine-inhibitable component of L-leucine uptake (Fig. 5). A small Na^+ dependent component of L-leucine uptake was resistant to inhibition by 5mM arginine, but this remained unchanged in the DEX group (Fig. 5). BCH-inhibitable L-leucine uptake in BLMV of DEX-treated rat kidneys is not different to the control value (Fig. 5).

Western blot analysis of membrane fractions using antibodies against 4F2hc and NBAT (Fig. 6) revealed induction of the 4F2hc protein in basolateral membranes of DEX-treated rat kidneys, whereas abundance of NBAT/rBAT protein (the heavy chain forming heterodimeric transporters in brush-border

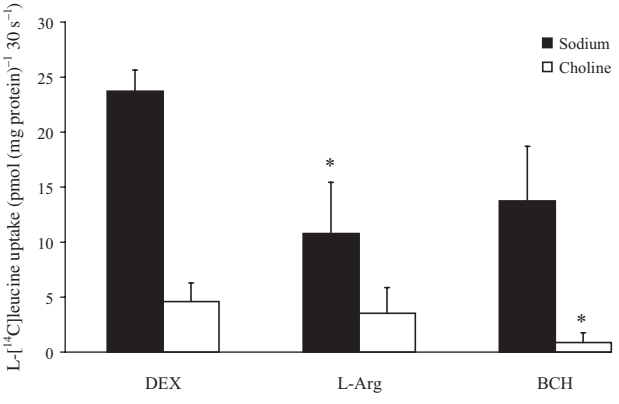

Fig. 5. Characteristics of dexamethasone stimulated L-leucine uptake into basolateral membrane preparations from rats. L-leucine uptake over 30s period into BLMV from kidneys of dexamethasone-treated rats in a medium containing L-[^{3}H]leucine (10μM final concentration) in the presence of Na^+ or choline, and in the absence (*DEX*) or presence of 5mM L-arginine (*Arg*), 5mM BCH. The data represent mean ± S.E.M. Experiments were performed in triplicate on four separate rat kidney preparations. *Significantly different from respective DEX values ($P \leq 0.05$)

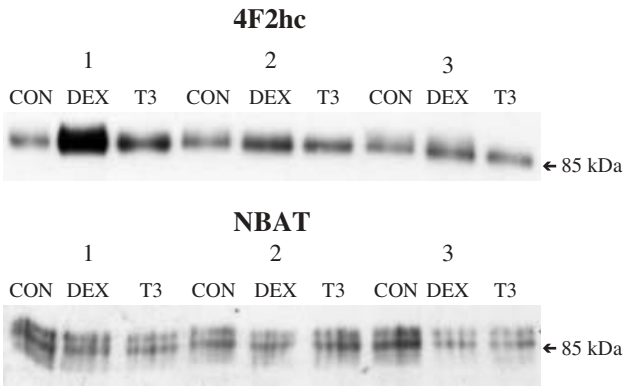

Fig. 6. Western blot of 4F2hc in BLMV, and NBAT in BBMV. Western blot for 4F2hc in BLMV and NBAT in BBMV after SDS-polyacryamide gel (7.5%) electrophoresis and transfer onto cellulose nitrate membranes of the kidney membrane preparations of NaCl (*CON*), DEX, and T3 treated rats (20 μg protein each). 4F2hc and NBAT proteins were detected by enhanced chemiluminescence. Experiments were performed in separate preparations from kidneys of six rats in each hormone-treated group. Results of 3 typical experiments are shown

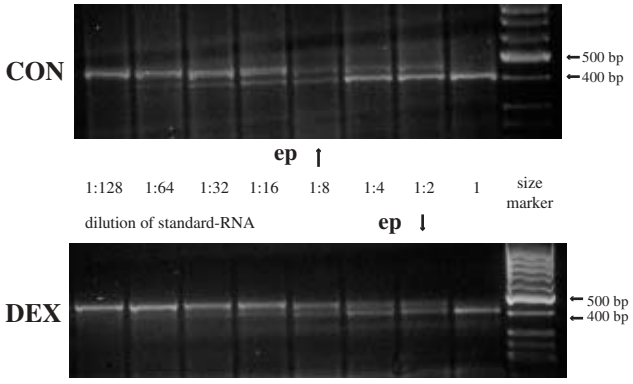

Fig. 7. y^+LAT1 – competitive RT-PCR in kidneys of controls and after dexamethasone treatment. Electrophoresis of 470 and 416 bp RT-PCR products corresponding to y^+LAT1 and internal standard-RNA, respectively. y^+LAT1 mRNA was isolated from rat kidney homogenate 12 hours after treatment with either NaCl or dexamethasone. At the equivalence point (*ep*) the concentration of the sample's RT-PCR product corresponds to the defined concentration of the standard-RNA RT-PCR product

Table 1. Densitometric analysis of Western blots for heavy chains

	4F2hc	NBAT
Control	1	1
Dexamethasone	1.46 ± 0.20*	1.03 ± 0.14
Triiodothyronine	1.08 ± 0.12	1.16 ± 0.10

Densitometric analysis of Western blot for relative protein abundance of 4F2hc in BLMV and NBAT in BBMV preparations (compare Figure 6). The data represent mean ± S.E.M. of total intensity of the signal relative to control. Experiments were performed in separate preparations from kidneys of six rats in controls and in each hormone-treated group. *Significantly different from respective control values ($P \leq 0.05$)

membrane) was unchanged by the glucocorticoid. Quantification of the Western blots by densitometric analysis revealed a 46% enhancement of 4F2hc protein abundance in response to DEX (Table 1), which is in good accordance with the functionally determined increase of 40% in total L-leucine uptake (Fig. 3). Extremely low abundances of 4F2hc in the BBMV fraction and NBAT/rBAT in the BLMV fraction were detected, confirming low cross-contamination of the two membrane fractions (data not shown).

Because of the lack of specific antibodies against rat y^+LAT1 (the light chain of system y^+L in kidney), we performed quantitative RT-PCR of y^+LAT1 mRNA abundance (Fig. 7). The results indicate an almost 4-times enhancement of renal y^+LAT1 mRNA after DEX treatment. The content of mRNA was increased from $2.3 \times 10^{-17} \pm 0.5 \times 10^{-17}$ in controls to $9.1 \times 10^{-17} \pm 2.5 \times 10^{-17}$ mol/μg total-RNA.

Discussion

We have shown previously that renal transport of large neutral amino acids such as L-leucine is regulated by the glucocorticoid analogue dexamethasone and probably by thyroid hormones *in vivo* (Schwertfeger et al., 2000). The present study was performed to detect the transport systems involved in L-leucine uptake in renal epithelium and their regulation by glucocorticoid and thyroid hormones at a molecular level.

Measurement of L-leucine uptakes in BBMV and BLMV of control, DEX-, and T3-treated rat kidneys revealed significant differences between the investigated groups only in basolateral membrane preparations of the glucocorticoid-treated group. The *in vivo* treatment of rats with 60 μg/100 g b.wt. DEX over three days led to an increase in L-leucine uptake of about 40%. The induced transport system was strictly sodium dependent for leucine, and the inhibition of Na^+ dependent L-leucine uptake by 5 mM L-arginine is a characteristic feature of system y^+L (Devés et al., 1992). This transport system is functionally related to 4F2hc and carries L-leucine in a Na^+ dependent way, whereas transport of L-arginine

is Na^+ independent (Fei et al., 1995; Bröer et al., 1995). The small Na^+ dependent component of L-leucine uptake that was not inhibited by high concentrations of L-arginine was unaffected by DEX. It possibly represents system ASC activity (Stevens et al., 1984; Utsunomiya-Tate et al., 1996). There was no evidence for participation of system A because L-leucine uptake remained unaffected in the presence of 5 mM MeAIB, a system A specific, non-metabolizable amino acid analogue (Christensen et al., 1965; Christensen, 1990). The sodium independent component of L-leucine uptake could be specified as system L activity because it was inhibited by BCH (Christensen, 1979). System L activity in basolateral membrane of the rat kidney is produced by 4F2hc with the light chain LAT2 and behaves as an exchanger (Bröer et al., 1997). Because of the high level of expression of the heterodimeric complex of LAT2 and 4F2 heavy chain in the intestine, kidney, and placenta, it is believed to be involved in the *trans*-cellular transport of neutral amino acids in epithelia (Pineda et al., 1999; Segawa et al., 1999; Rossier et al., 1999).

However, our functional data using BLMV suggest only system y^+L as the glucocorticoid regulated transport system in rat kidney, where this high-affinity transport system is produced from the heavy chain 4F2 and the light chain y^+LAT1 (Estévez et al., 1998; Torrents et al., 1998). System y^+L is expected to mediate preferentially under physiological conditions the cellular uptake of neutral amino acids and the efflux of cationic amino acids in the renal epithelium (Pfeiffer et al., 1999b). The pattern of expression of human y^+LAT1 and its chromosomal location identify y^+LAT1 as a candidate gene for lysinuric protein intolerance (Mykkänen et al., 2000). Lysinuric protein intolerance is an autosomal recessive disease in which transport of cationic amino acids lysine, arginine, and ornithine is defective. This effect has been localized at the basolateral membranes of epithelial cells in the renal tubules (Rajantie et al., 1981).

In Western blot analysis we found a glucocorticoid-induced upregulation of the 4F2 heavy chain in the rat kidney. The enhancement of 4F2hc protein abundance of about 45% was comparable to the increase of L-leucine uptake into BLMV and is also consistent with our previous observations (Schwertfeger et al., 2000) of a significant reduction in fractional leucine excretion by rat kidney *in vivo* after DEX treatment. These results raise the possibility that the functional properties of heterodimeric transporters are strongly dependent on the abundance and availability of heavy chain (in this case 4F2hc) protein. However, the question arises here as to why only system y^+L activity was induced after DEX treatment and not also system L activity.

In a few studies, regulation of 4F2hc and of different light chains has been investigated. Upregulation of 4F2hc mRNA levels and an enhanced system-L-like amino acid-transport activity after treatment with phorbol esters was shown previously in lymphocytes (Bröer et al., 1997). Coordinately induced 4F2hc and light chains were found in normal lymphocytes following mitogenic stimulation *in vitro* with concanavalin (Nakamura et al., 1999). Spindler et al. (1997) described upregulation of LAT1 while searching for early aldosterone-regulated gene products. Campell et al. (2000) found increased expression of TA1/LAT1 mRNA and system L activity in rat hepatic cell lines in response to arginine depletion, but no appreciable response of 4F2hc to the same stimulus. Kudo and Boyd (2000) found enhanced system L and y^+L activity following culture of human placental chorionic villi which correlated with increased expression of 4F2 heavy chain mRNA, but they found no changes in mRNA expression of the light chains.

Because of these conflicting reports, we investigated the mRNA abundance of the light chain y^+LAT1 using RT-PCR, and found a 4-times enhancement of its expression. Therefore, we suggest that functional expression of the heterodimeric transport system y^+L in kidney depends on the abundance of both the 4F2 heavy chain and the y^+LAT1 light chain, at least with respect to regulation by corticosteroid. The observation that only basolateral transport mechanisms for L-leucine are induced in the present study is consistent with the idea (e.g. as proposed by Cheeseman, 1992) that the basolateral membrane is the major locus for regulation of transepithelial transport of amino acids.

The lack of effect of T3 on expression of renal leucine transporters may indicate that effects of the hormone on renal leucine fluxes *in vivo* result from changes in transmembrane amino acid and/or ion gradients rather than abundance of transporter proteins.

In summary, we have shown the *in vivo* induction of the broad-scope amino acid transport system y^+L by glucocorticoids in rat kidney. We conclude that the abundance of 4F2hc in the basolateral membrane correlates with the overall activity of amino acid transport whereas the proportional abundance of the

light chains determines which heterodimeric transport systems were formed and inserted into the membrane. These results in connection with previous *in vivo* data of DEX-induced L-leucine reabsorption indicate an involvement of system y^+L in the regulatable tubular reabsorption of large neutral amino acids.

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