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Epidermal growth factor (EGF) increases the renal amino acid transport capacity in amino acid loaded rats

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Summary. In anaesthetized adult female rats, the influence of epidermal growth factor (EGF) on renal amino acid handling was investigated in glutamine, arginine (both 50 mg/100 g b.wt. per hour), or alanine (90 mg/100 g b.wt. per hour) loaded animals. Continuous infusions of the three amino acids were followed by an increase in the fractional excretion (FE) of the administered amino acids as well as of the other endogenous amino acids. Under load conditions (alanine, arginine or glutamine), EGF pretreatment (8 μg/100 g b.wt. subcutaneously for 8 days, twice daily 8 a.m. and 4 p.m.) was followed by a stimulation of renal amino acid reabsorption. The increase in the fractional excretion of the administered amino acids was significantly lower than in non-EGF-treated rats. These changes in amino acid transport were connected with a significant reduction of GFR after EGF pretreatment (0.96 ± 0.10 vs. 0.62 ± 0.07 ml/min × 100 g b.wt.) and a distinct increase in sodium excretion (2.98 ± 0.55 vs. 4.97 ± 0.71 μval/100 g b.wt. × 20 min). After loading with p-aminohippurate (PAH; 200 mg/100 g b.wt.), PAH excretion in EGF rats was increased by about 20%, whereas urinary protein excretion was lower in EGF pretreated rats (control: 0.45 ± 0.04 vs. EGF: 0.18 ± 0.03 mg/100 g b.wt. × 20 min). The PAH load reduced amino acid reabsorption as a sign of overloading of renal tubular transport capacity, but in EGF pretreated animals the amino acid excretion was only slightly increased under these conditions. Furthermore, EGF pretreatment depressed normal kidney weight gain significantly (874 ± 18 vs. 775 ± 32 mg/100 g b.wt.). EGF can improve the renal tubular transport capacity, but, compared to well-known stimulators of renal transport like dexamethasone or triiodothyronine, its effect is only of a moderate degree.

Key words: Amino acid transport – Kidney – Epidermal growth factor – Amino acid load – Alanine – Arginine – Glutamine – p-Aminohippurate – Rats

Introduction

Hormonal control of renal amino acid handling is poorly understood. Dexamethasone and triiodothyronine (T3) stimulate amino acid transport in

rats (Fleck et al., 1997). Furthermore, both dexamethasone and T3 increase the tubular secretion capacity for weak organic acids like p-aminohippurate (PAH; Bräunlich, 1984). The effect of other hormones or growth factors on the kidney is also rarely investigated. Epidermal growth factor (EGF) effects kidney function (Carpenter and Cohen, 1990). The kidney represents a major source of EGF (Konturek et al., 1990), EGF receptors could be found in renal tissue (Fischer et al., 1989), and EGF is transported within the kidney (Nielsen et al., 1989). It could be shown that EGF influences various renal functions:

- EGF induces diuresis and natriuresis (Breyer, 1988; Warden and Stokes, 1993),
- it modulates glomerular hemodynamics and renal metabolism (Harris, 1991),
- phosphorylation is stimulated and gluconeogenesis is inhibited in rat proximal tubules (Harris and Daniel, 1989),
- phosphate transport is stimulated in the rabbit proximal convoluted tubule (Quigley et al., 1995),
- the Na^+-dependent polyamine transport in renal epithelial cells is stimulated (Parys et al., 1990),
- the amino acid uptake into rat kidney cells increases (Boerner et al., 1985),
- the glucose transport in human renal proximal tubular cells is enhanced (Racusen et al., 1997).

EGF action on the kidney seems to be mediated and/or modulated by glucocorticoids and triiodothyronine. Dexamethasone increases EGF binding (Karasik et al., 1988) and EGF concentration in the kidney, but it reduces EGF plasma concentration (Tuomela et al., 1990). On the other hand, EGF receptor synthesis is reported to be reduced by dexamethasone (Oberg and Carpenter, 1991). T3 exerts synergistic control on the action of EGF (Fernandez-Pol et al., 1989a). After T3 treatment, EGF plasma concentration declined (Gresik and Maruyama, 1987), but T3 enhances renal tubular cell replication by stimulating EGF receptor gene expression in rat renal proximal tubular cells (Rogers et al., 1995).

We concluded that the stimulatory effects of dexamethasone and T3 on renal transport functions, measured in the above mentioned studies, could be evoked via EGF. To further characterize the effect of EGF on renal transport functions the influence of this growth factor on various renal transports was investigated in rats. Arginine, alanine, and glutamine were administered as continuous infusion to overload amino acid reabsorption carriers (Silbernagl, 1992). The three amino acids were chosen for the following reasons: The physiological concentrations of these amino acids are relatively high and their FE is relatively low. Thus, their renal reabsorption seems to be very effective. The amino acids are nearly non toxic. Therefore they can be administered in relatively high doses. Nevertheless, using amino acid loading, besides renal effects, metabolic actions of EGF (Fischer et al., 1989) must also be taken into consideration in *in vivo* experiments.

Material and methods

Animals

Investigations were performed in female Wistar rats (Han:Wist) of our institute's own out-bred stock. At the beginning of the experiments the animals were 2 months old, and the average body weight was 158 ± 6 g. Rats were fed a standard diet (Altromin 1316) and tap water ad libitum. Animals were housed under standardized conditions in plastic cages, light-dark cycle 12/12 hours, temperature 22 ± 2°C, humidity 50 ± 10%.

Experimental design

The rats were anaesthetized with ketamine (Ursotamin® Serumwerk Bernburg, F.R.G., 7.5 mg/100 g b.wt.) and xylazine (Ursonarkon® Serumwerk Bernburg, F.R.G., 1.2 mg/100 g b.wt.). Both substances were administered intramuscularly. A catheter was placed in a tail vein. The animals were then infused isotonic saline containing 4 g/l fluorescein isothiocyanate (FITC)-inulin (Bioflor, Uppsala, Sweden) at 4 ml/100 g b.wt. per hour for the remainder of the experiment. Thereafter a polyethylene catheter was inserted into the urinary bladder via urethra. To minimize urine collecting periods for the determination of GFR and fractional amino acid excretion (FE), urine was collected in 20-minutes periods for 3 hours. In previous experiments it could be shown that under these experimental conditions both hematocrit (Fleck et al., 1992) and blood pressure (Fleck and Bräunlich, 1986) remain nearly constant during the clearance study. In the middle of each period and at the end of the experiment blood was collected from the retrobulbar plexus.

The renal excretion of PAH (E. Merck, Darmstadt) was measured under the same experimental conditions after the intravenous administration of 200 mg PAH/100 g b.wt., dissolved in 2 ml distilled water. This dose saturates reliably the transport capacity in adult rats (Bräunlich et al., 1983).

Glomerular filtration rate (GFR) was determined by inulin clearance. Inulin concentration was measured fluorometrically using FITC-inulin in blood and urine samples (Sohtell et al., 1983).

Amino acid load

Rats were loaded with glutamine or arginine (each 50 mg/100 g b.wt.) or alanine (90 mg/100 g b.wt.). Amino acid doses were determined in previous experiments. The amino acids were administered as a continuous infusion together with inulin in 4 ml normal saline per 100 g b.wt. × hour. It was the goal of the experimental schedule to enhance amino acid plasma concentration distinctly, whereas toxic effects of amino acids should be prevented. The injection solutions of alanine, arginine, and glutamine had osmolarities of 380, 365, and 400 mosmol, respectively, and were adjusted at neutral pH. Control animals received the same volume of normal saline containing FITC-inulin.

Treatment with epidermal growth factor

Epidermal growth factor ([Des-Leu26/Cys(Acm)20/31]EGF (20–31) = EGF; BACHEM Bubendorf, Switzerland) was administered in a dose of 8 μg/100 g b.wt. subcutaneously for 8 days, twice daily at 8 a.m. and 4 p.m. EGF was dissolved in normal saline (1 ml/100 g b.m.). This EGF dose, according to literature, influences various organ functions effectively (Norman et al., 1990; Yamamoto et al., 1993; Wang et al., 1996). The subcutaneous route of administration is suitable to obtain sufficient EGF plasma levels in the rat (Vinterjensen et al., 1996). Controls received normal saline only.

Determination methods

Amino acid determination

The determination of amino acids by column chromatography with fluorescence (Roth and Hampai, 1973) has been described in detail elsewhere (Silbernagl, 1983). Proteins

were removed from urine and plasma samples by administration of trichloroacetic acid. After centrifugation, the supernatant was neutralized by adding NaOH. Then the samples were diluted with citrate buffer and analyzed by HPLC on an amino acid analyzer (Knauer, Berlin, F.R.G.) with o-phthalaldehyde as a fluorescent amino ligand (Roth, 1971). Calibration runs were performed with freshly prepared amino acid solutions composed of analytical grade amino acids (Serva, Heidelberg, F.R.G.)

Inulin in blood and urine

Fluorescence was measured at 480 nm excitation and 520 nm emission wave lengths in a HITACHI F-2000 spectrofluorometer (Hitachi Ltd., Tokyo, Japan).
PAH in urine was determined using the method of Bratton and Marshall (1939).
Protein in urine was estimated by a protein-dye binding technique (Bradford, 1976).
Sodium and potassium in urine were measured by flame photometry.

Statistics

The results are given as means ± S.E.M. with n = 5–6 in each group. The level of significance was $p \leq 0.05$ (Mann-Whitney-test).

Results

Under alanine, arginine or glutamine infusions both plasma amino acid concentration and fractional excretion increased significantly as a sign of overloading of reabsorption capacity (Fig. 1). Interestingly, for glutamine the increase in FE is the lowest, despite a distinct increase in its plasma concentration.

In control rats without amino acid load, EGF pretreatment caused a significant reduction in GFR (Fig. 2, lower part). However, after amino acid infusions GFR raised in EGF pretreated animals and was not further different in controls and EGF pretreated rats. With the exception of alanine infusion in non-EGF-pretreated controls, neither amino acid infusion nor EGF pretreatment influenced urine flow rates (Fig. 2, upper part).

During continuous infusion of alanine, arginine or glutamine, the fractional excretion of these three amino acids remained distinctly reduced in EGF pretreated rats during the whole clearance period (Fig. 3). But the stimulation of amino acid reabsorption, that means, the decrease in FE is not uniform after the three amino acids: under arginine infusion the FE drops down to 1/3 of controls, under glutamine FE is diminished to $^1/_2$ of controls, but under alanine infusion the EGF effect became distinct only during the first clearance period (only this value is given in Fig. 1).

Under amino acid load the tubular reabsorption of the physiological amino acids, not administered in the coutinuous infusions, increased distinctly for most amino acids. During saline infusion and, more pronounced under amino acid load conditions, the FE of most amino acids is lower in EGF pretreated animals than in controls without EGF pretreatment (not shown).

EGF retarded the normal kidney wet weight gain during the eight days of pretreatment (Table 1) whereas the body weight was not affected by EGF.

To further clarify EGF effects on the kidney, additional experiments were performed measuring the basolateral transport of PAH and the influence of a

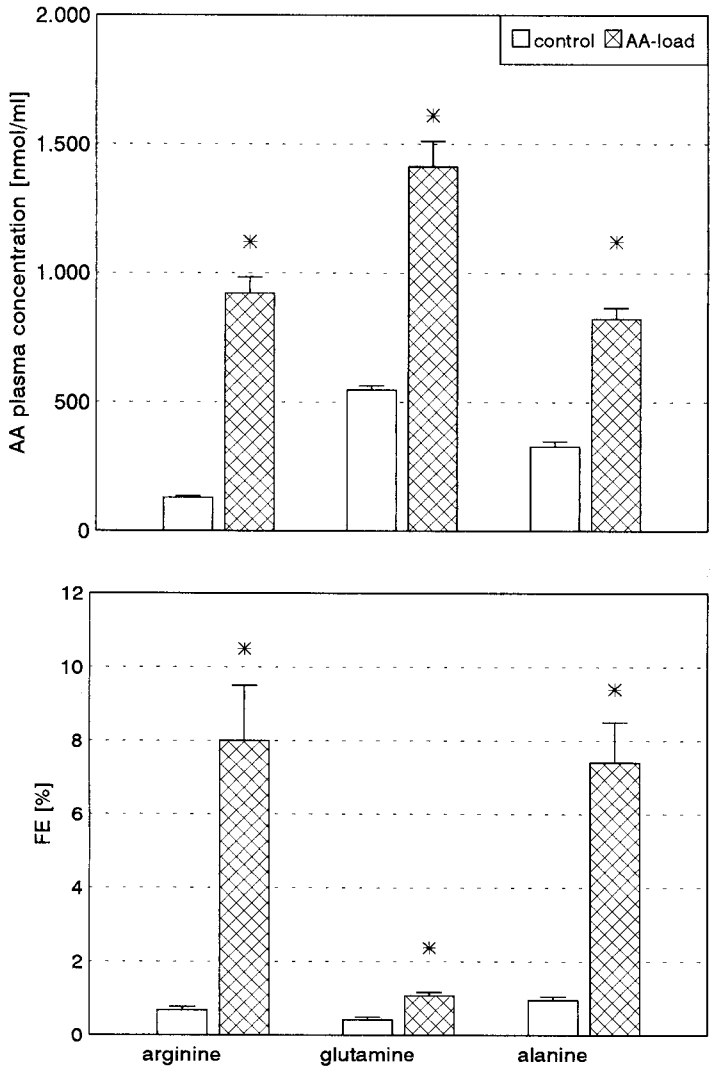

Fig. 1. Influence of continuous infusion of arginine, glutamine, or alanine (cp. method) on their plasma concentrations (upper part) and fractional excretions (*FE*; lower part) in adult rats. Arithmetic means ± S.E.M., n = 6. * – significantly different from saline loaded controls ($p \leq 0.05$)

PAH load on the other renal transport processes. EGF pretreatment enhanced PAH excretion significantly by about 20% (Fig. 4). PAH administration was followed by a significant osmotic diuresis, which disappeared already 40 minutes after PAH injection (Fig. 5). EGF had no influence on urine flow before and after PAH bolus, whereas it depressed GFR both before (see also Fig. 2) and after PAH load.

As expected, the urinary excretion of sodium and potassium increased significantly after loading with PAH-sodium salt (Table 1). Under load conditions, electrolyte excretion was similar in controls and EGF pretreated rats whereas sodium excretion was significantly higher in EGF-rats before PAH

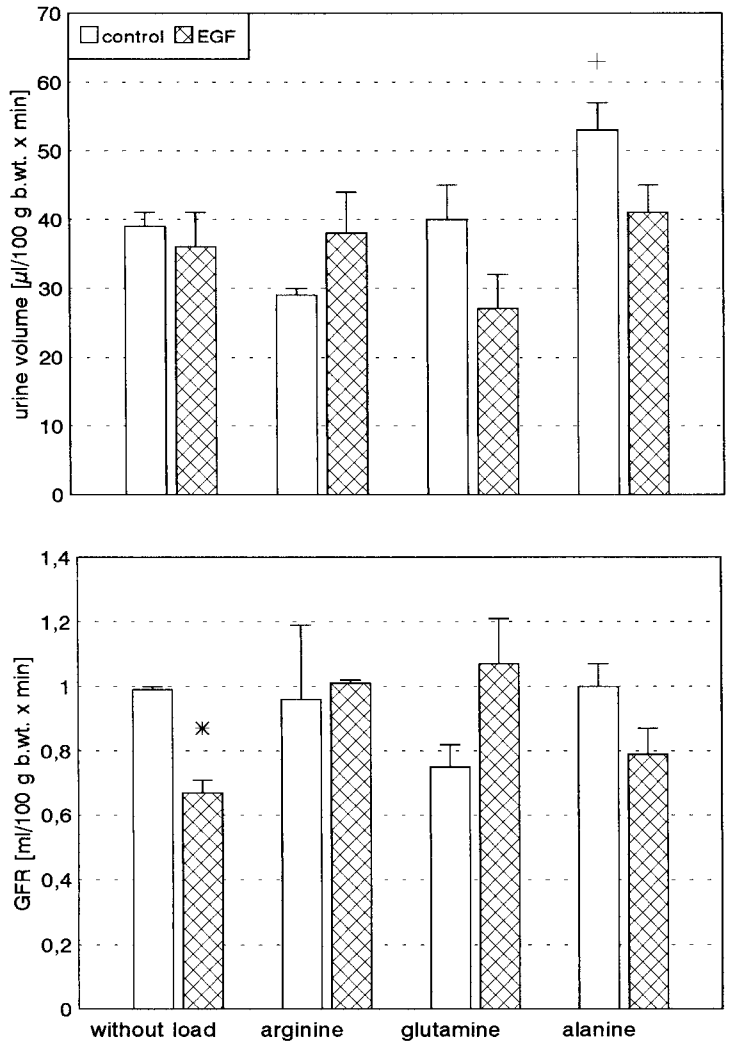

Fig. 2. Influence of continuous infusion of arginine, glutamine, or alanine and/or of pretreatment with EGF (cp. method) on urine volume (upper part) and GFR (lower part) in adult rats. Arithmetic means of nine 20-min-clearance periods ± S.E.M., n = 6. * – significantly different from NaCl-pretreated rats ($p \leq 0.05$) + – significant effect of amino acid infusion ($p \leq 0.05$)

bolus injection. The protein excretion into urine increased significantly in control animals after PAH load, but in EGF pretreated rats the increase in urinary protein excretion was prevented.

In control rats, PAH load was followed by a distinct reduction of the tubular amino acid reabsorption: for all amino acids the fractional excretion was enhanced; in 12 of 20 cases this increase was significant (Table 2). In the EGF pretreated group, the renal amino acid handling was nearly not influenced by EGF before PAH bolus injection, but under PAH loading the fractional excretion of most amino acids was increased only to a lower degree compared to PAH loaded controls without EGF pretreatment.

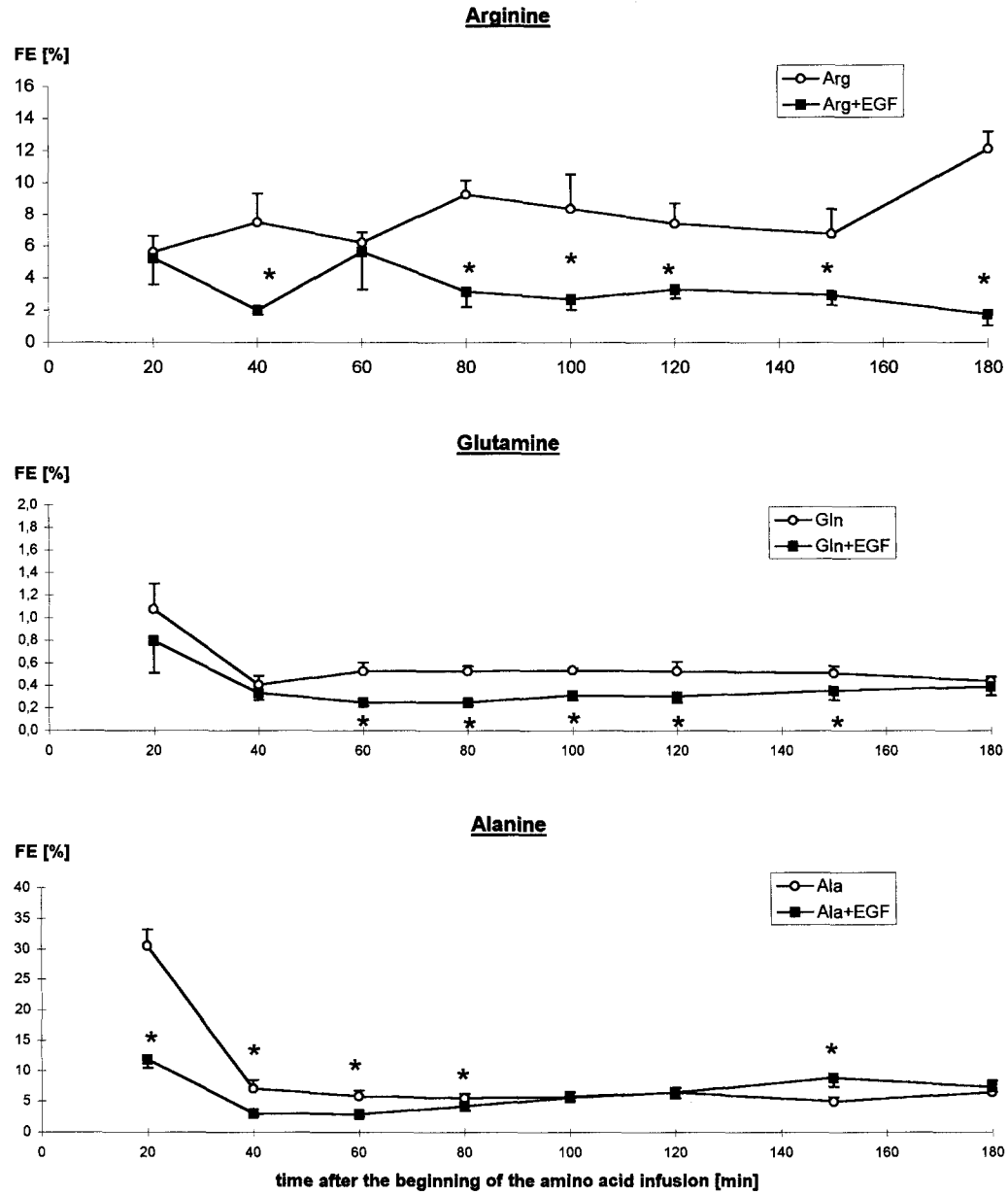

Fig. 3. Influence of EGF pretreatment (cp. method) on the time course of the fractional excretions (*FE*) of arginine, glutamine, and alanine, respectively, under continuous infusion of these amino acids. Arithmetic means ± S.E.M., n = 6. * – significantly different from NaCl-pretreated rats ($p \leq 0.05$)

Discussion

EGF is involved in the regulation of cell proliferation and modulates growth (Boonstra et al., 1995). A possible regulatory role for EGF in the membrane transport in the nephron has been suggested more than ten years ago (Salido et al., 1986). Furthermore, the effect of EGF at the cellular level is reported to

Table 1. Influence of pretreatment with EGF on renal excretion of electrolytes and protein and on body and kidney weights in rats before and immediately after loading with PAH (200 mg/100 g b.wt.)

	Before PAH load		After PAH load	
	control	EGF	control	EGF
Electrolyte excretion [μval/100 g b.wt. × 20 min]				
sodium	2.98 ± 0.55	4.97 ± 0.71*	20.42 ± 3.13°	24.11 ± 2.67°
potassium	1.39 ± 0.12	1.55 ± 0.07	2.34 ± 0.21°	2.52 ± 0.32°
$Q_{Na/K}$	2.06 ± 0.20	3.12 ± 0.36*	8.72 ± 0.95°	8.45 ± 0.73°
Protein excretion [mg/100 g b.wt. × 20′]	0.18 ± 0.02	0.17 ± 0.01	0.45 ± 0.08°	0.18 ± 0.03*
Body weight [g]			185.3 ± 20.1	201.2 ± 18.4
Kidney weight [mg/100 g b.wt.]			874 ± 18	775 ± 32*

Arithmetic means ± S.E.M.; n = 6.
*– significant difference between controls and EGF pretreatment ($p \leq 0.05$); °– significant effect of PAH load ($p \leq 0.05$).

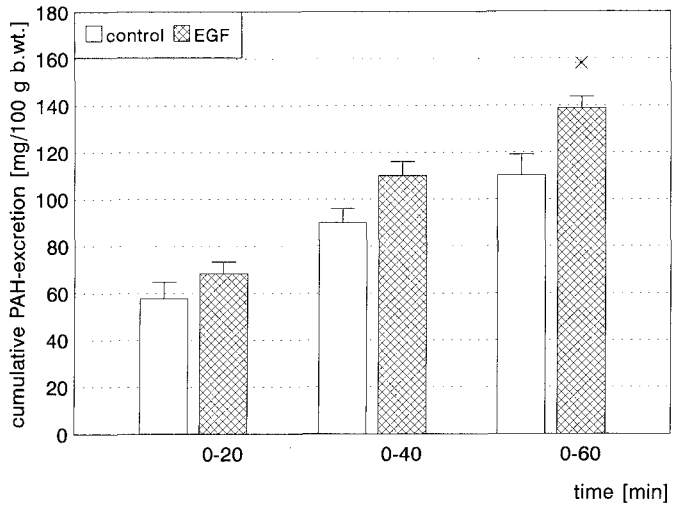

Fig. 4. Cumulative excretion of para-aminohippurate (*PAH*) in NaCl- and EGF-pretreated rats. Arithmetic means ± S.E.M., n = 6. * – significant EGF effect ($p \leq 0.05$)

be mediated or to be modified by glucocorticoids or T3 (Scott et al., 1995; Rogers et al., 1995). Therefore the hypothesis should be proven whether or not EGF is able to influence renal tubular transport processes as found for dexamethasone or T3.

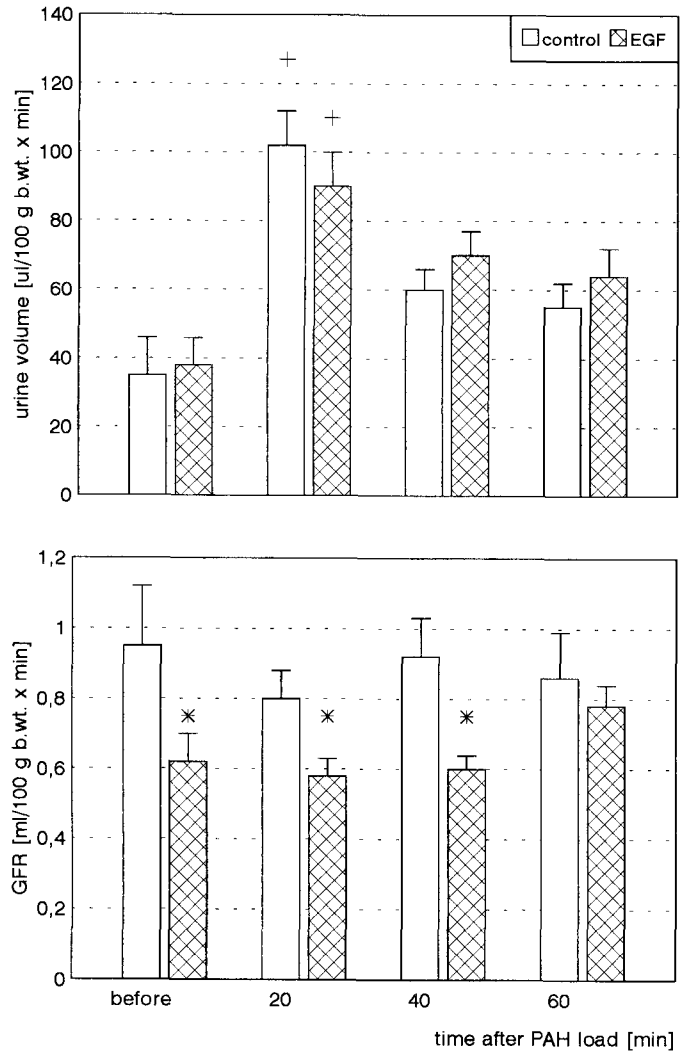

Fig. 5. Influence of EGF pretreatment (cp. method) on the time course of urine volume (upper part) and GFR (lower part) in rats administered a bolus injection of para-aminohippurate (*PAH*, 200 mg/100 g b.wt.). Arithmetic means ± S.E.M., n = 6. * – significantly different from NaCl-pretreated rats ($p \leq 0.05$); + – significant effect of the PAH bolus ($p \leq 0.05$)

From our results it can be concluded that

- EGF stimulates the tubular reabsorption of amino acids,
- it enhances the tubular secretion of PAH, and
- it reduces GFR, sodium reabsorption, and kidney wet weight.

The latter point can be explained as follows: It could be shown in previous studies that EGF decreases sodium reabsorption in the renal tubules (Vehaskari et al., 1991). Furthermore, EGF is described to act as a diuretic (Gow and Phillips, 1994). These two reasons could be responsible for a reduction in GFR as a consequence of the tubuloglomerular feedback regula-

Table 2. Influence of pretreatment with EGF on the fractional excretions (%) of amino acids in rats before and immediately after loading with PAH (200 mg/100 g b.wt.)

	Control		EGF	
	before PAH	after PAH	before PAH	after PAH
Acidic amino acids				
Asp	9,46 ± 1,71	16,00 ± 2,92	14,36 ± 2,88	11,13 ± 0,52
Glu	2,88 ± 0,68	3,38 ± 0,49	2,81 ± 0,57	0,83 ± 0,14*+
Basic amino acids				
Arg	1,70 ± 0,35	1,97 ± 0,24	0,47 ± 0,11+	0,79 ± 0,07*+
Lys	0,35 ± 0,08	0,97 ± 0,14*	0,42 ± 0,06	0,55 ± 0,05
His	1,18 ± 0,23	3,00 ± 0,43*	0,86 ± 0,10	2,10 ± 0,16*
1MH	7,48 ± 1,94	16,19 ± 1,86*	1,96 ± 0,45+	3,58 ± 0,37*+
Neutral amino acids: aliphatic				
Gly	5,36 ± 0,98	9,21 ± 1,12*	3,81 ± 0,68	5,95 ± 0,43
Ala	1,58 ± 0,29	3,24 ± 0,41*	1,68 ± 0,36	1,79 ± 0,10
Ser	2,44 ± 0,53	4,97 ± 0,57*	1,46 ± 0,21	1,98 ± 0,13
Thr	1,46 ± 0,25	3,26 ± 0,43*	0,52 ± 0,05	1,27 ± 0,09*+
Val	1,81 ± 0,54	3,16 ± 0,38	1,21 ± 0,23	3,31 ± 0,24*
Leu	1,20 ± 0,23	3,05 ± 0,35*	0,81 ± 0,14	1,71 ± 0,13*
Ile	1,26 ± 0,25	3,39 ± 0,46*	0,65 ± 0,12	1,25 ± 0,08*+
Met	3,75 ± 0,74	5,65 ± 0,83	0,55 ± 0,14	3,48 ± 0,36*
	aromatic			
Phe	2,26 ± 0,53	4,11 ± 0,51*	6,06 ± 0,91	5,42 ± 0,51
Tyr	1,02 ± 0,1	1,80 ± 0,31	1,30 ± 0,27	1,10 ± 0,22
	ω-amides			
Asn	1,44 ± 0,29	2,59 ± 0,35*	2,39 ± 0,32	2,55 ± 0,23
Gln	0,84 ± 0,19	1,46 ± 0,18	0,75 ± 0,21	0,74 ± 0,13+
Others				
β-Ala	14,15 ± 3,58	14,05 ± 1,06	10,58 ± 2,18	4,48 ± 0,55*+
Tau	15,93 ± 2,46	23,60 ± 1,95*	22,67 ± 4,00	18,69 ± 1,83

Asp aspartic acid; *Glu* glutamic acid; *Arg* arginine; *His* histidine; *1MH* 1-methylhistidine; *Gly* glycine; *Ala* alanine; *Thr* threonine; *Leu* leucine; *Ile* isoleucine; *Met* methionine; *Phe* phenylalanine; *Tyr* tyrosine; *Asn* asparagine; *Gln* glutamine; *β-Ala* β-alanine; *Tau* taurine. Arithmetic means ± S.E.M.; n = 6. + – significant difference between controls and EGF pretreatment ($p \leq 0.05$); * – significant effect of PAH load ($p \leq 0.05$).

tion (Thurau, 1975). Beside these renal effects, a prolonged decrease in blood pressure found in conscious rats treated subchronically with EGF (Keiser and Ryan, 1996) could contribute to the fall in GFR. In contrast to the latter paper, reporting an increase in kidney weight after 4 weeks pretreatment with EGF, in our study the kidney weight was reduced, but only to a slight degree. This discrepancy can be explained by the different times of pretreatment: 4 weeks vs. 8 days.

As expected, under amino acid load, plasma concentrations and fractional excretion of the administered amino acids increased significantly. Therefore, the capacity of the transport carriers for alanine, arginine, and glutamine

seems to be employed to capacity after administration of the respective amino acid.

The amino acid load, however, also interferes with the transport of endogenous amino acids which were not administered. The reason for this phenomenon could be

- a direct competition at the amino acid carrier sites in the kidney between the amino acids administered and endogenous amino acids using the same carriers (Christensen, 1990),
- a decrease of ATP, and, therefore, a reduction of the Na^+-gradient in the renal tubuli (Gutmann et al., 1993),
- the reduced contact time in the nephron because of the increased urine flow.

A three-days-administration of T3 or dexamethasone significantly reduces the fractional excretions of leucine (T3 and dexamethasone) and glutamine (dexamethasone only) in amino acid loaded rats (Fleck et al., 1997). Obviously the various amino acid carriers are differently influenced by the two hormones. Basing on these results, the influences of EGF on the tubular reabsorption of amino acids and on the tubular secretion of PAH were investigated in this study. The stimulation phenomenon seems to be a sign of improved transport capacity in the renal tubules after pretreatment with EGF. As mentioned in the introduction, EGF can influence a variety of transport processes. Boerner et al. described already in 1985 a stimulation of the amino acid uptake in rat kidney cells. Quigley et al. (1995) found a dose-dependent stimulation of proximal convoluted tubule phosphate transport by EGF. Moreover, the transport of polyamines and glucose is reported to be increased after EGF (Parys et al., 1990; Racusen et al., 1997). Also in liver cells, EGF increases the transport capacity of the cell membrane: it activates the Na^+/H^+-antiport in adult rat hepatocytes (Incerpi et al., 1996). Few reports in the literature are contradictory, e.g. dexamethasone acts as a negative regulator of EGF receptor synthesis (Oberg and Carpenter, 1989), and, therefore, dexamethasone and EGF are counteracting concerning metallothionine gene expression (Moffatt et al., 1995). On the other hand, T3 is reported to down-regulate EGF receptors, too (Vesey et al., 1993), whereas T3 seems to act synergistically with various growth factors like EGF (Fernandez-Pol et al., 1989b). Altogether, the findings mentioned above, suggest that EGF can enhance transport capacities at various cell membranes. Using PCR techniques, it should be possible to clarify, whether or not the stimulatory effect of EGF is mediated by an increase in carrier protein synthesis. Compared to the renal effects of dexamethasone and T3, the effects of EGF on renal transport functions resembles more that of dexamethasone than of T3. However, a reason for this coincidence between the two effects can not yet reliably be given.

A further interesting result of this study is the influence of PAH loading on tubular amino acid reabsorption: after PAH bolus injection the fractional excretion of amino acids increased as a result of reduced amino acid reabsorption capacity. The opposite is described as well: alanine perfusion of

the isolated rat kidney decreases net PAH secretion by 50% (MacDougall and Wiegmann, 1988). This interaction could reflect a shortage of energy rich substrates within the proximal tubular cell caused by increased PAH transport and followed by diminution of amino acid reabsorption.

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