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Influence of an extracellular volume expansion (ECVE) on renal amino acid- and sodium handling in patients with autosomal dominant polycystic kidney disease (ADPKD)

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Summary. Although ADPKD is one of the first kidney diseases to be understood from the gene to the pathogenesis of clinical abnormalities, there were no data concerning the renal handling of amino acids and possible disorders of amino acid (AA) pattern in these patients. Therefore, in 9 patients suffering from ADPKD and in 8 healthy normal persons (NP) renal amino acid excretion was measured before and after extracellular volume expansion (ECVE) (2l of physiological electrolyte solution). Renal function was stable in both groups (serum creatinine: ADPKD: 85.1 ± 18.4 vs. NP 84.4 ± 13.5 µmol/l; GFR: 93.8 ± 16.4 vs. 104.4 ± 9.4 ml/min/1.73 m^2). Mean blood pressure was higher in ADPKD patients than in NP (99.4 ± 2.6 vs. 85.5 ± 2.4 mmHg), but did not change after ECVE. After ECVE in both groups, urine volume increased distinctly, whereas GFR was only slightly enhanced. The plasma concentrations of leucine, glycine, valine, threonine, glutamine, and alanine were significantly higher in controls than in ADPKD patients. The amino acid reabsorption capacity was reduced in ADPKD patients in 12 of 21 amino acids before ECVE. After ECVE, the fractional excretion of amino acids (FE_{AA}) increased only in NP. In parallel with changes in amino acid handling, the FE_{Na} (%) after ECVE increased both in ADPKD patients and in NP (before ECVE – ADPKD: 1.22 ± 0.23 vs. NP: 1.53 ± 0.23; after ECVE: 3.17 ± 0.25 (ADPKD) vs. 2.74 ± 0.22/NP; (ADPKD $p \leq 0.01$, NP $p \leq 0.02$) whereas FE_{Li} (%) increased significantly only in ADPKD ($p \leq 0.045$) range (before ECVE – ADPKD: 25.8 ± 8.9 vs. NP: 20.5 ± 4.0; after ECVE: 41.4 ± 5.4 vs. 25.2 ± 3.9). Furthermore, concentrations of cGMP (pmol/ml) in plasma increased after ECVE (before ECVE – ADPKD: 5.31 ± 0.56 vs. NP: 6.65 ± 0.79; after ECVE: 11.31 ± 1.66 vs. 11.30 ± 1.91; $p \leq 0.05$). Na^+-dependent and, perhaps, NO-mediated processes in the reabsorption of AA in the proximal tubule seem to be different in ADPKD and may be related to different distributions of receptors and ATP-dependent transport systems with pathogenetic impact on abnormal transtubular fluid transport in ADPKD.

Keywords: Amino acids – ADPKD – Renal failure – Renal amino acid-/sodium handling – Hypertension – Nitric oxide – Cyclic GMP – Na^+/K^+-ATPase

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary disease occurring in one out of 200 to 1,000 individuals. ADPKD is characterized by cystic lesions in the kidneys and often by structural abnormalities in the gastrointestinal tract and cardiovascular system (Zeier et al., 1988; Fick, 1992; Zerres, 1992).

In recent years there has been a flurry of investigative activity with the identification of two chromosomes (4 and 16) carrying a putative gene (Reeders et al., 1985; Kimberling et al., 1988; Fick, 1992), with description of the early phases of the disorder (Gabow et al., 1984), and with broadening of the concept of the disorder into that of a systemic disease.

ADPKD has been shown to be associated with a greater than 50 % incidence of hypertension prior to deterioration in renal function as assessed by glomerular filtration rate. Thus it appears that hypertension is an early event in the natural history of ADPKD (Zeier et al., 1988).

A further observation is an abnormal distribution of epidermal growth factor receptor and of the Na^+/K^+-ATPase in tubular epithelial cells. Normally located at the basolateral membrane of the collecting duct there is a persistence of apical membrane Na^+/K^+-ATPase in patients with ADPKD (Arner et al., 1992; Fick, 1992; Grünfeld et al., 1992; Saggar-Malik et al., 1994). This finding may have pathogenetic importance in abnormal transtubular fluid transport; i.e. secretion instead of reabsorption of sodium into the cysts lumen, due to altered polarity of Na^+/K^+-ATPase (Wilson et al., 1991; Ritz et al., 1993). With a deterioration of kidney function, both proximal and distal tubular reabsorption of sodium is reduced and the accompanying changes of atrial natriuretic peptide and urinary excretion of prostaglandin E_2 may be compensatory phenomena counteracting declining GFR (Sørensen et al., 1990). Furthermore a volume expansion in ADPKD-patients yielded an increased fractional excretion of sodium with a significant shift of the pressure-natriuresis regression line to the right (Torres et al., 1989).

To the best of our knowledge there are no data regarding the management of amino acids and possible disorders of aminoaciduria or amino acid patterns in patients with ADPKD.

Patients and methods

Experimental design

1. Patients

A total of 9 patients (3 male, 6 female) with ADPKD and 8 healthy normal persons (NP; 3 male, 5 female) sex- and age-matched (34.6 ± 13.0 vs. 32.7 ± 11.4 years) were examined. No significant difference in body-mass-index was existing between the two groups (23.7 ± 4.0 vs. 22.1 ± 2.3). Renal function was stable in both groups (serum creatinine: ADPKD:

85.1 ± 18.4 vs. NP 84.4 ± 13.5 μmol/l; GFR: 93.8 ± 16.4 vs. 104.4 ± 9.4 ml/min/1.73 m^2). 24-h-ambulatory blood pressure measurement (Space Labs® model 90207, Redmond Inc., Washington, USA) yielded MAP of 84.5 ± 5.9 mm Hg in NP vs. 95.1 ± 5.7 mm Hg in ADPKD ($p < 0.01$).

Both groups were instructed to keep a strict sodium-reduced diet of 20 mmol/d sodium supplemented by 80 mmol/d of "slow-release-salt-tablets" (Ciba® Lab's, Horsham, England) for 7 days. Dietary protein intake was 1 g/kg body weight. During the 5 days preceding the study, compliance was monitored by 24-hour-urine collections (mean urine sodium: 100 mmol/d, mean urine urea: 300 mmol/d). All antihypertensive medication was withdrawn 3 days before the first examination.

2. Treatment regimen

On days 6 and 7, patients were admitted to the ward. All measurements were carried out in fasting patients, starting at 7 a.m. after a 30 min rest in supine position in a quiet room. Both groups were hydrated with 600 ml of tea in the first and 400 ml in the following hours. Continuous blood pressure measurements were carried out at 20-min intervals during the day and at 30-min intervals during the night (10 p.m. to 6 a.m.) or every 6 min during volume expansion. Blood samples were collected to detect baseline levels of electrolytes and creatinine at 7 a.m. On day 7, cGMP in plasma, FE_{Na} and amino acids in plasma and urine were estimated. Two clearance measurements were performed between 9 and 10 a.m. (OSO) and between 12 a.m. and 1 p.m. (IISO). Between 10.30 and 11.30 a.m., 2 l of a physiological electrolyte solution (E 153®, Serumwerk Bernburg, Germany) were administered intravenously within two hours under standardized conditions via an ultraprecise infusion pump (imed 960®, San Diego, USA) to produce an extracellular volume expansion (ECVE). Hydration status was monitored by measuring the right atrium diameter – two-chamber-view (TCV) – before and 30 min after ECVE using echocardiography (ving med 800®, Sonotron, Magdeburg, Germany).

Determination methods

1. GFR and ERPF-measurements

Clearance measurements were performed as previously described in detail (Schmidt et al., 1990). In brief, patients received a priming injection of 1,500 mg inulin/m^2 (Inutest®, Laevosan GmbH, Linz, Austria) and 500 mg PAH/m^2 body surface area (BSA) (Nephrotest®, Biologische Arbeitsgemeinschaft Lich, Germany), respectively. Subsequently, the patients received an infusion of inulin (10 mg/m^2 BSA/min) and PAH 8,8 mg/m^2 BSA/min) via a perfusion pump (Perfusor®, Braun, Melsungen, Germany) at a rate of 4.6 ml/min. Urine was collected at 30-min intervals. Inulin was measured enzymatically with inulinase (Boehringer Mannheim, Germany) in accordance with Schmidt et al. (1990) and PAH by colorimetry in accordance with Bratton and Marshall (1939). GFR and ERPF measurements were performed before and directly after ECVE.

2. Amino acid determination

The determination of amino acids by column chromatography with fluorescence detection was done according to Roth and Hampai (1973) and has been described in detail elsewhere (Silbernagl, 1983). In brief, proteins were removed from urine and plasma samples by administration of trichloroacetic acid. After centrifugation, the supernatant was neutralized by adding 0.4 N NaOH. Then the samples were diluted with citrate buffer and analyzed by HPLC on an amino acid analyzer (Knauer, Berlin, Germany) 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, Germany).

3. Determination of cGMP

Cyclic GMP was determined radiochemically in accordance with Domino et al. (1991) using a RIA (IBL Gesellschaft für Immunochemie und Immunobiologie mbH, Hamburg, Germany).

4. Measurement of the fractional excretion of sodium and lithium

FE_{Na} (%) was calculated from clearances of creatinine and sodium. Creatinine was determined in accordance with the Jaffé method (Lustgarten and Wenk 1972); sodium concentrations in urine and plasma were detected by flame photometry. FE_{Li} (%) was calculated as previously described in detail (Thomsen, 1984; Strazzullo et al., 1988) after 450 mg oral lithium-carbonate load (= 12.2 mmol of elemental lithium) measured by atomic absorption spectrophotometry (AAS 5 EA®, Analytic Jena GmbH, Germany).

Statistical analysis

Arithmetic means ± S.E.M. were calculated; differences between the means were statistically analyzed using the MANN-WHITNEY-test.

Results

As shown in Fig. 1, urine volume and GFR were comparable in ADPKD patients and healthy normal persons (NP) before volume load. ERPF (ml/min/1.73 m^2) did not change significantly in both groups before and after ECVE (Cl_{PAH} before ECVE: ADPKD: 484.2 ± 112.4; NP: 573.4 ± 115.3; after ECVE: ADPKD: 540.3 ± 123.8; NP: 621.1 ± 124.6).

The baseline MAP was different in ADPKD patients (99.4 ± 2.6 mm Hg) and NP (85.5 ± 2.4 mm Hg), but did not change after ECVE. After infusion of 2 l of electrolyte solution, urine volume increased distinctly in both groups, with GFR beeing significantly enhanced.

There were differences between amino acid plasma concentrations of ADPKD patients and controls (Fig. 2); leucine, glycine, valine, threonine, glutamine, and alanine concentrations were significantly higher in controls. The ECVE had no effect on amino acid plasma concentrations in both groups. The amino acid reabsorption capacity was reduced for 11 of 20 AA before ECVE in ADPKD patients, i.e. the fractional excretion of these amino acids (FE_{AA}) is distinctly higher (Fig. 3). On the other hand, the FE_{AA} of leucine, alanine, tryptophane, glutamic acid, taurine, arginine, glutamine, and histidine is not significantly different from controls. Surprisingly, FE_{AA} increased after ECVE, preferentially in the control group. Therefore, fractional excretion after ECVE is not longer different in controls and ADPKD patients (Fig. 4). The changes of fractional excretion before and after ECVE are

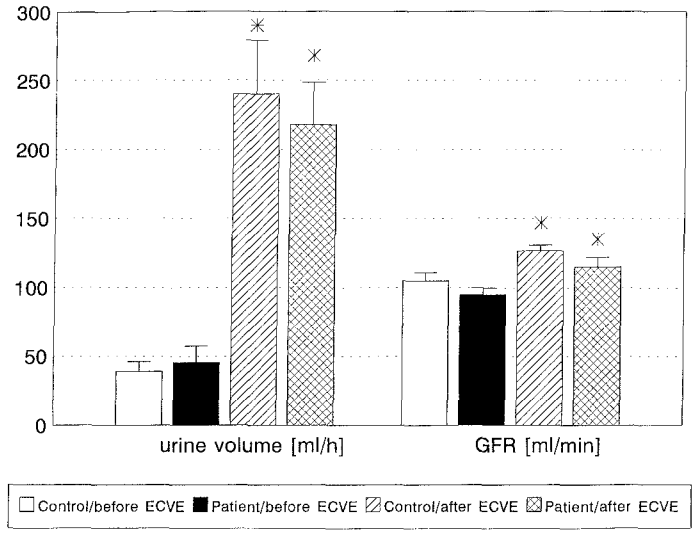

Fig. 1. Urine volume and GFR before and after ECVE in patients with ADPKD and in healthy controls (cf. methods). Arithmetic means ± S.E.M.; n = 8–9. Asterisks indicate significant changes after ECVE ($p \leq 0.05$)

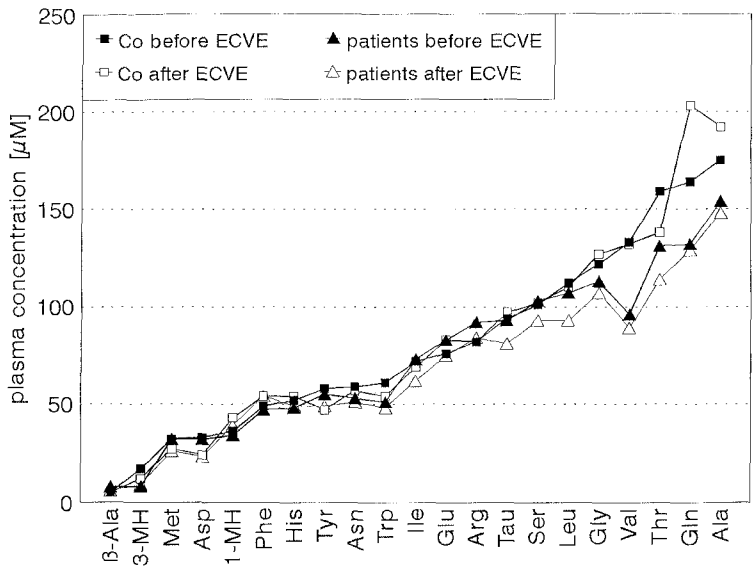

Fig. 2. Amino acid plasma concentrations in patients with ADPKD and in healthy controls before and after ECVE (cf. methods). Amino acids are arranged in increasing order for controls before loading. Arithmetic means are given; n = 8–9

shown in Fig. 5. In ADPKD patients, FE_{AA}-values are mostly unchanged or entirely doubled. However, with the exception of alanine, glutamic acid and valine, FE_{AA} increased about 3- to 8-fold, with a mean of 5.2-fold after ECVE in healthy controls.

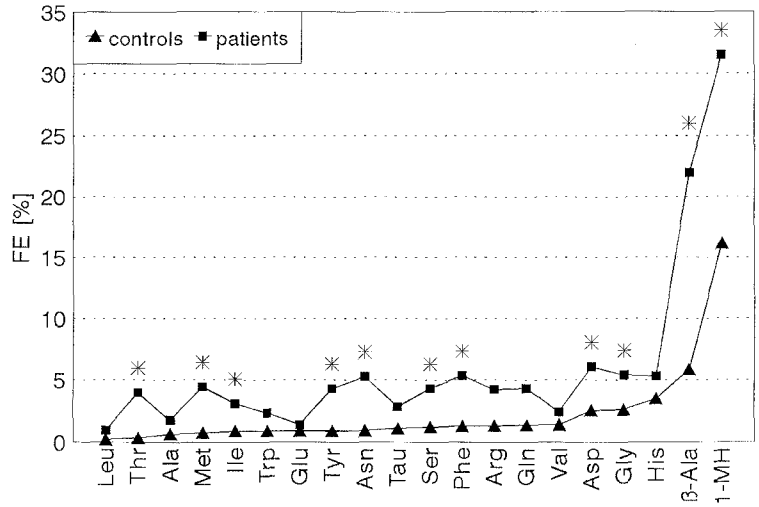

Fig. 3. Fractional excretion (*FE*) of amino acids before ECVE in ADPKD patients and in healthy controls (cf. methods). Arithmetic means are given; n = 8–9. Asterisks indicate significant differences between both groups ($p \leq 0.05$)

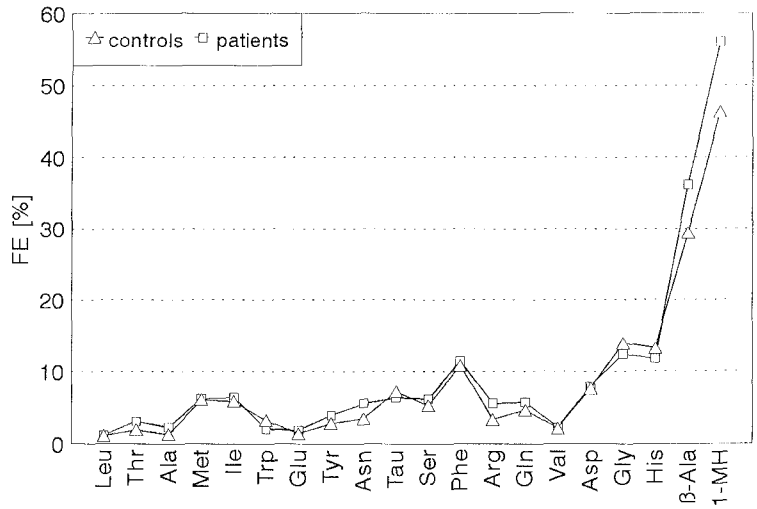

Fig. 4. Fractional excretion (*FE*) of amino acids after ECVE in ADPKD patients and in healthy controls (cf. methods). Arithmetic means are given; n = 8–9

In parallel to changes in amino acid handling, the FE_{Na} (%) after ECVE increased both in ADPKD patients and in NP (before ECVE – ADPKD: 1.22 ± 0.23 vs. NP: 1.53 ± 0.23; after ECVE – ADPKD: 3.17 ± 0.25 vs. 2.74 ± 0.22, ADPKD $p \leq 0.01$; NP $p \leq 0.02$) supported by the FE_{Li}(%) with a significant ($p \leq 0.05$) increase in ADPKD after ECVE only (before ECVE – ADPKD: 25.8 ± 8.9 vs. 20.5 ± 4.0; after ECVE –

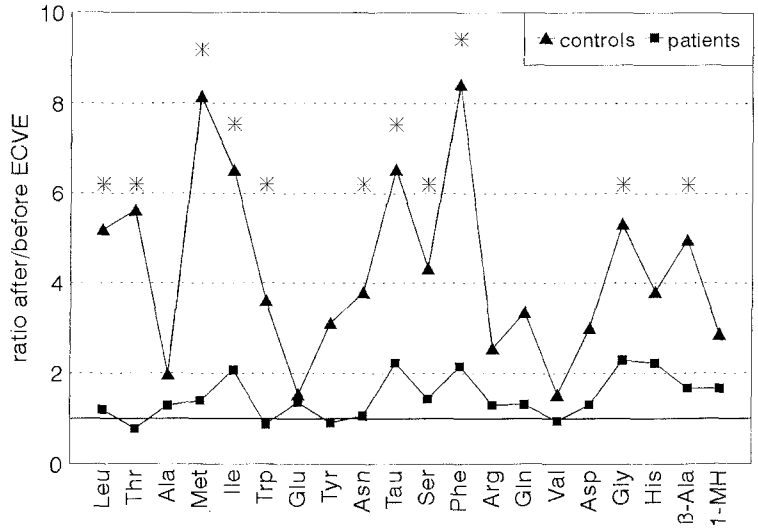

Fig. 5. Relationship between fractional excretions of amino acids after and before ECVE in patients with ADPKD and in controls (cf. methods). Arithmetic means are given; n = 8–9. Asterisks indicate significant differences between ADPKD patients and controls ($p \leq 0.05$). 1-value before ECVE for ADPKD patients and controls, respectively

Table 1. Influence of ECVE (2l of physiological electrolyte solution) on mean arterial blood pressure (MAP), fractional excretion of sodium (FE_{Na}), and concentration of cGMP in plasma of normal persons (NP) and patients suffering from ADPKD

	before ECVE		after ECVE	
	NP	ADPKD	NP	ADPKD
MAP [mmHg]	85.5 ± 2.4	99.4 ± 2.6	86.1 ± 1.8	100.1 ± 3.6
FE_{Na} [%]	1.53 ± 0.23	1.22 ± 0.23	*2.74 ± 0.22	*3.17 ± 0.25#
FE_{Li} [%]	20.5 ± 4.0	25.8 ± 8.9	25.2 ± 3.9	*41.4 ± 5.4#
$cGMP_{plasma}$ [pmol/ml]	6.65 ± 0.79	5.31 ± 0.56	*11.30 ± 1.91	*11.31 ± 1.66

Arithmetic means ± S.E.M.; n = 8–9. Asterisks indicate significant changes after ECVE ($p \leq 0.05$). "#" indicate significant change between NP and ADPKD after ECVE ($p \leq 0.0003$).

ADPKD: 41.4 ± 5.4 vs. 25.2 ± 3.9). Furthermore, concentrations of cGMP (pmol/ml) in plasma increased after fluid load (before ECVE – ADPKD: 5.31 ± 0.56 vs. NP: 6.65 ± 0.79; after ECVE: 11.31 ± 1.66 vs. 11.30 ± 1.91) (Table 1).

Hydration status in both groups was not different at baseline and not significantly increased after ECVE (TCV right atrium diameter [mm]- before ECVE – ADPKD: 36.9 ± 6.6 vs. NP: 34.5 ± 8.4; after ECVE – ADPKD: 34.0 ± 9.0 vs. NP: 35.9 ± 7.4).

Discussion

The present study has demonstrated differences in the handling of amino acids (AA) in ADPKD patients in comparison to normal subjects. Normally largely reabsorbed by the proximal tubule at a reabsorptive rate exceeding 95 to 98% of the filtered load, the selective aminoaciduria of essential AA (threonine, methionine, isoleucine, phenylalanine) and nonessential AA (glycine, tyrosine, asparagine, serine, aspartic acid, alanine and 1-methyl-histidine), respectively, was increased in our patients. Ultrastructural examination shows that this part of the tubule has large numbers of mitochondria (Mansbach et al., 1973). The abnormalities observed seemingly do not agree with the concept of group-specific or individual specific AA-transporter defects which are well known in renal tubular disorders as DeToni-Debré-Fanconi-syndrome, Hartnup disease or cystinuria, respectively. Oxidative phosphorylation dysfunction (OXPHOS) may impair the activity of sodium-coupled transport processes in the proximal tubule. The analysis of 20 amino acids in adults with OXPHOS-disease provides evidence that proximal renal tubule AA-reabsorption is the most common identified abnormality and is characterized by increased FE of neutral AA (Shaffer et al., 1995).

The pathogenic factors responsible for a possible defect in tubular AA-handling are not definitely clarified by the described structural abnormalities of the collecting ducts in ADPKD. Furthermore, the AA-pattern differences in plasma between the 2 groups are not explicable by interindividual differences in systemic AA-levels related to nonequivalent dietary protein intake. Moreover, for most AA-levels and AA-ratios, there is no correlation with both protein and energy intake (Laidlaw et al., 1994).

Plasma levels of several AA are known to be altered in end stage renal disease (Tizianello et al., 1980a). Various reasons for these alterations have been assumed. It has been suggested, primarily from animal studies, that with the loss of functioning renal parenchyma an increasing loss of the renal metabolic function occurs, including the metabolism of AA (Kopple, 1983). The Modification of Diet in Renal Disesase-study-group (MDRD) (Klahr, 1989) examined 78 patients (21 of them with ADPKD) with chronic renal failure and moderate to nearly end-stage renal failure (i.e. 3 groups with GFR of 11.5 to 37.5 ml/min/1.73 m^2). They found altered plasma AA-profiles in symptomatic patients. It has not been well documented whether such uremia-like symptoms only influence the AA-profile at certain stages of renal function loss or whether they progressively influence the course of the disease. If the renal function is diminished, some alterations in plasma AA's tend to manifest themselves later in the course of the disease (Pechar et al., 1978). In contrast to these observations and, surprisingly, in patients who still exhibited significant reduced renal function (i.e. GFR of 25 to 60 ml/min/1.73 m^2), many of the alterations in plasma AA-levels characteristic of chronic renal failure were already present. Some AA-abnormalities that are useful markers of renal metabolic capacity loss (e.g. increased citrulline or decreased serine) were often seen in patients with only mild renal failure (Laidlaw et al., 1994).

Our data have shown a wide variance in plasma AA-concentrations. Considering the fact that our patients do not differ greatly from normal subjects regarding age and nutritional status it is obvious that there are different plasma AA-patterns and/or AA-metabolism in ADPKD. Whether alterations in liver metabolism as described elsewhere (Tizianello et al., 1980b) are responsible for this differences remains to be investigated.

According to our results, different variants and/or defects of transepithelial transport mechanisms can be expected in ADPKD and would explain different reabsorptive capacities in 12 of 21 AA's. This is supported by NMR spectroscopy findings relating to the cyst fluid of patients with ADPKD. Foxall et al. (1992) have revealed a combination of biochemical features of the cystic fluid showing to be distinctly different from both plasma and urine. Some AA's (isoleucine, lysine, threonine, valine) were present at mM-concentrations in cyst fluid. In some cases their levels are up to two times higher than those in normal plasma or urine. The constancy of this biochemical composition probably reflects the chronic nature of cyst fluid accumulation and a long turnover of cyst fluid. This is unique among the other body fluids and indicates that the unusual composition may be related to the epithelial polarity reversal of the cyst epithelium which could also contribute to the growth of cysts.

It seems, however, more relevant that sodium and fluid reabsorption within the proximal tubule varies in proportion to spontaneous alterations in the GFR so that the fractional reabsorbed amount remains roughly constant. A reduction in or a lack of AA (and of other organic substances) in the tubular fluid decreases proximal reabsorption by reducing active sodium transport (Silbernagl, 1992). Apical uptake of most AA's occurs by Na^+-AA-cotransport driven by both chemical and voltage components. The energy maintaining the Na^+-gradient depends on Na^+/K^+-ATPase activity; Na^+-AA symport depends on secondary active transport mechanisms because its energy is derived from the Na^+-electrochemical gradient rather than direct coupling to a metabolic process (Schäfer and Barfuss, 1980). The net flux of Na^+ from lumen to peritubular fluid varies among the different nephron segments and correlates with the activity of Na^+/K^+-ATPase in the basolateral cell membrane (Berry et al., 1996). The abnormal distribution of Na^+/K^+-ATPase to the apical membrane may play a role in the different renal handling of AA in ADPKD. Renal Na^+-handling may constitute a pathophysiological link between the molecular/functional change in Na^+/K^+-ATPase and hypertension. However, the underlying cellular and molecular mechanisms responsible for increased Na^+-reabsorption leading to high blood pressure are unknown in essential hypertension and hypotheses that have been advanced are controversial (Gabow, 1993; Braun et al., 1996). Our observations of lower fractional sodium excretion rates in ADPKD than in normal subjects under basal conditions (1.22 % vs. 1.53 %) and the higher percentage increase after ECVE in ADPKD (3.17 % vs. 2.74 %) and in particular the increase of FE_{Li} after ECVE in ADPKD (25.8% vs. 41.4%) seem to corroborate this relationship, regardless of the obvious increase of GFR. This is supported by the additonal finding of a significant increase of

cGMP plasma levels after ECVE in the two groups. Cyclic GMP seems to be a potent marker of intrarenal nitric oxide (NO)-activity. An increase in NO synthesis reduces or limits tubular sodium reabsorption, whereas NO synthesis blockade elicits an antinatriuresis (Alberola et al., 1992). The results of raising plasma levels of cGMP after volume expansion accompanied by increased diuresis and FE_{Na} provide evidence that the action of intrarenal NO is mediated both at the glomerular and probably at the renal tubule side. Arginine as a well-known precursor in the generation of NO showed no correlation both in healthy individuals and ADPKD in plasma as well as in urine. Whether the decarboxylated product resulting in the formation of agmatine (Morrissey and Klahr, 1996) plays a role in NO-mediated changes in tubular AA handling of ADPKD cannot be clarified yet.

In summary, our study indicates different AA patterns in ADPKD patients as compared to normal subjects. The known Na^+-dependent and furthermore NO-mediated processes in the resorption of AA in the proximal tubule seem to be different in ADPKD and may be related to a different distribution of receptors and/or ATP-dependent transport systems resulting in abnormal transtubular fluid transport in ADPKD. The postulation of a cofactor in the genesis of the early-onset hypertension in ADPKD is therefore certainly somewhat speculative. NO (EDRF)-mediated hormonal mechanisms may play a role in this context, but this is not clear as yet. The observation of an increased FE_{AA} in NP after ECVE resulting from a disruption of proximal glomerular-tubular balance is probably without pathogenetic validity.

Further studies should focus on that problem until the remaining questions regarding the pathogenic source of hypertension in ADPKD will be elucidated.

References

Albeola A, Pinilla JM, Quesada T, Romero JC, Salom MG, Salazar FJ (1992) Role of nitric oxide in mediating renal response to volume expansion. Hypertension 19: 780–784

Arner ED, Swemey WE, Nelson WJ (1992) Abnormal sodium pump distribution during renal tubulogenesis in congenital murine polycystic kidney disease. Proc Natl Acad Sci USA 89: 7447–7451

Berry CA, Ives HE, Rector FC jr (1996) Renal transport of glucose, amino acids, sodium, chloride and water. In: Brenner BM, Rector FC (eds) The kidney. WB Saunders Comp, Philadelphia, pp 334–347

Bratton AC, Marshall EK jr (1939) A new coupling component for sulfanilamide determination. J Biol Chem 128: 537–550

Braun C, Lüdicke C, Rebsch W, Gretz N, van der Woude FJ, Rohmeiss P (1996) Autoregulation of renal blood flow and pressure-dependent renin release in autosomal dominant polycystic kidney disease of rats. Nephrol Dial Transplant 11 [Suppl]6: 52–57

Domino SE, Tubb DJ, Garbers DL (1991) Assay of guanylyl cyclase catalytic activity. Methods Enzymol 195: 345–355

Fick G (1992) Autosomal-dominante polycystische Nierenerkrankung. Dtsch Med Wochenschr 117: 1160–1164

Foxall PJ, Price RG, Jones JK, Neild GH, Thompson FD, Nicholson JK (1992) High resolution proton magnetic resonance spectroscopy of cyst fluids from patients with polycystic kidney disease. Biochim Biophys Acta 1138: 305–314
Gabow PA (1993) Autosomal dominant polycystic kidney disease. N Engl J Med 329: 332–342
Gabow PA, Ikle DW, Holmes JH (1984) Polycystic kidney disease: prospective analysis of nonazotemic patients and family members. Ann Intern Med 101: 238–247
Grünfeld JP, Chauveau B, Knebelmann A (1992) Autosomal dominant polycystic kidney disease: from molecular genetics to the patients. Clin Invest 70: 791–793
Kimberling WJ, Fain PR, Kenyon JB, Goldgar D, Sujansky E, Gabow PA (1988) Linkage heterogeneity of autosomal polycystic kidney disease. N Engl J Med 319: 913–918
Klahr S (1989) The modification of diet in renal disease study. N Engl J Med 320: 864–866
Kopple JD (1983) Amino acid metabolism in chronic renal failure. In: Blackburn GL, Grant JP, Young VR, John Wright (eds) Amino acids – Metabolism and medical applications. John Wright PSG Inc., Boston, MA, pp 327–332
Laidlaw AS, Berg RL, Kopple JD, Naito H, Walker WG, Walser M (1994) Patterns of fasting plasma amino acid levels in chronic renal insiffuciency: results from the feasibility phase of the modification of diet in renal disease study. Am J Kidney Dis 23: 504–513
Lustgarten JA, Wenk RE (1972) A simple rapid kinetic method for serum creatinine measurement. Clin Chem 18: 1419–1422
Mansbach AB (1973) Ultrastructure of the proximal tubule in renal physiology. In: Orloff J, Berliner RW (eds) American Physiology Society, Washington D.C., p 31
Morrissey JJ, Klahr S (1996) Effects of agmatine, an active metabolite of arginine metabolism, on the kidney. Nephrol Dial Transplant 11: 1217–1219
Pechar J, Malek P, Dobersky P, Shala J, Jirka J, Schück O, Nadvornikova H, Reneltova J (1978) Influence of protein intake and renal function on plasma amino acids in patients with renal impairment and after kidney transplantation. Nutr Metab 22: 278–287
Reeders ST, Brenning MH, Davies KE, Nicholls RD, Jarman AP, Higgs DR, Pearson PL, Weatherhall DJ (1985) A highly polymorphic DNA marker linked to adult polycystic disease on chromosome 16. Nature 317: 542–544
Ritz E, Zeier M, Geberth S, Waldherr R (1993) Autosomal dominant polycystic kidney disease (ADPKD)-mechanisms of cyst formation and renal failure. Aust N Z J Med 23: 35–41
Roth M (1971) Fluorescence reaction for amino acids. Anal Chem 43: 880–882
Roth M, Hampai A (1973) Column chromatography of amino acids with fluorescence detection. J Chromatogr 83: 353–356
Saggar-Malik AK, Jeffery S, Patton MA (1994) Autosomal dominant polycystic kidney disease. Br Med J 308: 1183–1184
Schäfer JA, Barfuss DW (1980) Membrane mechanisms for transepithelial amino acid absorption and secretion. Am J Physiol 238: F335–F346
Schmidt M, Mann JFE, Stein G, Herter M, Nussberger J, Kleinbeil A, Ritz E (1990) Natriuresis-pressure relationship in polycystic kidney disease. J Hypertens 8: 277–283
Shaffner JM, Voljavec AS, Dixon J, Kaufman A, Wallace DC, Mitch WE (1995) Renal amino acid transport in adults with oxidative phosphorylation diseases. Kidney Int 47: 1101–1107
Silbernagl S (1983) Kinetics and localization of tubular resorption of "acidic" amino acids. A microperfusion and free flow micropuncture study in rat kidney. Pfluegers Arch 396: 218–224
Silbernagl S (1992) Amino acids and oligopeptides. In: Seldin DW, Giebisch G (eds) The kidney, vol. 2. Raven Press, New York, pp 2889–2920
Sørensen SS, Glud TK, Sørensen PJ, Amdisen A, Pedersen EB (1990) Change in renal tubular sodium and water handling during progression of polycystic kidney disease: relationship to atrial natriuretic peptide. Nephrol Dial Transplant 5: 247–257

Strazzullo P, Iacoviello L, Iacone R, Giorgione N (1988) Use of lithium clearance in clinical and epidemiological investigation: a methodological assessment. Clin Sci 74: 651–657

Thomsen K (1984) Lithium clearance: a new method for determinig proximal and distal tubular reabsorption of sodium and water. Nephron 37: 217–223

Tizianello A, De Ferrari G, Garibotto G, Giovanna G, Robando C (1980a) Renal metabolism of amino acids and ammonia in subjects with normal renal function and in patients with chronic renal insufficiency. J Clin Invest 65: 1162–1173

Tizianello A, De Ferrari G, Garibotto G, Robando C (1980b) Amino acid metabolism and the liver in renal failure. Am J Clin Nutr 33: 1354–1362

Torres VE, Wilson DM, Offord KP, Burnett JC jr, Romero JC (1989) Natriuretic response to volume expansion in polycystic kidney disease. Mayo Clin Proc 64: 509–515

Wilson PD, Sherwood AC, Palla K, Du J, Watson R, Norman JT (1991) Reversed polarity of Na^+-K^+-ATPase: mislocation to apical plasma membranes in polycystic kidney disease epithelia. Am J Physiol 260: F420–F430

Zeier M, Geberth S, Ritz E, Jaeger T, Waldherr R (1988) Adult dominant polycystic kidney disease – clinical problems. Nephron 49: 177–183

Zerres K (1992) Autosomal recessive polycystic kidney disease. Clin Invest 70: 794–801

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