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In vitro stimulation of renal tubular *p*-aminohippurate transport by dexamethasone in rat kidneys and in intact kidney tissue of patients suffering from renal cell carcinoma

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Abstract The aim of this study was to test whether or not the accumulation of *p*-aminohippurate (PAH) can be increased in intact human renal cortical slices obtained from tumor-bearing kidneys of patients suffering from renal cell carcinoma (RCC). Tissue slices were incubated for 24 h in Williams medium E containing 0.01–50 μM dexamethasone. Thereafter slices were placed in PAH-containing Cross-Taggart medium and PAH uptake into kidney tissue was measured for 2 h. In both rat and human renal tissue slices, PAH uptake capacity increased significantly in a concentration-dependent manner after 24 h of incubation in dexamethasone-containing medium (rat, 136%; man, 156%). The stimulatory effect was already significant after 12 h of incubation. In additional experiments it was shown that incubation in triiodothyronine (T_3)-containing medium has different effects: in man, T_3 does not influence the PAH accumulation capacity of renal cortical slices whereas in rats PAH accumulation is significantly lower after 24 h of incubation with T_3. Thus stimulation of tubular transport capacity can be performed in vitro in human renal cortical slices. Discrepancies between the effects of dexamethasone and T_3 indicate different modes of action of the two hormones at the cellular level.

Key words Renal tubular transport · *p*-aminohippurate · Stimulation · Renal cell carcinoma · Dexamethasone · Triiodothyronine

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Introduction

p-Aminohippurate (PAH) is transported across the basolateral membrane of the renal proximal tubule in exchange for intracellular alpha-ketoglutarate, by a mechanism indirectly coupled to sodium via Na^+/alpha-ketoglutarate cotransport [38]. It is known that the renal excretion capacity of PAH can be stimulated in rats and rabbits after repeated administration of dexamethasone or triiodothyronine (T_3) and other suitable substances in vivo (for review see [16] and in experiments on renal cortical slices after the addition of, for example, dexamethasone to the incubation medium in vitro [14]. The stimulatory effect can be shown in rats after 3 days of pretreatment: for example PAH excretion into urine increases after T_3 treatment by about 60% [7]. An increase in de novo protein synthesis could be one of the reasons for the stimulation of renal PAH transport [3]. Both renal transport capacity and the effect of stimulatory pretreatment on renal function are age-dependent [6]: renal excretion capacity of 10-day-old rats is immature; however, in young rats the stimulation of renal transport processes is often higher than in adult animals [15]. Further possible mechanisms responsible for the stimulation phenomenon (increase in glomerular filtration rate, rise in renal blood flow, extrarenal hormone effects, metabolic changes after pretreatment) could not be differentiated unambiguously [9, 10, 17].

For better understanding of the processes involved in the stimulation of renal PAH transport at the cellular level, a new experimental approach has been developed to stimulate the tubular transport of PAH in vitro. For this reason renal cortical slices were incubated for 24 h in a modified Williams medium E under carbogen gassing. Thereafter slices were placed in Cross-Taggart buffer containing PAH, and the uptake of PAH from the medium into the slices was measured in 2 h accumulation experiments. Under these experimental conditions, the slice-to-medium ratio for PAH decreases from about 12 in freshly prepared rat kidney cortical slices to about

6 in slices incubated for 24 h. Nevertheless, after this long incubation period the PAH accumulation process is an active one, because under nitrogen atmosphere PAH uptake is completely abolished. By adding dexamethasone to the incubation medium it is possible to increase the slice-to-medium ratio by 30–50% in rat kidney.

On the basis of these results in rats, the renal PAH transport capacity should be stimulated in human kidney tissue also.

Without doubt, uptake of PAH into renal cells is quite different from that of chemotherapeutic agents. Nevertheless, the final goal of these experiments is the stimulation of the uptake of cytostatics in human kidney tissue to overcome the so-called multidrug resistance in renal cell carcinoma (RCC) [27, 30]. It is a well-known clinical finding that RCC and its metastases are quite insensitive to any kind of cancer chemotherapy [25, 26]. This multidrug resistance is reported to be caused by expression of the MDR1 gene and is characterized by broad-spectrum cross-resistance to many chemotherapeutic agents [4, 11, 28, 33, 39]. The favorite hypothesis to explain the phenomenon of multidrug resistance is an increase in transport of chemotherapeutic agents out of renal carcinoma cells [23, 24, 31]. We have also shown previously that the accumulation capacity of RCC tissue is significantly reduced [18]. Therefore, stimulation of the uptake of cytostatic drugs could be beneficial in the treatment of renal cancer and its metastases. The investigation of the stimulation of the well-known PAH transport in human renal cortical slices is a first step in this direction. For this reason, experiments were performed in this study on intact renal tissue of RCC-bearing human kidneys.

Materials and methods

Patients

Between December 1995 and May 1996 in vitro accumulation experiments were performed on human kidney slices. Intact renal cortical tissue of tumor-bearing kidney was obtained from 15 patients undergoing radical tumor-nephrectomy in the Department of Urology, Jena. Intact kidney tissue is defined as macroscopically inconspicuous material from the tumor-bearing kidney. Tumors were classified as pT_1 to pT_3 without multifocal localization. The distance between intact renal tissue and RCC was ≤4 cm. The patients were 9 men and 6 women with an average age of 63.1 ± 10.8 years. Tissue samples (about 1 g) were stored in normal saline on ice (4°C) immediately after kidney removal. After transportation (30 min) slices were prepared as described below.

Animals

The animal experiments were carried out on female Wistar rats (Han: WIST) of our institute's own out-bred stock. In 10-day-old animals both sexes were used; 55-day-old animals were females. The litters were reduced to six animals. Young animals were nursed by their dams. Adult rats were fed a standard diet (Altromin 1316) and tap water ad libitum, and were housed under standardized conditions in plastic cages with a light-dark cycle of 12/12 h, temperature of 22 ± 2°C and humidity of 50% ± 10%. Animal care and treatment were conducted in conformity with institutional guidelines that are in compliance with international laws and policies (EEC Council Directive 86/609, OJL 358, December 1987; NIH *Guide for the Care and Use of Laboratory Animals*, NIH publication no. 85–23, 1985)

Stimulation of PAH excretion capacity in vivo

Dexamethasone 60 µg/100 g body weight (b.w.) (Fortecortin Mono, E. Merck, Darmstadt) was given intraperitoneally for 3 days, once daily. Following this dose, glucocorticoid receptor sites are completely saturated by dexamethasone [32]. Dexamethasone was dissolved in normal saline (1 ml/100 g b.w.). Controls received the solvent only.

Clearance experiments were performed 24 h after the last treatment with dexamethasone. Renal excretion of PAH (E. Merck, Darmstadt) was measured after the administration of doses that can reliably saturate the transport capacity in rats of different ages: 100 (10-day-old) or 200 (55-day-old) mg PAH/100 g b.w. dissolved in distilled water (2 ml/100 g b.w.) intraperitoneally [8]. Animals were placed in metabolic cages for 1 h and urine was collected and measured volumetrically. The urine of three 10-day-old rats was pooled to obtain a sufficient amount for PAH determination.

Accumulation experiments

Dexamethasone-treated and control rats were anesthetized with ether, exsanguinated and the kidneys removed and stored in normal saline on ice. Kidney tissue samples of RCC patients were prepared in the same way. Renal cortical slices with pool sizes of about 100 mg (1 mm thick) were incubated in 50-ml Erlenmeyer flasks with PAH with bidirectional shaking (about 100 r.p.m.) in 3 ml Cross-Taggart buffer [pH 7.4; 30°C; oxygen gassing (2.5 l/h per sample); incubation time 120 min; concentration of PAH: 8.5×10^{-5} M]. Following incubation, PAH was determined in the supernatant fraction of the homogenate and in the incubation medium. The active uptake of PAH was expressed by the ratio between PAH concentration in the tissue and in the medium after the end of incubation (slice-to-medium concentration ratio = $Q_{S/M}$) according to Stopp and Bräunlich [36].

Additional experiments were performed with 2-, 6-, 12- and 24-h-incubation of renal cortical slices (100 mg) of untreated rats or RCC patients in 50-ml Erlenmeyer flasks loaded with 10 ml Williams medium E (WME, Bio Whittaker) supplemented with L-glutamine (292 mg/l), insulin (1 µM), gentamicin (50-mg/l), and different concentrations of dexamethasone or triiodothyronine (T_3; Sigma, St. Louis, Mo.) under carbogen gassing (95% O_2/5% CO_2; 2.5 l/h per sample) adjusted to pH 7.4 and 25°C. Chemicals were commercially available and of analytical grade. Thereafter slices were placed in Cross-Taggart-buffer (pH 7.4) containing PAH, and the uptake of PAH from the medium into the slices was measured in 2-h accumulation experiments as described above.

Concentrations of PAH were determined using the colorimetric method introduced by Bratton and Marshall [5].

Determination of glutathione and potassium content of renal cortical slices

Reduced (GSH) and oxidized (GSSG) glutathione were determined after Kretzschmar et al. [22] and Hissin and Hilf [20], respectively, with slight modifications. In brief: three parts of each homogenate were denatured and diluted with 4 parts of metaphosphoric acid (25% w/v) and 9 parts of 0.2 M sodium phosphate EDTA buffer, pH 8.0, and stored at −80°C until determination. After thawing and centrifugation at 20 000 *g* at 4°C for 30 min, glutathione was measured in the supernatant. Potassium was measured by flame photometry in the same supernatant after further dilution (1:20) with water.

Statistics

The results are given as arithmetic means ± SEM of four to six independent slice preparations. In each patient, four slice preparations were used for each concentration to minimize methodological variances. In the PAH excretion experiments $n = 6$ adult rats were included; in 10-day-old rats urine of three animals was pooled. Statistically significant differences between various experimental groups were tested for using the Mann-Whitney test ($P \leq 0.05$).

Results

In Fig. 1, the influence of in vivo administration of dexamethasone for 3 days on renal PAH excretion and PAH accumulation capacity of renal cortical slices is compared with the effect of dexamethasone added in vitro to the incubation medium on PAH accumulation during 24 h of incubation. In 10-day-old rats, the renal PAH excretion was significantly lower than in adult animals, but it increased significantly after in vivo pretreatment with dexamethasone; in 55-day-old rats this stimulatory effect was not significant. The PAH accumulation capacity of slices was not influenced by the in vivo pretreatment with dexamethasone, either in immature or in adult rats. However, after 24 h of incubation of renal cortical slices in medium containing dexamethasone (0.5 μM) the PAH *accumulation* capacity was increased significantly in both age groups. This increase paralleled the rise in renal PAH *excretion* after in vivo pretreatment with dexamethasone.

To further clarifiy the in vitro effect of dexamethasone, the PAH accumulation in renal cortical slices was investigated in more detail after 24 h of incubation in dexamethasone-containing WME. The PAH accumulation capacity increased in adult rats by 36% (Fig. 2). This stimulatory effect was concentration-dependent, with an optimal dexamethasone concentration in the medium of 0.5 μM. There was no influence of dexamethasone in 2-h incubation experiments in Cross-Taggart medium (not shown). During 24 h of incubation neither GSH nor GSSG concentrations changed significantly. Also the potassium content in the renal cortical slices did not decrease after 24 h of incubation (not shown). PAH accumulation in renal cortical slices of adult rats in WME after 24 h of incubation reached about two-thirds of the values of acute incubation experiments (11.4 ± 0.3 vs 7.1 ± 0.2). Under a nitrogen atmosphere the concentration of PAH in slices and in medium was nearly the same ($Q_{S/M}$ about 1; not shown); this indicates that PAH accumulation under oxygen gassing is an active process after both 2 and 24 h of incubation time.

Quite similar results were obtained in renal cortical slices of human kidney. In acute PAH accumulation experiments (2 h of incubation in PAH-containing Cross-Taggart medium) on intact human kidney tissue, slice-to-medium ratios of 3.12 ± 0.24 were measured (cf. reference value in Fig. 3). Sex differences did not

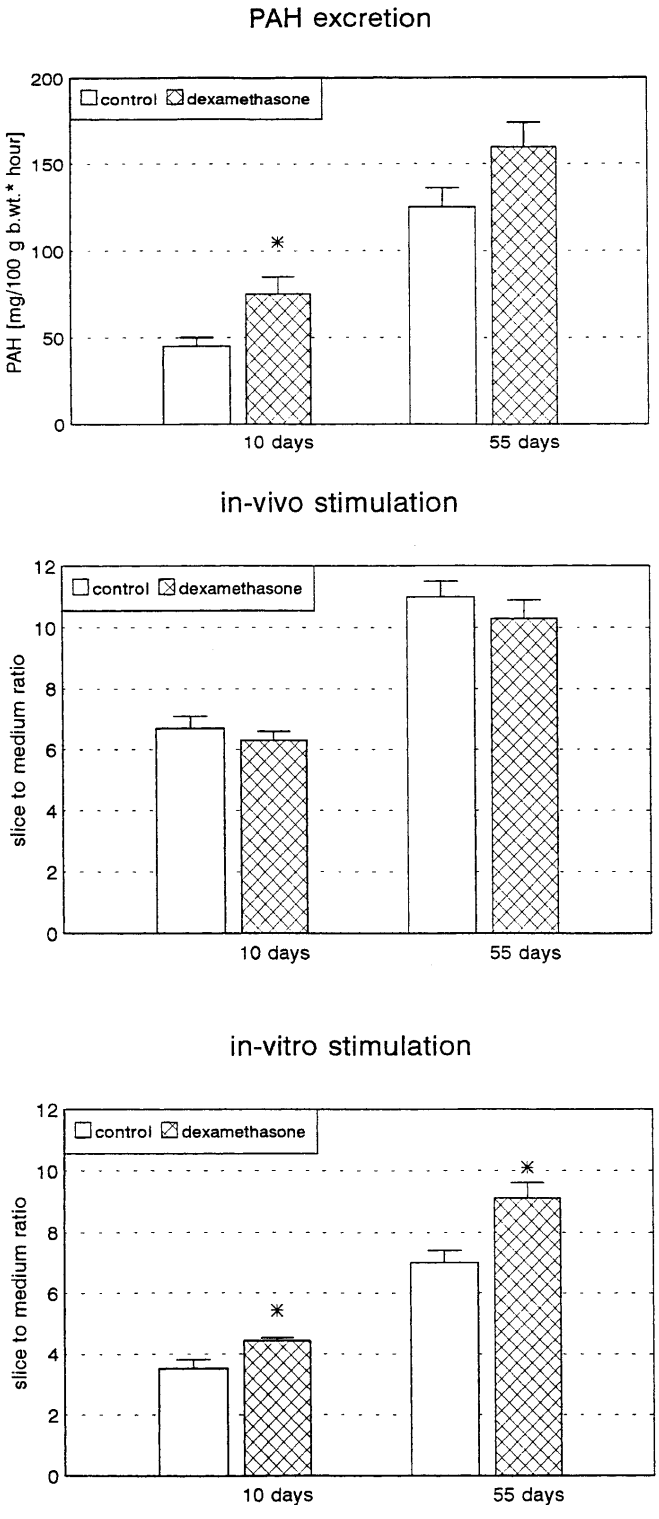

Fig. 1 Influence of in vivo dexamethasone treatment (see Materials and methods) on urinary *p*-aminohipporate (PAH) excretion and PAH accumulation in renal cortical slices of 10- and 55-day-old rats compared with the effect of a 24 h of in vitro stimulation (0.5 μM dexamethasone) on PAH accumulation capacity. Arithmetic means ± SEM; $n = 4$; *asterisks* mark significant dexamethasone effects ($P \leq 0.05$)

exist: men 3.28 ± 0.55 versus women 2.74 ± 0.38. After 24 h of incubation the PAH accumulation capacity decreased to 2.68 ± 0.42. However, distinct interindividual differences between 1.86 and 5.41 could be found (Fig. 3). The addition of dexamethasone in different concentration to the incubation WME increased the PAH accumulation of intact human renal tissue after 24 h significantly (56% increased; Fig. 4). This stimulatory effect is concentration-dependent also, but with a maximum at 1 μM dexamethasone in the medium.

As shown in Fig. 5, the stimulatory effect of dexamethasone was time-dependent. After 2 h (cf. reference value in Fig. 3) and after 6 h of incubation in dexamethasone-containing medium, no stimulation of PAH accumulation occurred in intact human kidney tissue. But after 12 h and, even more noticeably, after 24 h a significant increase in PAH accumulation capacity could be demonstrated. These results parallel those of experiments on rat renal cortical slices [14].

To further characterize hormone effects on renal accumulation capacity, additional experiments were done using T_3 for incubation (Fig. 6). Twenty-four-hour incubation of renal cortical slices in T_3-containing WME was followed by different effects in rat and man: in rats the PAH accumulation dropped concentration-dependently down to 20% of the control value whereas in man no influence of T_3 could be found.

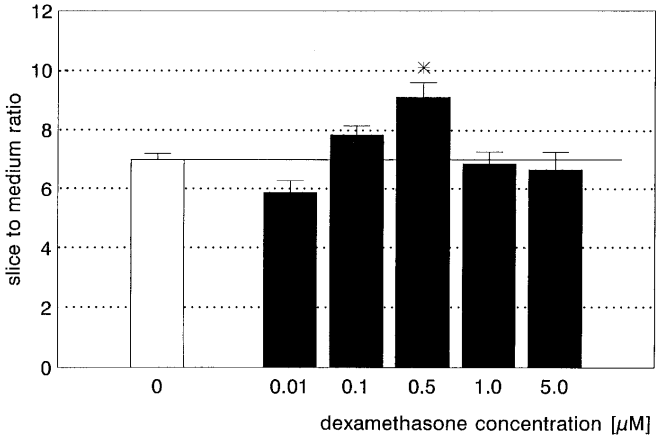

Fig. 2 Influence of 24 h of incubation in dexamethasone-containing Williams medium E on PAH accumulation (slice-to-medium ratio) in renal cortical slices of adult rats. Arithmetic means ± SEM; $n = 4$; *asterisk* indicates significant dexamethasone effect ($P \leq 0.05$)

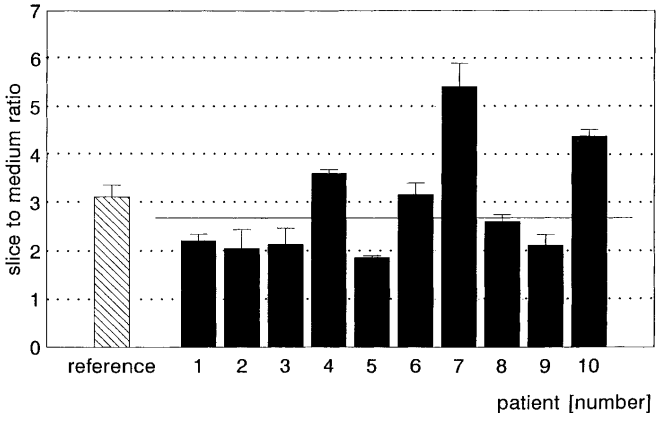

Fig. 3 PAH accumulation (slice-to-medium ratio) in renal cortical slices of intact renal tissue of 10 patients suffering from renal cell carcinoma during 24 h of incubation in Williams medium E. *Reference* value measured in a previous study for 2 h of incubation in Cross-Taggart medium; $n = 25$ [18]. Arithmetic means ± SEM of four to six patients, four samples of each patient per concentration; *line* mean value of the 10 patients

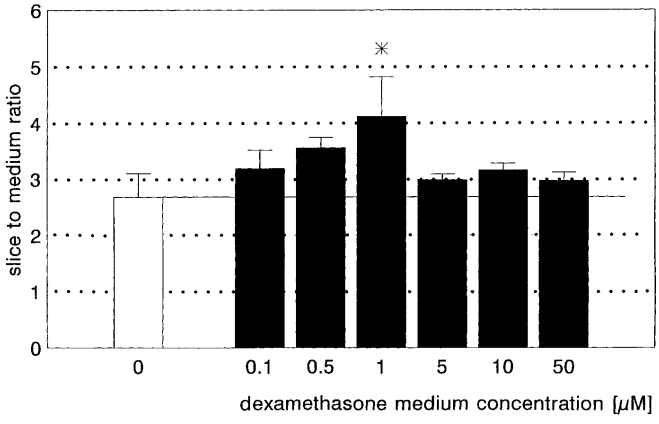

Fig. 4 Influence of 24 h of incubation in dexamethasone-containing Williams medium E on PAH accumulation (slice-to-medium ratio) in renal cortical slices of intact renal tissue of 10 patients suffering from renal cell carcinoma. Arithmetic means ± SEM; $n = 4$ slice preparations per patient and each concentration; *asterisk* indicates significant dexamethasone effect ($P \leq 0.05$)

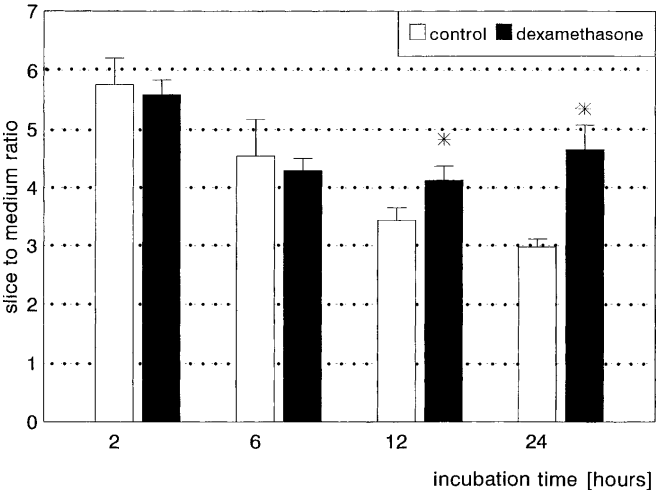

Fig. 5 Influence of dexamethasone (1 μM) in the incubation medium (Williams E) on PAH accumulation (slice-to-medium ratio) in renal cortical slices of intact renal tissue of patients suffering from renal cell carcinoma in dependence on the incubation time. Arithmetic means ± SEM; $n = 3$ patients, each four samples per time; *asterisks* mark significant dexamethasone effects ($P \leq 0.05$)

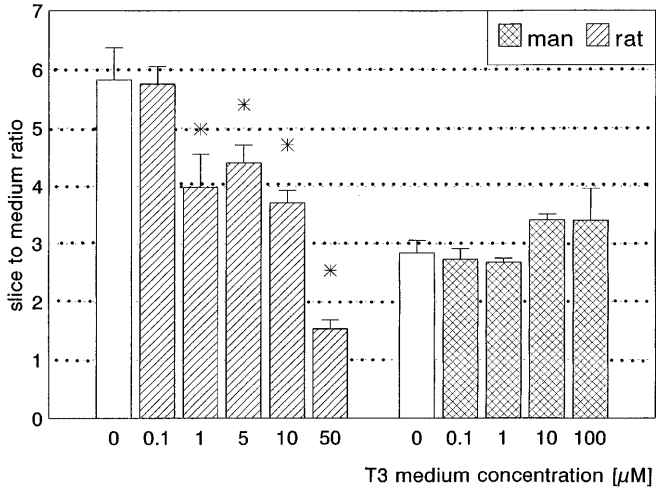

Fig. 6 Influence of 24 h of incubation in triiodothyronine (T_3)-containing Williams medium E on PAH accumulation (slice-to-medium ratio) of renal cortical slices of adult rats and of intact renal tissue of five patients suffering from renal cell carcinoma. Arithmetic means ± SEM; $n = 4$ (rats); in each of the five patients four slice preparations per concentration were used; *asterisk* indicates significant T_3 effect ($P \leq 0.05$)

Discussion

The most interesting result of this study was to prove in principle the possibility of stimulating in vitro the renal tubular transport capacity in human kidney tissue. As shown previously for rat kidney tissue slices [14], it is possible to increase the PAH accumulation of human renal cortical slices significantly during 12-h and, more pronounced, after 24 h of incubation of the slices in dexamethasone-containing buffer solution. In the rat the optimal dexamethasone concentration was 0.5 μM, whereas in man it was 1.0 μM. Age differences concerning the stimulatory dexamethasone effect, found in rats, are of minor importance for man, because in RCC patients the age was between 55 and 70 years, so that an influence of age could not be detected. There are few results in the literature describing stimulation of renal tubular secretion capacity in man; for example, the repeated administration of sulfamerazine stimulates the renal excretion of PAH in man [37]. To the best of our knowledge, in vitro investigations of the accumulation capacity of human kidney tissue after stimulatory treatment in vivo are not described in the literature.

In the near future the general possibility of stimulating the renal uptake of drugs such as PAH will be proven for (a) other substances (anticancer drugs) and (b) RCC tissue. Preliminary experiments have shown the stimulation phenomenon in RCC tissue and the possibility of stimulating the accumulation of anticancer drugs such as methotrexate (Fleck et al., unpublished data). By this approach it could be possible to overcome the multidrug resistance of RCC against cytostatics such as 5-fluorouracil, leucovorin, zidovudine or methotrexate [13, 35]. Multidrug resistance represents a major obstacle to the successful therapy of neoplastic diseases. Although the P-glycoprotein is expressed in many cell lines and occurs in patient tumors, its expression is not a universal feature of multidrug resistance, suggesting that other mechanisms are operating also [2]. The enhanced export of glutathione xenobiotic conjugates which is coupled to the multidrug resistance associated gene no. 1 (MRP1) product [4, 21], could be antagonized by an increased uptake of these xenobiotics. Other export routes out of the RCC cell could even be diminished as shown for cholesterol transport: the reduced release of free cholesterol from RCC cells may, in part, be responsible for the accumulation of cholesterol in human renal cancer [12]. Many approaches to counteract multidrug resistance are published in the literature. Well-qualified candidates seem to be calcium channel blockers such as verapamil [28] and dexverapamil, which has reduced cardiac toxicity [29]. A study by Amato et al. [1] showed that the co-administration of dexamethasone improves the tolerance of intermittent high-dose interferon. Perhaps this strategy might be combined with the stimulation of the uptake of anticancer drugs by dexamethasone to obtain benefits in RCC therapy.

Despite the optimistic interpretation of our results a few critical points must be considered. First, while without doubt the accumulation capacity of rat and human kidney tissue can be stimulated in vitro, it remains open whether the stimulation phenomenon, measured after incubation in dexamethasone-containing WME, is the consequence of an increased de-novo synthesis of carrier proteins as assumed by Berkhin and Varshavsky [3] or results from a better nutritional state of the renal cortical slices. To this purpose polymerase chain reaction experiments are under way, but final conclusions can not yet be drawn. Second, dexamethasone is a substrate to the MDR1 gene product [34]. Therefore, it cannot be ruled out that in vitro dexamethasone pretreatment does not stimulate uptake but simply interferes with the known transport proteins removing PAH that has diffused into the cell passively. Nevertheless, the effectiveness of dexamethasone in stimulating renal PAH excretion after in vivo pretreatment in rats, indicates the practicability of this approach in patients also. Finally, anticancer chemotherapy is focused on RCCs and their metastases. It remains open to question whether metastases are susceptible in the same way as the original RCC. Furthermore, the administration of high-dose dexamethasone could attack healthy cells in the organism as well. Therefore, the effect of anticancer drugs could be enhanced in intact tissues also. Nothing is known about the sensitivity to dexamethasone of intact cells compared with RCC and its metastases. Finally, general side effects of high-dose dexamethasone therapy should be kept in mind.

A last aspect of this study consists of the different effect of dexamethasone in comparison with T_3. We interpret the lack of effect of T_3 on renal PAH accumulation in human kidney tissue and the significant reduction of PAH accumulation in rat renal cortical

slices as follows: T_3 has a catabolic effect and, therefore, the so-called house-keeping of the tubular cells increases. In rats, this metabolic stimulus is responsible for enhanced energy consumption and PAH uptake is diminished as shown previously for renal amino acid reabsorption [19]. Perhaps this phenomenon is of minor importance in human kidney tissue and the T_3 effect on transport capacity could be masked in man.

Altogether, investigations of the renal tubular transport involved in the uptake of anticancer drugs into RCC may lead to a better understanding of the mechanisms governing the expression of multidrug resistance and provide a new approach to overcoming this phenomenon.

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