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IN VITRO ANALYSIS OF DRUG-INDUCED STIMULATION OF RENAL TUBULAR *p*-AMINOHIPPURATE (PAH) TRANSPORT IN RATS

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Abstract—After repeated administrations of PAH, sulfamethoxypyridazine, cyclopenthiazide, or phenobarbital to rats, the apparent Michaelis constant for PAH uptake in renal cortical slices was not changed, whereas maximum PAH uptake was markedly increased. In addition, PAH efflux was not affected by the pretreatment of rats. Protein concentration in kidney cortex was significantly increased after all pretreatments. Consequently, an increase in *de novo* synthesis or a decrease in the degradation of the carrier fraction can be supposed.

After repeated administrations of various drugs such as PAH, probenecid, phenol red, penicillin, sulfaclomid, sulfamethoxypyridazine, cyclopenthiazide [1], and phenobarbital [2], the renal excretion of PAH was accelerated in rats of different ages, except in the first 2 weeks of life. In accordance with these findings, an enhancement of PAH accumulation was observed in renal cortical slices from adult, but not from newborn and infant rats[3]. Using such an *in vitro* technique, modifications of glomerular filtration rate, renal blood flow, tubular reabsorption rate and extrarenal factors can be excluded. Consequently, the reason for the drug-induced stimulation must be searched for in the tubular mechanism for secretion of organic acids in general, and in the carrier-mediated transport rates across the contraluminal membrane and/or in the permeation rates across the luminal membrane of the tubular cells in particular. A passive intra-cellular retention of PAH can be neglected [4]. Furthermore, for interpretation of the stimulation phenomenon, a proliferation of renal proximal tubuli must be taken into consideration [5].

The aim of the present study was to determine the kinetics of PAH accumulation in renal cortical slices from saline-treated control rats as well as from rats repeatedly pretreated with some drugs producing a stimulatory effect. In addition, it was studied whether or not a proliferation of kidney occurs after the pretreatment of rats with these drugs.

MATERIALS AND METHODS

Wistar rats (Jena) of our institute's colony breed were used. Newborn rats[a] of both sexes and adult female rats[b] were pretreated with saline or various drugs in doses correlating with elimination velocity and physico-chemical properties, respectively. Doses in mg/100 g body wt i.p. are given in brackets: PAH (100[a] or 300[b], twice a day), sulfamethoxypyridazine (15, once a day), cyclopenthiazide (5, twice a day) and phenobarbital (3[a] or 6[b], once a day), respectively, for 3 or 4 days [1, 2]. Sixteen to twenty-four hours after the last administration, renal cortical slices were prepared from 5- and 55-day-old rats as previously described [6].

Pooled renal cortical slices (200 mg) were always incubated in 3.0 ml Krebs–Ringer phosphate buffer (pH 7.4) containing PAH in increasing concentrations from 3.3×10^{-5} to 2.0×10^{-4} M unless otherwise stated. All incubations were carried out in a Warburg apparatus at 25° under a gas phase of 100% oxygen for 100 min. After the incubation the slices were removed from the medium, blotted, homogenized, and denatured with 10% trichloroacetic acid. PAH was determined in the supernatant fraction as well as in the incubation medium using Bratton–Marshall reaction [7]. Active PAH uptake was calculated as the difference between PAH concentration per g tissue (wet weight) and final PAH concentration per ml incubation medium. Apparent Michaelis constant (K_m) and apparent maximum intracellular PAH concentration (V_{max}) were calculated for the uptake curve constructed from the plot of the actively accumulated PAH concentrations vs initial PAH concentrations in the incubation medium using a Hewlett–Packard calculator (HP 20) [8].

PAH efflux from renal cortical slices of 55-day-old rats back into the incubation medium was measured in additional experiments. The slices were first incubated in Krebs–Ringer phosphate buffer (pH 7.4) containing 8.5×10^{-5} M PAH at 25° under a gas phase of 100% oxygen for 100 min. At the end of the loading period, the slices were rapidly separated from the medium, blotted, and placed in a medium initially free of PAH and bubbled with nitrogen. The following incubations were carried out under a gas phase of nitrogen for 5–25 min. Anaerobic conditions were chosen in order to block the active PAH transport. The efflux was thus measured in the absence of simultaneous re-uptake of PAH released from renal cortical slices. At 5 min intervals the slices were separated from the medium, blotted, homogenized, and denatured with 10% TCA. PAH was measured

Table 1. Apparent Michaelis constant (K_m), maximum PAH uptake (V_{max}), efflux half-life of PAH ($t_{\frac{1}{2}}$) and efflux rate constant (k)*

Kinetic parameters of PAH uptake and efflux				
	Apparent K_m (mM)	Apparent V_{max} (μmoles/g wet wt for 100 min)	Efflux $t_{\frac{1}{2}}$ (min)	k_{efflux}
Control (saline)	0.39 ± 0.07	1.52 ± 0.18	6.06 ± 0.41	0.114 ± 0.008
Pretreatment:				
PAH	0.41 ± 0.02	2.24 ± 0.06†	6.63 ± 0.85	0.104 ± 0.013
Sulfamethoxypyridazine	0.37 ± 0.03	2.22 ± 0.11†	6.15 ± 0.36	0.113 ± 0.007
Cyclopenthiazide	0.40 ± 0.02	2.35 ± 0.08†	5.99 ± 0.39	0.116 ± 0.008
Phenobarbital	0.49 ± 0.05	2.76 ± 0.23†	5.85 ± 0.42	0.118 ± 0.008

* These kinetic parameters were determined in renal cortical slices from 55-day-old rats repeatedly pretreated with saline (control), PAH, sulphamethoxypyridazine, cyclopenthiazide or phenobarbital. Each value represents the mean ± S.E.M. of four sets of experiments.

† Values significantly different from respective control ($P \leqslant 0.05$).

as described above. The efflux of PAH was quantified by calculating the half-life ($t_{1/2}$) as well as the first order rate constant (k) for the efflux curve constructed from the plot of the differences between PAH concentration per g tissue (wet wt) and the final PAH concentration per ml efflux medium vs efflux time using a Hewlett–Packard calculator (HP 20).

In further studies, kidney weight/body weight ratio, DNA and protein concentrations of kidney cortex were determined in 55-day-old rats repeatedly pretreated with saline, PAH, sulfamethoxypyridazine, cyclopenthiazide and phenobarbital, respectively. DNA was determined as described by Leyva and Kelly [9]. Calf thymus DNA was used as standard [received from ZIMET (AdW)]. Protein was determined using a modified Biuret method [10].

Arithmetic means ± S.E.M. are given. Differences between means were statistically analysed using Student's t-test ($p \leqslant 0.05$).

RESULTS

Table 1 shows the kinetic parameters of PAH accumulation in renal cortical slices from 55-day-old rats. After repeated administrations of PAH, sulfamethoxypyridazine, cyclopenthiazide or phenobarbital, apparent Michaelis constant (K_m = index of the carrier–substrate affinity) is not changed, whereas maximum PAH uptake (V_{max} = index of the transport capacity or transport velocity) is significantly increased in comparison to slices from saline-treated rats. PAH efflux from renal cortical slices back into the incubation medium is not affected by the drug pretreatments of rats.

In analogous experiments the kinetic parameters were quantified in renal cortical slices from 5-day-old rats. For PAH uptake an apparent K_m of 0.40 ± 0.04 mM and an apparent V_{max} of 0.64 ± 0.06 μmole/g (100 min incubation), as well as an efflux $t_{\frac{1}{2}}$ of 14.66 ± 0.69 min, were calculated. Appropriate pretreatments of newborn rats are without effect on the kinetic parameters. Therefore, the findings are not presented here.

Table 2 demonstrates kidney weight/body weight ratio in untreated as well as pretreated 55-day-old rats. After PAH and cyclopenthiazide pretreatments, kidney weight/body weight ratio is slightly increased ($p \leqslant 0.05$) in comparison to saline-treated controls, whereas sulfamethoxypyridazine and phenobarbital pretreatments are without effect.

Figure 1 demonstrates DNA and protein concen-

Table 2. Comparison of kidney weight/body weight ratio in 55-day-old untreated rats, saline-treated controls and in rats pretreated with various drugs*

Kidney weight/body weight ratio				
	Kidney wt (both kidneys) (g)	Body wt (g)	$\frac{\text{Kidney wt}}{\text{Body wt}} \times 100$	
Untreated rats	1.25 ± 0.10	158 ± 11	0.79 ± 0.02	(6)
Control (saline)	1.18 ± 0.06	150 ± 6	0.79 ± 0.05	(12)
Pretreatment:				
PAH	1.31 ± 0.06	153 ± 4	0.86 ± 0.05†	(12)
Sulfamethoxypyridazine	1.19 ± 0.03	152 ± 5	0.79 ± 0.09	(10)
Cyclopenthiazide	1.26 ± 0.03	145 ± 4	0.87 ± 0.02†	(12)
Phenobarbital	1.16 ± 0.14	141 ± 5	0.82 ± 0.06	(12)

* Values represent mean ± S.E.M.; N is given in brackets.

† Values significantly different from control ($P \leqslant 0.05$).

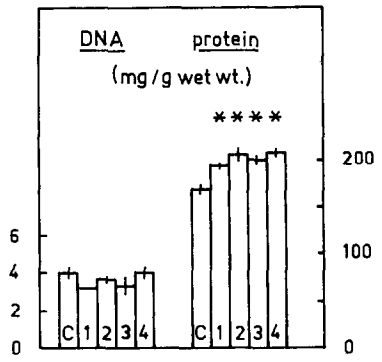

Fig. 1. DNA and protein concentrations (mg/g wet wt) in renal cortical slices from 55-day-old rats pretreated with saline (C), PAH (1), sulphamethoxypyridazine (2), cyclopenthiazide (3) or phenobarbital (4). Means ± S.E.M. are given (N = 3–5). Asterisks indicate values significantly different from their respective control ($P \leqslant 0.05$).

trations in kidney cortex from saline-treated controls as well as from rats repeatedly treated with PAH, sulfamethoxypyridazine, cyclopenthiazide and phenobarbital, respectively. After all pretreatments, DNA concentration in kidney cortex is identical with controls, whereas a small increase in total protein concentration is statistically significant.

DISCUSSION

The cell membrane is involved in the control of cell proliferation [11]. A cascade of morphological, physiological and biochemical changes follows the initial triggering of cell activation and precedes DNA synthesis and cell division. However, not all causally related events will ultimately lead to mitosis [12].

The kidney responds to irritations, such as unilateral nephrectomy [13], temporary ischemia [14], mercury necrosis [15], the administration of a single high dose of folic acid [5, 16, 17], and of suramin [8-(3-benzamido-4-methyl-benzamido)-naphtalene-1,3,5-trisulfonic acid] [18], with a marked increase in weight, DNA and RNA synthesis, followed by an intensive mitosis. Our findings presented here indicate that the stimulation of renal PAH transport induced by repeated administrations of PAH, sulfamethoxypyridazine, cyclopenthiazide or phenobarbital is not associated with such a generalized renal hyperplasia. Neither a marked increase in kidney weight nor an alteration in DNA concentration of kidney cortex was observed after the pretreatment. In this regard, after repeated administrations of PAH, cyclopenthiazide (Table 2), and penicillin [19, 20], the enhancement of PAH transport was associated with a slightly increased kidney weight/body weight ratio, whereas sulfamethoxypyridazine, phenobarbital (Table 2) and 3-methylcholanthrene [21] had no such effect. The small increase in kidney weight/body weight ratio occurring after PAH and cyclopenthiazide pretreatment could be explained by an increase in the volume of tubular cells, since a decrease in body weight was not observed. However, after PAH pretreatment of rats, the water content of kidney cortex was not altered in comparison to saline-treated controls [22].

Electron microscopic examinations of renal tissue from controls and penicillin-pretreated rabbits did not reveal morphological alterations which could explain the stimulatory effect [23]. In addition, penicillin-induced stimulation of PAH transport was not attributed to an increase in the length of the proximal straight tubule, as recently demonstrated [24]. However, small changes in the degree of basolateral membrane infoldings which may result in significant enhancement of surface area cannot be fully excluded.

On the other hand, the reason for the stimulation phenomenon must be searched for in the tubular transport process. Therefore, it was of interest to elucidate whether or not the enhancement of PAH accumulation in renal cortical slices can be characterized by changes in transport kinetics, as already demonstrated for kidney maturation [25]. As demonstrated in Table 1, the apparent K_m of PAH uptake is always the same, whereas the apparent V_{max} is significantly increased after pretreatment of rats with PAH, sulfamethoxypyridazine, cyclopenthiazide and phenobarbital. Furthermore, PAH efflux is not affected by the pretreatments. An efflux alteration was reported for phlorizin [26]. Consequently, an increased carrier capacity or mobility of the carrier–substrate complex can be supposed, whereas the carrier–substrate affinity is not changed. A change in the mobility of the carrier–substrate complex could be affected by variation in OH^-/H^+ ratio [27] which could be attributed to the repeated administrations of organic anions. Thus, it could have a direct effect by changing the conformation of the carrier or other membrane components. In addition, it could also act indirectly through alterations in cellular functions, such as Na^+–K^+–ATPase, that may be involved in renal PAH transport [28]. On the other hand, the stimulation could also be attributed to an increased synthesis or a reduced degradation of the carrier fraction. After all pretreatments, protein concentration in kidney cortex was significantly increased.

Furthermore, renal cortical slices from rats treated with penicillin incorporated more glutamine and leucine than did slices from controls [29]. Finally, the stimulation can be prevented by inhibitors of protein synthesis [3, 30, 31]. These findings permit the assumption that an enhanced *de novo* synthesis of carrier proteins occurred [29].

In agreement with previous findings [3], no stimulation was induced in newborn rats (see Results). In contrast, a marked stimulation was repeatedly documented in 2-week-old, but not in 4-week-old rabbits pretreated with penicillin, phenobarbital or 3-methylcholanthrene [20, 21, 24, 30, 32]. In our opinion, this discrepancy in the findings can be explained by time differences in kidney maturation between the species. The rat kidney cortex is characterized by a high degree of nephrogenesis during the first 15 days of life [33, 34]. In this phase of cell differentiation a stimulation of PAH transport does not seem possible. However, after the 20th day of age a stimulation occurred [35], although the ability to accumulate PAH is as yet immature in comparison

to 33-day-old rats. In contrast, nephrogenic tissue in rabbit kidney is no longer present at 10 days of age [36].

In summary, a stimulation of renal tubular transport mechanism for organic anions was observed after repeated administrations of various organic acids [1–3], as well as of phenobarbital and 3-methylcholanthrene, two classical inducers of microsomal enzyme activities in liver cells [37, 38]. It seems likely that a common mechanism exists which could explain the stimulation phenomenon. Further ways and means must be found to characterize this phenomenon in detail.

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