Enhancement of p-aminohippurate accumulation in renal cortical slices after repeated administrations of various organic anionic drugs to rats of different ages

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Summary. After repeated administrations of various organic anionic drugs to rats of different ages, an enhancement of p-aminohippurate accumulation was observed in renal cortical slices from adult but not from newborn and infant rats. The effect can be interpreted as specific substrate-induced stimulation of the organic anion transport.

After repeated administrations of anionic drugs, such as p-aminohippurate (PAH), probenecid, phenol red, penicillin, sulfaclomide, sulfamethoxypyridazine, and cyclopenthiazide, the renal excretion of PAH was accelerated in rats of different ages, except in the first 2 weeks of life[2]. This effect is not caused by an increase in glomerular filtration rate[3]. Furthermore, changes in tubular reabsorption rate can be neglected, because only 1% of PAH is undissociated in urine[4].

The enhancement of PAH accumulation in renal cortical slices from rats having repeatedly received various weak organic acids could prove a substrate-induced stimulation of the organic anion transport system. Under these in vitro-conditions differences in glomerular filtration rate or renal blood flow can be excluded. The aim of the present study was to investigate whether or not the tubular PAH accumulation in vitro can be increased by repeated administrations of various organic anionic drugs to newborn, infant, and adult rats. In addition, it was studied whether or not the substrate-induced stimulation of PAH accumulation can be prevented by simultaneous administration of an inhibitor of the protein synthesis to adult rats[5,6].

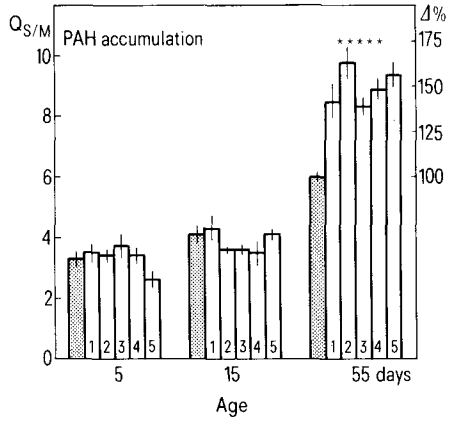
Material and methods. Wistar rats (Jena) of our colony breed were used. Newborn rats of both sexes, and female adult animals were pretreated with different drugs (doses are given in brackets (mg/100 g b.wt i.p.)): PAH (100 or 300), probenecid (20), phenol red (2), sulfamethoxypyridazine (15, once daily), and cyclopenthiazide (5), respectively, twice daily for 3 or 4 days[2]. At 24–16 h after the last administration, renal cortical slices were prepared from 5-, 15- and 55-day-old rats, as previously described[7]. Kidney slices were incubated in Krebs-Ringer phosphate buffer (pH 7.4), which contained 8.5×10^{-5} M PAH. All incubations were carried out in a Warburg apparatus at 25 °C under a gas phase of 100% oxygen for 120 min[7–9]. PAH accumulation was measured and compared with PAH accumulation in renal cortical slices from untreated rats.

In order to estimate the specific effect of the pretreatment on the transport system for organic anions, the PAH accumulation was compared in renal cortical slices from untreated rats, from rats having received 5 ml/100 g b.wt saline, or from rats repeatedly administered 94 mg/100 g b.wt tris-hydroxymethyl aminomethane (THAM), a weak organic base[9].

In further studies, the PAH accumulation was measured in renal cortical slices from untreated rats, from animals administered 0.175 mg/100 g b.wt cycloheximide (inhibitor of protein synthesis), from rats having received 15 mg/100 g b.wt sulfamethoxypyridazine, and from rats simultaneously administered sulfamethoxypyridazine plus cycloheximide for 2 days. PAH was determined by the Bratton-Marshall reaction[10,11]. PAH accumulation is expressed as slice to medium ratio ($Q_{S/M}$). Arithmetic means±SE are given. Differences between means were statistically analysed using Student's t-test ($p \leq 0.05$).

Results. PAH accumulation in renal cortical slices is significantly different depending on age. The slice to medium ratio ($Q_{S/M}$) is 3.3±0.3 for 5-day-old untreated rats and 6.0±0.2 for 55-day-old ones, respectively. After repeated administrations of organic anions, such as PAH, probenecid, phenol red, sulfamethoxypyridazine, and cyclopenthiazide to newborn, infant, and adult rats, an enhancement of PAH accumulation was observed only in renal cortical slices from adult, but not from 5- and 15-day-old animals (figure).

Furthermore, repeated administrations of saline or of the organic cation THAM to adult rats have no effect on the accumulation of the organic anion PAH (table 1).



Effect of repeated administrations of PAH (1), phenol red (2), probenecid (3), sulfamethoxypyridazine (4), and cyclopenthiazide (5) on PAH accumulation in renal cortical slices from rats of different ages. Columns represent means ±SE of 4–12 samples. Asterisks indicate values significantly different from respective control (▨). ($p \leq 0.05$).

Table 1. Comparison of PAH accumulation in renal cortical slices from untreated adult rats and from animals pretreated with saline or THAM. Means±SE are given (n = 4)

PAH accumulation in renal cortical slices from adult rats ($Q_{S/M}$)	
Untreated	6.1±0.2
Pretreated with: Saline	6.2±0.2
THAM	5.6±0.5

Table 2. Effect of cycloheximide on sulfamethoxypyridazine-induced stimulation of PAH accumulation in renal cortical slices

PAH accumulation in renal cortical slices from adult rats ($Q_{S/M}$)	
Untreated	5.9±0.4
Pretreated with:	
Cycloheximide	5.6±0.1
Sulfamethoxypyridazine	*9.1±0.3
Sulfamethoxypyridazine Plus cycloheximide	5.8±0.1

Experiments were performed as described in methods. Each value represents the mean ±SE of 4 samples. Asterisk indicates the value significantly different from respective control ($p \leqq 0.05$).

Table 2 demonstrates the inhibitory effect of cycloheximide on sulfamethoxypyridazine-induced stimulation of PAH accumulation in renal cortical slices from adults. The normally observed PAH accumulation is not affected by cycloheximide. A similar effect of cycloheximide on probenecid-induced stimulation was noted.

Discussion. In principle, a PAH slice to medium ratio > 1 measured in renal cortical slices is an index of the ability of the proximal tubular cells to maintain a concentration gradient. Under steady-state conditions, PAH accumulation is the result of a carrier-mediated transport into the tubular cells, a possible intracellular retention, and of the efflux from the cells back into the incubation medium. Energy is also required to keep the transported PAH inside the cellular border[8]. A passive PAH uptake cannot be stated[8,12]. The intracellular PAH portion is in a free form[13].

Enhancement of PAH accumulation in renal cortical slices from rats repeatedly administered various organic anions could prove a substrate-induced stimulation of the transport system of organic anions. Changes in glomerular filtration rate, renal blood flow, or in extrarenal factors can be excluded.

In our experiments repeated administrations of PAH, probenecid, phenol red, sulfamethoxypyridazine, and cyclopenthiazide to newborn, infant, and adult rats produced an enhancement of PAH accumulation in renal cortical slices from adult, but not from 5- and 15-day-old animals. Similar results were found for THAM accumulation following repeated THAM administrations[9]. There is a good agreement of results obtained under in vitro- and in vivo-conditions[2,14]. On the other hand, repeated administrations of saline or of the organic cation THAM to adult rats has no effect on the accumulation of the organic anion PAH. Consequently, enhancement of renal tubular PAH transport can be interpreted as substrate-induced stimulation.

After repeated administrations of PAH, probenecid, phenol red, sulfamethoxypyridazine, and cyclopenthiazide, no symptoms for nonspecific toxic effects of these drugs occured[15].

Stimulation of the organic acid transport system by penicillin, PAH, and gentamicin, respectively[16-19] was already documented. Treatment of rats with nontoxic doses of organic cationic substrates, such as N-methylnicotinamide or tetraethylammonium, did not result in stimulatory effects, whereas treatment of rats with nephrotoxic agents, such as uranyl nitrate or potassium dichromate specifically enhanced N-methylnicotinamide accumulation in renal cortical slices[20,21].

The observed enhancement of the carrier-mediated PAH transport could be produced by an increased concentration of carrier protein or by an increased turnover rate of the carrier. Consequently, it was studied whether or not the stimulation of PAH accumulation in renal cortical slices can be prevented by simultaneous administration of an inhibitor of protein synthesis[6]. Cycloheximide significantly inhibits the stimulatory effects induced by sulfamethoxypyridazine as well as probenecid. An effect on the de novo-synthesis of carrier proteins can be supposed, as already postulated[22]. Furthermore, after repeated administrations of various organic anions the apparent Michaelis constant (i.e. the affinity of PAH for the transport sites) is unchanged, whereas the maximum PAH concentration (i.e. the transport capacity or transport velocity) is increased in renal cortical slices[18,23]. Finally, the PAH efflux from the proximal tubular cells back into the incubation medium is not affected by the pretreatment of rats with PAH, cyclopenthiazide, and sulfamethoxypyridazine[23]. Further ways and means must be found to characterize the functional transport sites as well as the energy availability.

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Radioimmunological determination of prostaglandin D_2 synthesis in human thrombocytes[1]

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Summary. A specific radioimmunoassay for prostaglandin D_2 was developed. Using the radioimmunoassay, prostaglandin D_2 synthesis by human thrombocytes was measured. While the cyclooxygenase inhibitor indomethacin inhibits formation of prostaglandin D_2, increased formation of prostaglandin D_2 was observed in the presence of the thromboxane synthetase inhibitor imidazole.

Prostaglandin (PG) D_2 has been considered as a biological inactivation product of the PG endoperoxide PGH_2[2] from which it can be formed by enzymatic or non-enzymatic isomerization[2-4]. Recently, however, a number of interesting pharmacological effects of PGD_2 have been described, such as inhibition of platelet aggregation[5,6], increase of