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KINETICS OF *p*-AMINOHIPPURATE TRANSPORT IN RENAL CORTICAL SLICES FROM NEONATAL AND ADULT RATS

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Abstract—PAH accumulation in renal cortical slices of rats is age-related different. Kinetic parameters were quantified using the Lineweaver–Burk analysis. The Michaelis constant for PAH uptake is 0.50 mM in all age groups, i.e., the affinity of PAH for the transport sites is the same. The maximum PAH concentrations (steady-state) in renal cortical slices from 5-, 20-, 33-, and 55-day-old rats are 0.91, 1.72, 2.57, and 1.72 mM, respectively. This finding suggests that the number of transport sites is increasing during the postnatal development of the kidney.

At birth the renal function is immature in most species. The clearances of inulin and *p*-aminohippurate (PAH) increase significantly with age [1, 2]. Likewise, a reduced ability of renal cortical slices from newborn animals to accumulate PAH or *N*-methylnicotinamide *in vitro* has been observed [3–8].

In principle, the accumulation of foreign compounds in renal cortical slices is an index of the ability of the proximal tubular cells to maintain a concentration gradient. Under steady-state conditions the accumulation process is the sum of the influx into the tubular cells, a possible intracellular retention, and of the efflux from the cells back into the incubation medium. PAH influx is the result of an oxygen-requiring transport, a passive PAH uptake cannot be stated. Furthermore, energy is also required to keep the transported PAH inside the cellular border [9]. The intracellular PAH portion ("secretory pool") is in a free form [10].

The reason for the age-related differences in PAH accumulation must be searched in different carrier-mediated transport rates across the peritubular membrane and/or in different permeation rates across the luminal membrane of the tubular cells.

In the present study we have investigated whether the affinity of PAH for the carrier of organic acids and/or the number of the transport sites differ during the postnatal development of the kidney. In addition, the ability of proximal tubular cells to maintain the intracellular PAH pool has been studied under various conditions.

MATERIALS AND METHODS

Wistar rats (Jena) of our institute's colony breed were used. 5-, 15-, and 20-day-old animals of both sexes were kept with their dams up to the time of experimentation. 33-, 55-, 105-, and 240-day-old female rats were fed with standard pellets (Type R, VEB Versuchstierproduktion Berlin–Lichtenberg) and tap water *ad lib.*

Animals were killed under ether anaesthesia by decapitation. Kidneys were removed immediately and placed in ice-cold Krebs–Ringer phosphate buffer (pH 7.4). Renal cortical slices were prepared as previously described [11]. 200 mg of pooled renal cortical slices were always incubated in 3.0 ml Krebs–Ringer phosphate buffer medium containing PAH in different concentrations. All incubations were carried out in a Warburg apparatus at 25° under a gas phase of 100% oxygen for 100 min unless otherwise stated. After incubation the slices were removed from the medium, blotted, homogenized, and denaturated with trichloroacetic acid (10%). PAH (VEB Berlin-Chemie) was determined by the Bratton–Marshall reaction [12, 13].

Age course of PAH accumulation in proximal tubular cells was determined by incubating the renal cortical slices from 5- to 240-day-old rats for 100 min. In the incubation medium the initial PAH concentration was 8.5×10^{-5} M. This concentration is most suitable for such studies as previously described [11]. Results are expressed as slice to medium ratio ($Q_{S/M}$) calculated by dividing PAH concentration per g tissue (wet weight) by PAH concentration per ml medium. Kinetic parameters of PAH transport during the postnatal development were quantified by incubating the renal cortical slices from 5-, 20-, 33-, and 55-day-old rats for 100 min at increasing PAH concentration from 3.3×10^{-5} to 2.0×10^{-4} M. The active PAH uptake was calculated as differences between PAH concentration per g tissue and final PAH concentration per ml medium. Results were plotted using the Lineweaver–Burk plot [14], where the reciprocal of PAH uptake (steady-state) was plotted against the reciprocal of initial PAH concentration in medium. Michaelis constant (K_m) and maximal PAH uptake were calculated.

Effect of a lack of oxygen on the ability of proximal tubular cells to maintain the intracellular PAH pool was determined as follows: (1) Aerobic and anaerobic PAH uptake in renal cortical slices from adult rats were compared after incubation for 40 and 100 min, respectively. In a 3rd group of samples O_2- was substituted by N_2-atmosphere after 40 min. Initial PAH concentration was 8.5×10^{-5} M. Results are

expressed as $Q_{S/M}$. (2) After incubation for 100 min the renal cortical slices from adult rats were separated from the medium, blotted and placed in a medium initially free of PAH. The efflux of PAH under aerobic and anaerobic conditions was compared. Results are expressed as PAH concentration per g tissue.

Arithmetic means ± standard error are given. Differences between means were statistically analysed using the Student's *t*-test ($P \leq 0.01$).

RESULTS

Figure 1 shows the age course of PAH accumulation in renal cortical slices of rats. In the first 15 days of age the PAH slice to medium ratio is low, increases rapidly to a maximum at 33 days and then declines gradually with age.

Figure 2 shows the Lineweaver–Burk analysis of PAH transport in renal cortical slices from rats of different ages. The slopes of the lines representing the net active PAH uptake are age-related different. All lines intersect the abscissa at the same point, whereas the ordinate is intersected at different points. The Michaelis constant (K_m) is 0.50 mM in all age groups. The maximal PAH uptake in renal cortical slices from 5-, 20-, 33-, and 55-day-old rats is 0.91, 1.72, 2.57, and 1.72 mM, respectively. The age-related differences of the maximal PAH uptake are in accordance with the age-related differences of PAH slice to medium ratio (Fig. 1).

Figure 3 demonstrates the effect of a lack of oxygen on the ability of the proximal tubular cells to maintain the intracellular pool of PAH. After anaerobic incubation for 40 min there is no PAH accumulation,

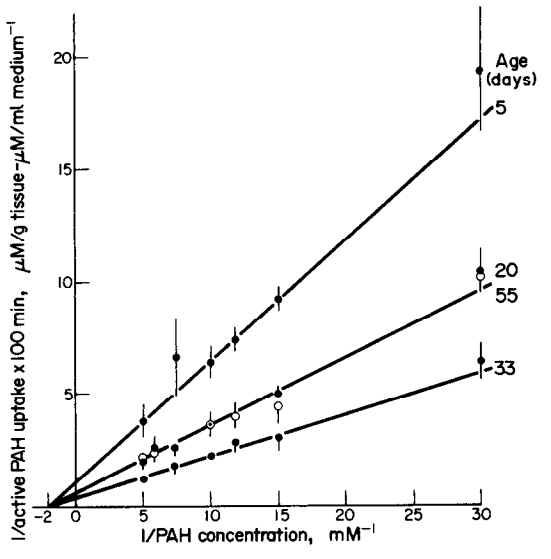

Fig. 2. Lineweaver–Burk analysis of PAH transport in renal cortical slices from 5-, 20-, 33-, and 55-day-old rats. The reciprocal of the difference between PAH concentration in renal cortical tissue (µM/g wet weight) and PAH concentration in incubation medium (µM/ml) was plotted against the reciprocal of initial PAH concentration. Experiments were performed as described in methods (n = 4–8). Kinetic parameters are given in the text.

whereas under aerobic conditions the $Q_{S/M}$ is 4.8 ± 0.1. In the 3rd group of samples, the oxygen atmosphere was substituted by nitrogen atmosphere only after this. Likewise, after anaerobic incubation for 100 min a PAH accumulation cannot be stated. On the other hand the $Q_{S/M}$ is increased from

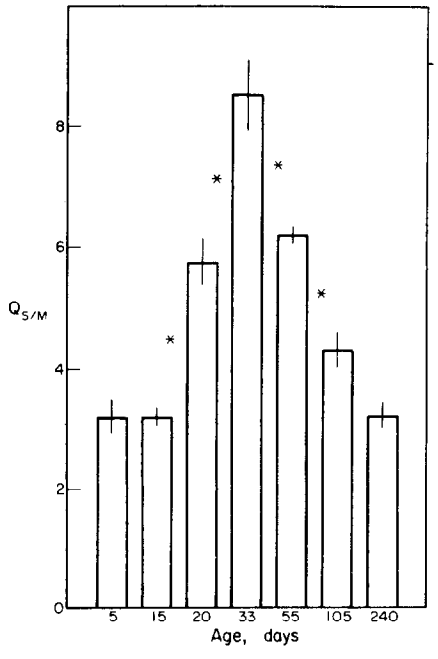

Fig. 1. Age course of PAH accumulation ($Q_{S/M}$) in renal cortical slices of rats. Experiments were performed as described in methods (n = 4–8). Asterisk indicates the value significantly different from the following age group ($P \leq 0.01$).

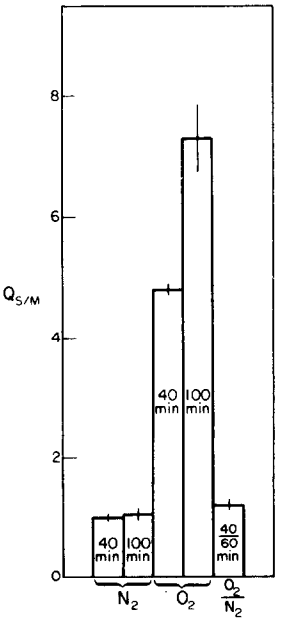

Fig. 3. Effect of a lack of oxygen on the PAH accumulation in renal cortical slices from adult rats. Experiments were performed as described in methods (n = 3–6). N_2 anaerobic incubation; O_2 aerobic incubation; O_2/N_2 after 40 min O_2- atmosphere was substituted by N_2-atmosphere.

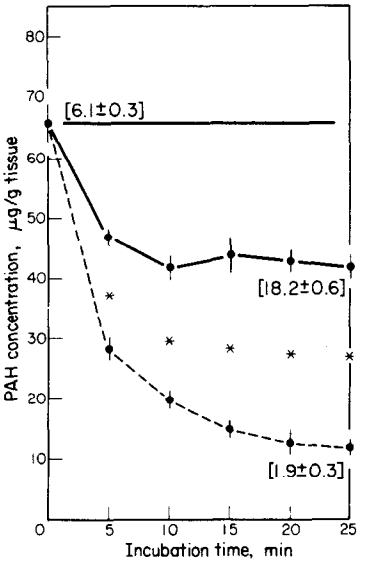

Fig. 4. Runout of PAH from renal cortical slices of adult rats into incubation medium initially free of PAH under aerobic and anaerobic conditions. ●——● Control; ●- - -● N_2-atmosphere. The slice to medium ratio is given in brackets. Experiments were performed as described in methods (n = 4–8). Asterisk indicates the value significantly different from the respective control ($P \leq 0.01$).

4.8 ± 0.1 to 7.3 ± 0.6 under aerobic conditions. In the 3rd group of samples the $Q_{S/M}$ is significantly ($P \leq 0.01$) declined from 4.8 ± 0.1 to 1.3 ± 0.1.

In Fig. 4 the runout of PAH from renal cortical slices back into a medium initially free of PAH is compared under aerobic and anaerobic conditions. After an initial decrease of PAH concentration in the renal cortical slices a new steady-state is maintained under aerobic incubation. In contrast to this finding a permanent runout is observed under anaerobic conditions.

DISCUSSION

The neonatal rat kidney is characterized by a high degree of nephrogenesis during the first week of age. At 15 days, nephrogenic tissue is no longer present and full development has been reported to occur at 28 days [15, 16]. From 1 month to 1 year very little change in structure is evident other than a thickening of the cortical layer and the development of the brush border to normal height [16]. A relationship between histological and physiological development in the neonatal rat kidney has been suggested [2, 7]. The age course of PAH accumulation in our experiments is in accordance with this pattern of kidney maturation.

The steady-state PAH accumulation contained a saturable component, i.e., a decrease in $Q_{S/M}$ with increasing medium concentration as already documented [11, 17]. The net oxygen-requiring PAH uptake follows the Michaelis–Menten kinetics, therefore the data were plotted using the Lineweaver–Burk plot. The strict interpretation of kinetic parameters in the light of enzyme kinetics is not altogether valid, because the transport in a slice/medium system is more complicated. In our opinion, the apparent Michaelis constant and the apparent maximal transport rate are suitable to characterize the PAH transport under comparable conditions. In the present study the K_m-value for PAH uptake in renal cortical slices from rats of different ages is 0.50 mM, i.e., the affinity of PAH for transport sites is the same in all ages. In contrast to K_m-value, the maximal PAH uptake differs during the postnatal development of the kidney, i.e., the transport capacity is increasing to a maximum at 33 days of age. It can be supposed that the age-related enhancement of the transport capacity is due to an increased synthesis of carrier protein as already postulated for the stimulation phenomenon [18]. Other authors have calculated a K_m-value of 0.586 mM for renal cortical slices of adult male rats [19], 0.54 mM for plasma membrane vesicles isolated from adult rat kidney cortex [20], and 0.544 mM for isolated renal tubules of rabbits [21]. It can be stated that K_m-values obtained with different methods are identical in rats and rabbits.

The decline of PAH accumulation that occurs after 4 weeks of age (see above) was also seen in renal tubules isolated from nonperfused kidneys of rabbits [22] as well as in tissue from puppies and piglets [7]. These *in vitro* findings might be explained by a greater runout of PAH from the proximal tubular cells [23]. This interpretation would correspond with the histological development of the brush border [16]. On the other hand, the decline in $Q_{S/M}$ can be explained by a depressed PAH uptake because there is an accordance with results obtained *in vivo* [1, 24]. A competitive inhibition of PAH transport by metabolic products associated with the age-related development of the liver enzyme systems or by dietary origins related with weaning [25] can be excluded in *in vitro* studies. In renal tubules isolated from perfused kidneys of rabbits the reduced ability to accumulate PAH was not observed after 4 weeks of age. Therefore endogenous inhibitors were suggested [22]. In previous studies a maximum of renal sodium excretion was also measured in 33-day-old rats after the administration of an extensive dose of 0.9% NaCl [26].

In accordance with data in the literature [27–29] the energy-requiring carrier transport is the most important factor in the PAH accumulation. If the oxygen supply is blocked, the proximal tubular cells are not able to keep the transported PAH inside the cellular border. Passive uptake or tissue protein binding of PAH cannot be stated.

Further ways and means must be searched to characterize the functional transport sites as well as the age-related energy availability.

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