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p-Aminohippurate (PAH)
growth
intrinsic transport capacity
kidney
penicillin
tubular length

p-Aminohippurate Transport in the Proximal Straight Tubule: Development and Substrate Stimulation

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Summary

***p*-Aminohippurate transport by the developing kidney has previously been shown to increase as a function of age and to be enhanced by pretreatment with penicillin. The relative contribution of increases in intrinsic cellular transport capacity and in tubular length was assessed in isolated perfused proximal straight tubules obtained from developing rabbits. The intrinsic capacity for transport (10^{-15} M/min · mm) increased 4.8-fold (y = −315 + 62.0x, $r = 0.69$, $P < 0.005$) from 8–19 days of age. Tubular length (microns) also increased (y = −38.5 + 52.3x, $r = 0.84$, $P < 0.001$) during the same period. Nine- to 13-day-old animals pretreated with procaine penicillin (400,000 U) divided over 3 days had an intrinsic capacity for transport of 684 ± 88 × 10^{-15} M/min · mm, which was 89% greater than the 361 ± 84 × 10^{-15} M/min · mm observed in age-matched, untreated controls ($P < 0.02$). In these groups tubular length was 630 ± 106 μm and 585 ± 37 μm, respectively ($P > 0.4$).**

Speculation

Most of the increase in absolute transport (intrinsic capacity × tubular length) seen with age is the result of the increment in the intrinsic transport capacity, while 33% is a consequence of the rise in the surface area of the transport membrane. Substrate stimulation augments the rate of maturation of the intrinsic transport mechanism without altering the age-related change in the length of the proximal straight tubule.

Uptake of *p*-aminohippurate (PAH) by renal cortical slices and secretion of this substance by the kidney have been shown to rise during development (7, 11, 15, 21, 24, 25). This may result from increases in intrinsic tubular transport capacity, the ratio of mass of the transporting segments to cortical mass, or both. Pretreatment of young animals with penicillin hastens the increase in uptake or in excretion of PAH, possibly through similar mechanisms (8, 9, 15). None of the methods thus far used can distinguish between these alternatives. Moreover, they are subject to a number of limitations. For instance, clearance measurements are influenced by the developmental changes in the intrarenal distribution of blood flow (2, 13, 22), which result in alterations in the delivery of substrate to the secreting segments of differing nephron populations; the slice technique detects only the intracellular accumulation of PAH and as a result gives information which corresponds only tenuously to net transepithelial transport (28). In addition, both methods relate transport to the weight of the tissue studied, yet the proximal tubule, the nephron segment responsible for PAH transport, comprises only a finite portion of the cortical or whole kidney mass. The isolated perfused tubule does not suffer from these limitations. Furthermore, it enables one to identify and measure the individual contribution made by changes in tubular length and intrinsic capacity to the maturation of the renal transport of organic anions.

MATERIALS AND METHODS

Sixteen suckling New Zealand White rabbits, aged 8–19 days, were studied for the changes in PAH transport with age. The effect of substrate stimulation on the maturation of the transport mechanism was investigated in an additional 8 rabbits, ranging between 10–13 days of age, which were pretreated with sc injections of procaine penicillin at a total dose of 400,000 U over a 2- to 3-day period. The results obtained were compared to those observed in age-matched controls.

The animals were killed by a blow to the head, and a 1- to 2-mm thick transverse section of the left kidney was transferred to a dish of chilled rabbit serum (Microbiological Associates, Bethesda, MD). As the result of technical difficulties encountered with the extremely thin cortex, we chose to dissect nephrons at random rather than to specifically select either superficial or juxtamedullary nephrons. Dissection of the entire length of a proximal straight tubule (PST), as well as its accurate identification, was assured by removing the terminal convolution of the proximal convoluted tubule and the tapered early descending thin limb. This is clearly demonstrated in Figure 1.

The tubules were transferred to a bathing chamber maintained at 37° and bubbled with 95% O_2–5% CO_2. Tubules were mounted on and perfused through concentric glass pipettes and insulated with Sylgard 184 (Dow Corning, Midland, MI) at both ends as previously described (5). The length of the PST was measured with an ocular micrometer. The holding pipettes and Sylgard seals enclosed less than 10–20 μm (about 5% of the PST at each end.

The perfusion fluid consisted of NaCl, 131 mM; $NaHCO_3$, 5 mM; KCL, 5 mM; Na_2HPO_4, 4 mM; Na acetate, 5 mM; $CaCl_2$, 1.8 mM; $MgSO_4$, 1 mM; was free of glucose and amino acids, and was titrated to a pH of 6.8 (16). The osmolality of the bath and that of the perfusate was measured with a Wescor vapor pressure osmometer (Logan, UT). Because of variations in the osmolality of different batches of serum, inequalities between bath and perfusate osmolality were corrected by the addition of small amounts of deionized water to the solution of higher osmolality (final osmolality 288–300 mOsm/kg H_2O). To replace evaporative losses, deionized water was added to the bath throughout the experiment at a rate which maintained conductivity constant (5).

After an initial 20- to 30-min equilibration period, three to five timed collections of tubular fluid were obtained in a precalibrated constriction pipette to serve as blanks. Collection rates ranged from 3.4–29 nl/min. [^{3}H]PAH (sp act 127 mCi/mM, New England Nuclear) was then added to the bath to provide a concentration of 2 × 10^{-4} M. After an additional 10- to 15-min equilibration period, three to five additional timed collections were obtained. The samples were placed directly into flasks containing 10 ml Aquasol liquid scintillation fluid (New England Nuclear) and counted on a Packard TriCarb liquid spectrometer. PAH secretion was calculated from the total counts in the collected fluid divided by the product of the specific activity and the collection rate.

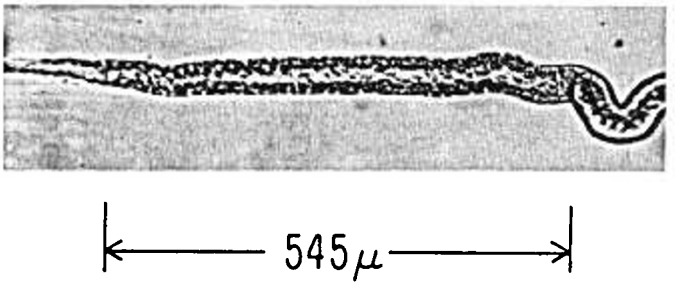

Fig. 1. Photomicrograph of a perfused proximal straight tubule. Note the tapered early descending thin limb of Henle's loop on the left and the terminal convolution of the proximal convoluted tubule on the right. The length of the straight segment is indicated below the figure.

RESULTS

The length of the PST ranged from 218–981 μm and, as expected, correlated well with age (Fig. 2). The equation describing the least squares regression is y (PST length μm) = −38.48 + 52.28x (age in days), $r = 0.84$, $P < 0.001$. According to the regression equation a 2.5-fold increase in PST length would be expected to occur within the age range studied. Close inspection of the data points reveals that two PSTs in 8-day-old animals were substantially shorter than the third and suggests that a curvilinear relationship may obtain during the early stages of development.

The intrinsic capacity (transport of PAH per mm tubular length) increased significantly with age (Fig. 3). The large degree of variability usually found in isolated perfused tubules is evident. The design of the study, however, precluded comparing each tubular segment to itself and thus limiting the scatter. Nevertheless, a significant regression was obtained by least square analysis y (10^{-15} mol/min · mm) = −314.61 + 62.04x, $r = 0.69$, $P < 0.005$. For the age range studied a 4.8-fold increase in intrinsic capacity can be calculated from the regression. Consequently, absolute PAH secretion, the product of intrinsic capacity and tubular length, had to increase since each of its components was found to rise with age. The effect of the increase in intrinsic capacity on absolute transport was nearly twice that of the rise in PST length.

There was no correlation between collection rate on the one hand and age, tubular length, or intrinsic transport capacity on the other.

Table I presents the data obtained from the 16 animals 9–13 days of age, half of which were pretreated with procaine penicillin. The mean age of the pretreated group was 11.3 ± 0.4 days, whereas the control group was 11.1 ± 0.4. The difference is due to only one pair where the control animal was studied 1 day later than the experimental animal. There was no significant difference between experimental and control animals in regard to collection rate (12.1 ± 1.6 nl/min and 15.3 ± 3.1 nl/min, respectively), $P > 0.3$. Tubular length in the penicillin-pretreated group (630 ± 106 μm) was more variable ($F = 8.18$, $P < 0.01$) than in the control group (585 ± 37 μm). However, there was no significant difference between the mean value observed when tested by either parametric (t-test adapted for unequal variance) or nonparametric statistics (Mann-Whitney U-test), $P > 0.4$ (3).

The intrinsic transport capacity was 684 ± 88 × 10^{-15} mol/min/mm after penicillin pretreatment whereas it was only 361 ± 84 in the control group. This is equivalent to an 89% increase in intrinsic transport capacity ($P < 0.02$).

DISCUSSION

PAH uptake by renal cortical slices (21, 24) and renal extraction in whole animal studies (11, 21, 25) have previously been shown to increase as a function of age and to be stimulated by pretreatment with penicillin (7–9, 15). Mechanisms suggested to account for the observed developmental changes and response to substrate stimulation include increases in intrinsic transport capacity, tubular mass, and availability of substrate. Another mechanism by which these changes might occur is genesis of nephrons with a greater transport capacity and/or length.

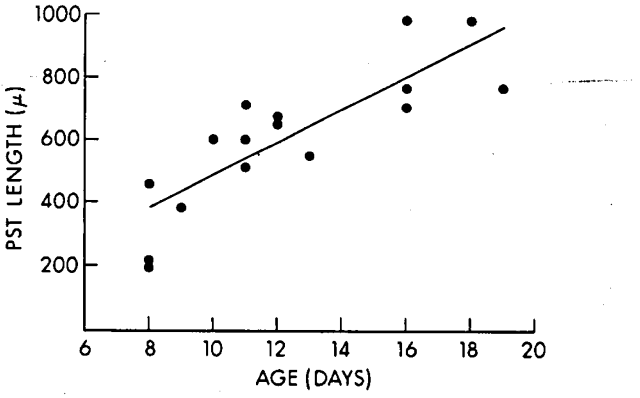

Fig. 2. Proximal straight tubule length (y) as a function of age (x). y = −38.5 + 52.3x, $r = 0.84$, $P < 0.001$.

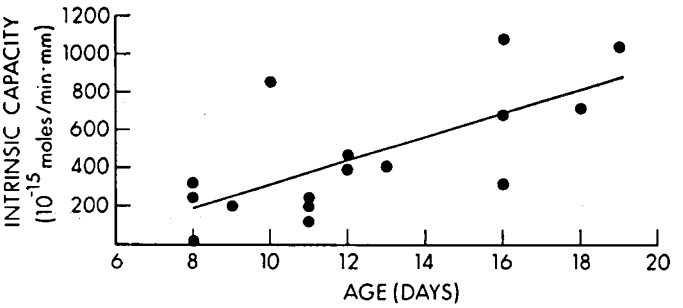

Fig. 3. Intrinsic capacity for PAH transport (y) as a function of age (x). y = −315 + 62.0x, $r = 0.69$, $P < 0.005$.

Table 1. *Data from 16 animals*

	Age, days	Intrinsic capacity, 10^{-15} mol/min·mm	PST length, μm	Collection rate, nl/min
Control ($n = 8$)				
Mean	11.1	361	585	15.3
SE	0.4	84	37	3.1
Penicillin ($n = 8$)				
Mean	11.3	684	630	12.1
SE	0.4	88	102	1.6
P	>0.8	<0.02	>0.6	>0.3

It is evident from these studies that both the intrinsic capacity and tubular length increase as a function of age during the period of development, and the increase in transport cannot be related to an increased substrate availability since the concentration of PAH (2×10^{-4} M) in the bath was constant. Unlike the studies which utilize cortical slices, these experiments do not suffer from problems involving the known changes in the ratios of extracellular/intracellular water content (6) or proximal tubular mass/cortical mass (4) which occur during development. Thus, these observations must be due to either a maturational phenomenon or the neogenesis of nephrons with a longer PST and a greater intrinsic transport capacity.

It has been clearly established that renal growth (10, 12, 19, 20, 23) and functional maturation (2, 13, 14, 17, 22) proceed centrifugally. This is associated with the development of a progressively more superficial population of nephrons, and presents a potential

obstacle to the investigation of maturation at the single nephron level. The problem derives from the fact that newly formed nephrons with specific morphologic and functional characteristics are added to the already existent population and might be randomly chosen for study. Moreover, the stage of maturation is probably variable even among the older nephrons. The problem is further magnified by nephron heterogeneity. In particular, it has been suggested that PAH transport may be greater in the superficial straight proximal tubule when compared to those occupying the deeper cortex (29). This difference may be related to the location and transport rates of specific cell types observed in the deep and superficial segments (30). It is therefore conceivable that the apparent change in measured intrinsic capacity which we observed could merely be a reflection of the differences in cell type or the site of origin of the proximal straight tubules studied, rather than a result of a maturational process which occurs on the level of a single nephron.

Several considerations lead us to reject the hypothesis that the results of this study might be explained solely on the basis of the selection problem which derives from nephronogenesis. First, the mean transport rate which we observed in 8-day-old animals (190×10^{-15} M/min · mm) is similar to that found by Woodhall in the adult rabbit juxtamedullary PST (253×10^{-15} M/min · mm), despite the fact that the concentration of PAH in the bath was 5-fold greater in our studies. This suggests that even if the nephrons selected were from the juxtamedullary region, their transport rate is less than that observed in adult animals. Second, the studies of the development of single nephron function in animals with completed nephronogenesis at birth, such as the guinea pig, have conclusively shown that development of the single nephron glomerular filtration rate of the superficial nephrons lags behind that of the deep nephrons (27). Similarly, a lag in the development of PAH uptake by outer cortical slices as compared to slices from the deep cortex has been reported by Rennick *et al.* (24). It is therefore likely that the study of newly formed superficial nephrons would result in lower values of intrinsic capacity rather than lead to erroneously high values. Third, PAH uptake by cortical slices has been shown to increase after nephronogenesis has been completed (7), which is inconsistent with the hypothesis that the increase in PAH transport derives solely from the neogenesis of "high transport capacity" nephrons. Finally, an approximation can be made to roughly assess the impact of nephronogenesis on the development of the intrinsic capacity. Several assumptions are required: 1) in contrast to the 200,000 nephrons present in the adult rabbit (18), only 100,000 are estimated to be present in the newborn of this species. This value is a gross approximation and is based on reports of nephronogenesis in the dog (22) and rat (1); 2) approximately 25% of the adult nephron population is considered to be "juxtamedullary"; 3) all of the nephrons formed after birth are superficial. Thus, in the adult rabbit, 50,000 nephrons are found in the deep cortex and by definition the same number is present in the newborn rabbit, leaving 50,000 superficial nephrons. The probability of randomly selecting a superficial nephron is therefore 50%, whereas in the adult it is 75%. Assuming that all the nephrons, including the newly formed superficial ones, function at the mature secretion rates of Woodhall *et al.* (29), the selection difference could account for a rise in transport capacity from $795–1068.5 \times 10^{-15}$ mol/min · mm, an increment of 34%. In contrast, our data demonstrate more than a 4-fold increase in intrinsic transport capacity. We therefore conclude that even if the superficial nephrons, shortly after their formation, were to function at rates equivalent to those observed in PST segments of the mature animal (an unlikely event), random selection of these nephrons cannot account for the pattern observed by us. However, as already mentioned, the maturation of the intrinsic capacity is only one of the factors responsible involved in this process. The increase in tubular length plays a lesser, but significant, role.

The studies on the effect of penicillin pretreatment show that the maturation of PAH transport mechanism can be stimulated, as originally shown in cortical slices by Hirsch and Hook (8), and subsequently confirmed by clearance studies in animals (15) and in a human subject (26). Our results demonstrate that this phenomenon is due solely to a rise in intrinsic transport capacity with no concomitant increase in tubular length. The finding appears to be inconsistent with the observation that penicillin pretreatment resulted in an increase in kidney weight (8, 15), the ratio of kidney to body weight, and the protein content of the kidney (9). However, since volume, hence weight, varies roughly with the cube of the whole kidney thickness, minimal changes in this dimension or renal water content, unrelated to changes in the length of the straight proximal tubule, may have profound effects on kidney weight. Similarly, small changes in the degree of basolateral membrane infoldings may result in large increases in surface area available for PAH transport and kidney protein content with no apparent alteration of tubular length. Our data do not permit a kinetic analysis to determine whether the substrate stimulated increase in PAH secretion resulted from increases in the number and/or affinity of transport sites, nor do they allow us to determine whether the membrane surface area available for transport was affected.

In conclusion, we have shown that the increase in PAH secretion which occurs during maturation is explained by a rise in the intrinsic capacity for transepithelial transport and by elongation of the tubule. The former mechanism is responsible for the bulk of the rise in PAH secretion by the PST during this particular age span and its rate of development is enhanced by pretreatment with penicillin.

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