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Substrate Stimulation of P-Aminohippuric Acid Transport: Effect on Uptake and Runout[1] (38847)

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Renal transport of organic anions is quantitatively immature in most neonatal animals. For instance, the clearance of *para*-aminohippuric acid (PAH) is less in newborn rats (1) and dogs (2, 3) than in adults. Several investigators have observed reduced accumulation of PAH by renal cortical slices from newborn (4–6). A great deal of evidence has accumulated which suggests that a primary factor in development of renal organic anion transport is the availability of substrate. Hirsch and Hook (7) demonstrated that pretreatment of newborn rats and rabbits with penicillin, a substrate of the organic acid secretory system, increased the ability of renal cortical slices to accumulate PAH. These studies were based on steady-state measurements when the rate of influx into and efflux from the slices was equal. Theoretically, penicillin might act to increase steady-state accumulation by enhancing active uptake, by decreasing efflux, or by a combination of the two. By determining early maximal uptake and runout of PAH, the effect of penicillin might be further delineated.

The specific objectives of this study were: (1) to quantify the effects of substrate stimulation with penicillin on active PAH uptake into renal cortical slices; (2) to analyze kinetically changes in the active transport process after penicillin; (3) to quantify the effect of penicillin on the rate of PAH efflux (runout) from preloaded slices.

Materials and Methods. Litters of New Zealand white rabbits were kept with their mothers until the time of experimentation. Beginning at 10 days of age, one half of each litter received 30,000 IU of procaine penicillin G (Duracillin; Eli Lilly Co., Indianapolis, IN) subcutaneously, twice daily for 3 days. Control littermates received saline. All animals were sacrificed by a blow to the head 24 hr after the final injection. The kidneys were quickly removed and placed in ice-cold saline. Thin renal cortical slices were prepared free-hand and incubated in the phosphate buffer devised by Cross and Taggart (8). After incubation, the slices were removed from the medium, blotted on gauze, and weighed. The tissue was treated as outlined by Cross and Taggart (8). PAH concentrations were determined by the methods of Smith *et al.* (9).

To estimate the maximal rate of uptake into slices, tissue was preincubated for 30 min. PAH was then added to achieve medium concentrations of 1.0, 2.0, and 4.0 $\times$ 10^{-4} M and the slices incubated for another 15 min. Duplicate tissue samples were incubated simultaneously in a two-chambered Dubnoff metabolic shaker, one under a gaseous phase of 100% oxygen and the other under 100% nitrogen. The oxygen-requiring component of PAH transport was determined by calculating the difference between PAH uptake under oxygen and nitrogen. The rate of transport was expressed as micrograms PAH taken up per gram of tissue per minute of incubation.

Runout of PAH was determined using the method of Farah *et al.* (10) with some of the modifications devised by Berndt (11). Slices were preloaded by incubating 300–600 mg of tissue in 6 ml of medium containing 6.3 $\times$ $10^{-4}M$ PAH for 90 min. Tissue was removed from the incubation medium, rinsed, and placed in a net fashioned of nylon mesh. The tissue was transferred at 1-min intervals through a series of 20 beakers each containing 4.0 ml of PAH-free medium. At the con-

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clusion of the runout experiment, the tissue was removed from the net, blotted, weighed, and treated as before. Tissue and runout beakers were assayed for PAH and the results expressed as μg PAH remaining in the slices per 100 mg tissue.

Data were analyzed statistically using a randomized complete block analysis of variance. The 0.05 level of probability was used as the criterion of significance (14).

Results. The rate of PAH uptake increased with increasing substrate concentration. Renal cortical slices from penicillin-treated 2-wk rabbits took up 2.50 $\pm$ 0.14, 3.65 $\pm$ 0.10, and 5.51 $\pm$ 0.09 μg PAH g^{-1} min^{-1} at medium concentrations of 1.0, 2.0, and 4.0 $\times$ $10^{-4}M$, respectively (Fig. 1). The rate of PAH uptake in these slices was significantly greater at each of the medium concentrations than in slices from control animals (1.90 $\pm$ 0.10, 3.02 $\pm$ 0.17, and 4.87 $\pm$ 0.17 μg g^{-1} min^{-1}). The rate of PAH uptake under nitrogen was not affected by penicillin pretreatment (Fig. 1). The difference in uptake between oxygen and nitrogen incubation, the best estimate of the oxygen-requiring, active

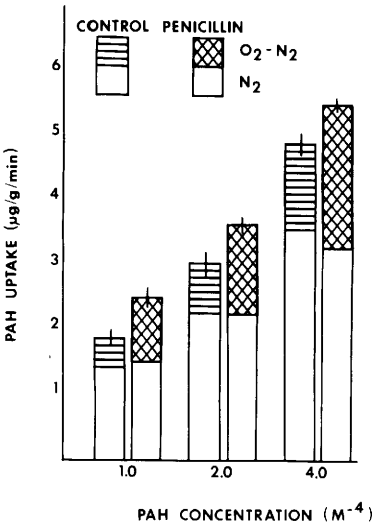

FIG. 1. *P*-Aminohippurate (PAH) uptake in 2-wk control and penicillin-pretreated rabbit renal cortical slices. In each experiment, slices from control and treated animals within a litter were pooled and distributed into 12 beakers, four at each concentration of PAH and two under each gaseous phase. Duplicate values were averaged. Each bar represents means ($\pm$SE) of pups from eight litters. Vertical bars represent SE of O_2-N_2 uptake.

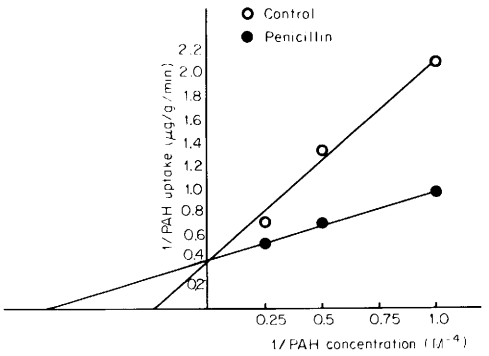

FIG. 2. Double-reciprocal plot of the oxygen-dependent *p*-aminohippurate (PAH) uptake from data in Fig. 1.

component of uptake, was significantly enhanced by penicillin at each concentration (Fig. 1).

A double-reciprocal plot of the oxygen-requiring component of PAH uptake resulted in two lines, representing control and penicillin-treated rabbits, which intersected the ordinate at the same point (Fig. 2). Both lines, however, intersected the abscissa at significantly different points. Because PAH transport is a multistep process, the validity of applying classical Michaelis–Menten kinetical analysis is open to criticism. The data presented in this case, however, are adequately described by such a transformation, allowing at least qualitative comparisons to be made. The results suggest that penicillin treatment did not alter the theoretical maximal rate of transport but rather affected the apparent affinity of this transport mechanism for PAH.

The runout of PAH from preloaded slices exhibited an initial fast component followed by a slower component. The data were linear when plotted on a semilogarithmic scale (Fig. 3). First-order rate constants were calculated for the linear portion of the curve. The runout constant for control tissue (0.022 $\pm$ 0.002 min^{-1}) was not significantly different from that for penicillin-treated tissue (0.023 $\pm$ 0.003 min^{-1}). Farah *et al.* (10) suggested that the rapid component of PAH runout was due to a loosely bound intracellular pool and the slow, linear component to a more tightly bound pool. Contributions to enhanced PAH accumula-

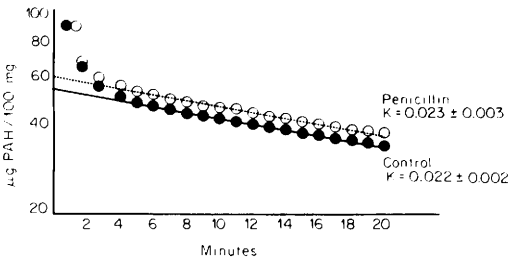

FIG. 3. Runout of *p*-aminohippurate (PAH) from renal cortical slices from control and treated littermates. Slices were preloaded with PAH for 90 min, rinsed, and transferred through a series of beakers containing no PAH at 1-min intervals. Concentration of PAH in the slices as a function of runout time was determined and a first-order rate constant (K) calculated. Points and regression lines represent means from four litters. Constants represent means ± SE and are not significantly different from each other ($P < 0.05$).

tion following penicillin might be made by either decreased runout or enhanced intracellular binding. Both phenomena would result in a slower rate of PAH runout. These possibilities may be excluded since an alteration in runout was not observed (Fig. 3).

Discussion. Hirsch and Hook (7) suggested that the action of penicillin on PAH accumulation was the result of an increased entry into the cell. This could result from higher turnover or more transport protein. Alternatively, increased entry could reflect a change in the affinity of the transport system for substrate. Kim *et al.* (5) and Ecker and Hook (12) investigated apparent kinetic changes in the PAH transport process with development in the rat and rabbit, respectively. In both species the apparent maximal velocity of uptake increased with age, whereas the apparent affinity of the mechanism for PAH was unchanged. Since penicillin appeared to alter apparent affinity and not maximal velocity (Fig. 2), it might appear that the effect of stimulating development with penicillin is different from normal growth. However, several alterations from previous techniques were included in this study. Ross and Weiner (13) demonstrated that freshly prepared renal cortical slices exhibit decreased adenylate energy charges, probably due to hypoxia during preparation. Aerobic incubation for 30 min allowed the slices to regain normal adenylate energy charges and presumably normal levels of tissue constituents necessary for maximal rates of transport. Previous kinetic analyses (5, 12) did not include preincubation and were in reality measuring transport under less than optimal conditions. Therefore, after preincubation, rates of PAH uptake were estimated for only 15 min to insure that influx into the cell would be the major component of PAH movement. Lastly, incubation of tissue slices under 100% nitrogen results in a PAH S/M ratio[4] of 1. PAH uptake under nitrogen, therefore, was taken to represent that portion of uptake due to passive diffusion. Previous kinetic analyses were done under oxygen only. Thus no attempt is made to compare quantitatively the data in Fig. 2 to previous work. The data are meant only for illustrative purposes, not as a rigorous kinetic analysis of the transport system.

Summary. The ability of penicillin pretreatment to increase PAH accumulation by slices of newborn rabbit renal cortex was dissected into two components, uptake and runout. The oxygen-requiring component of the uptake process was significantly enhanced by penicillin treatment, whereas runout was unaffected. Kinetically, the data suggest that penicillin alters the affinity of the transport system for PAH. Due to the limitations of such a kinetic analysis, no conclusions may be drawn from such a suggestion. However, it may be concluded that penicillin pretreatment increases renal accumulation of PAH solely by stimulating the uptake process. Elucidation of the molecular changes involved will require techniques more sophisticated than uptake into renal cortical slices.

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[4] S/M ratio represents steady-state concentrations of ([PAH]/g slice)/([PAH]/ml medium).

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