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KINETICS STUDIES ON THE RENAL TRANSPORT OF PROBENECID IN VITRO

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Summary

1. The kinetic parameters of renal transport of probenecid have been assessed by studying the uptake of the drug in rabbit kidney tubules incubated in an electrolyte medium under various conditions.

2. The added compounds inhibited the uptake of probenecid both by kidney cortical slices and separated renal tubule preparations in the following order: *p*-aminohippurate < phenol red < bromophenol blue < bromocresol green. A reversible competitive inhibitory effect of these organic anions on the renal accumulation of the drug was observed.

3. The K_m for renal uptake of probenecid in separated tubules (0.04 mM) and the K_I values calculated in this system for *p*-aminohippurate (0.5 mM), phenol red (0.09 mM), bromophenol blue (0.02 mM) and bromocresol green (0.015 mM) were found to be in good agreement with the corresponding K_I value of probenecid and K_m values of these compounds previously observed in various kidney tissue preparations.

4. On the basis of above mentioned findings, it is concluded that probenecid, *p*-aminohippurate and various phenolsulphonphthalein dyes are transported by the common renal organic anion transport system.

Probenecid (di-*n*-propyl sulfamyl benzoic acid) has been recognized for more than a decade as inhibitor of renal transport of organic anions [1—4]. Earlier attempts to characterize the inhibitory action of this drug indicated that the influence of this compound on the renal uptake of organic anions may be non-specific in nature, since it has been reported that probenecid also reduced the renal excretion of compounds that are not organic acids [5—9]. Moreover, the results on the mechanism of transport of probenecid by kidney slices reported by Berndt [10—12], raised further doubt as to whether this drug can be considered as true substrate of organic anion transport system. However, a recent report from our laboratory showed that probenecid is taken up by

rabbit kidney cortical slices by an energy-requiring system susceptable to metabolic inhibition [13]. It also appeared from this study that steady-state renal accumulation of probenecid was inhibited by addition of various organic anions to the incubation medium.

In the present communication, we report experiments in which we have examined further the mechanism of uptake of probenecid by separated renal tubules. Furthermore, an attempt has been made to elucidate the nature of inhibition of renal accumulation of probenecid caused by the presence of *p*-aminohippurate or various phenolsulphonphthalein dyes in the medium.

Separated renal tubules were prepared by modification of the method of Burg and Orloff [14]. Anesthetized male or femal rabbits, weighing about 2.5—4.0 kg were killed by exsanguination. In order to remove plasma albumin from the tissue preparation, the excised kidneys were first perfused with 20 ml physiological saline. 5 ml freshly prepared solution of 0.3% collagenase dissolved in an electrolyte medium of 110 mM NaCl/5 mM KCl/10 mM sodium-acetate/1.2 mM NaH_2PO_4/1.2 mM $MgSo_4$/1.0 mM $CaCl_2$/25 mM $NaHCO_3$ and gassed with 95% O_2 plus 5% CO_2, were used for the injection into the renal artery after ligating the renal vein and ureter. 10 min after intraarterial injection of collagenase, the renal cortex was dissected and minced. The minced cortex tissue was incubated in 20 ml 0.15% collagenase dissolved in the above mentioned electrolyte medium for 1 h at 25°C under continuous O_2 supply. The loosened tubular suspension was filtered through a single layer of surgical gauze and the filtrate was centrifuged at $120 \times g$ for 1 min. The sediment thus obtained was treated with three alternating repetitions of washing with the same solution and with centrifugation in order to remove collagenase. Then the renal tubules were suspended in the electrolyte medium containing 0.015 mM ^{14}C-labelled probenecid together with non-labelled probenecid in desired concentrations, to give a 5% (wet wt./v) suspension. *p*-Aminohippurate, phenol red, bromophenol blue or bromocresol green was added to the incubation medium as indicated in the figures. Small portions of the suspension (5 ml) were incubated for 45 min at 25°C in the special flask described by Burg and Orloff [14] and during this period stirring and oxygenation of the samples were achieved by bubbling 95% O_2 and 5% CO_2. After the incubation period, the contents of the flask were quickly transferred to centrifuge tubes and centrifuged for 15 min at $15\,000 \times g$ and 0°C. The radioactive contents of supernatants and sediments were analyzed as previously described [13]. Inulin space was determined in a separate series of experiments as described elsewhere [15].

In a series of experiments the effect of *p*-aminohippurate and various phenolsulphonphthalein dyes on the accumulation of probenecid in cortical slices were studied. Slices were prepared and incubated as recently described [13].

Table I shows the results of a series of experiments in which reversible competitive effect of different inhibitors on the uptake of probenecid in kidney slices was examined. The cortical slices prepared from the same kidney were divided into two groups, A and B, and the incubation was carried out as described in the legend to Table I. It is seen that *p*-aminohippurate and various phenolsulphonphthalein dyes inhibit the renal accumulation of probenecid, either added from the start of the incubation period (group A) or added after

TABLE I

REVERSIBLE COMPETITIVE EFFECT OF VARIOUS INHIBITORS ON THE UPTAKE OF PROBENECID IN KIDNEY CORTEX SLICES

The slices prepared from the same kidney were divided into two groups, A and B. In group A slices were incubated for 2 h in a medium containing [^{14}C]probenecid (0.015 mM) together with one of the following inhibitors: *p*-aminohippurate (0.5 mM), phenol red (0.2 mM), bromophenol blue (0.1 mM) or bromocresol green (0.1 mM). In group B slices were first preincubated in inhibitor-free, but probenecid-containing medium for 2 h, in order to obtain steady-state accumulation of probenecid. After preincubation an appropriate amount of inhibitor was added to Warburg cups, so as to give the same final medium concentration of inhibitor as mentioned above and incubation was further carried out for two more hours. The results given in the table are the mean values ± S.D. of twelve experiments.

Inhibitor	T/M_{prob} *	
	Group A	Group B
None	27.6 ± 3.2	28.4 ± 4.3
p-Aminohippurate	14.3 ± 1.8	15.6 ± 1.6
Phenol red	9.4 ± 1.9	10.5 ± 1.7
Bromophenol blue	10.1 ± 1.3	11.6 ± 1.5
Bromocresol green	8.7 ± 1.4	9.5 ± 1.8

* T/M_{prob} is the ratio between the concentration of probenecid in tubule water and medium.

preincubation of the slices in medium containing probenecid, in order to obtain first the steady-state accumulation of the drug (group B). These findings strongly suggest that probenecid, *p*-aminohippurate and various phenolsulphonphthalein dyes compete for the same transport system or recognition site(s) at the renal cell membrane.

In order to test the above mentioned hypothesis, the rate of uptake of probenecid in separated renal tubules in the presence of inhibitors was studied. Since we intended to examine the action of the inhibitors in a quantitative

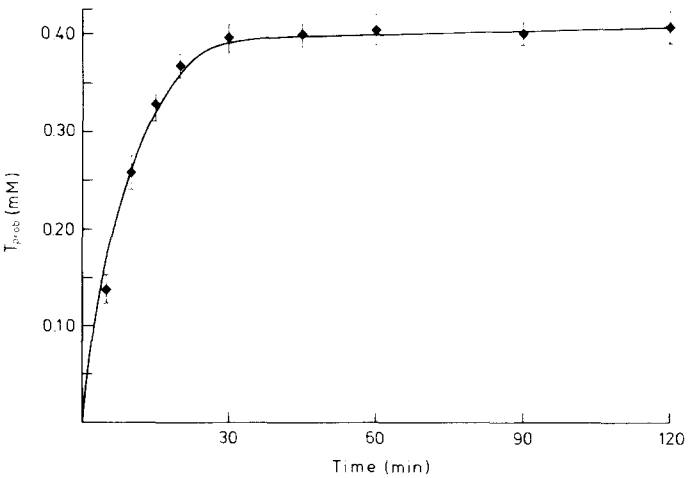

Fig. 1. Time course of uptake of probenecid under aerobic conditions by separated renal tubules. 5 ml of a 5% (wet wt./v) suspension were incubated in electrolyte medium, containing 0.015 mM probenecid. Ordinate: T_{prob}, concentration of probenecid in tubule water. Abscissa: time of incubation. Figure shows mean values of five experiments. Vertical bars indicate S.E.

manner, it was desirable to establish conditions for the attainment of equilibrium values for the uptake of probenecid in tubules.

Fig. 1 shows the results of experiments in which the uptake pattern of [^{14}C]-probenecid in separated renal tubules as a function of time was studied. It can be seen that probenecid is rapidly taken up by the renal tubules during the initial period of incubation, and accumulation of the drug approaches steady-state value already after an incubation period of 30 min. In all experiments to be reported below the separated renal tubules were kept in the incubation medium for 45 min to ensure steady-state conditions for the uptake of probenecid.

The effect of relatively low concentrations of *p*-aminohippurate and various phenolsulphonphthalein dyes on the renal uptake of [^{14}C]probenecid at increasing medium concentrations of non-labelled probenecid is shown in Fig. 2. Here curve A functions as a control, and curves B, C, D and E refer to the addition of *p*-aminohippurate, phenol red, bromophenol blue and bromocresol green, respectively. It appears from the figure that the presence of these compounds results in various degrees of reduction of probenecid concentration in the tubules. The compounds inhibit the uptake of probenecid by separated renal tubules with the following order of effectiveness: *p*-aminohippurate < phenol red < bromophenol blue < bromocresol green. Further analysis of these results indicates that a linear relation can be obtained by plotting reciprocal values of the points pertaining to probenecid uptake curves (A—E). K_m for

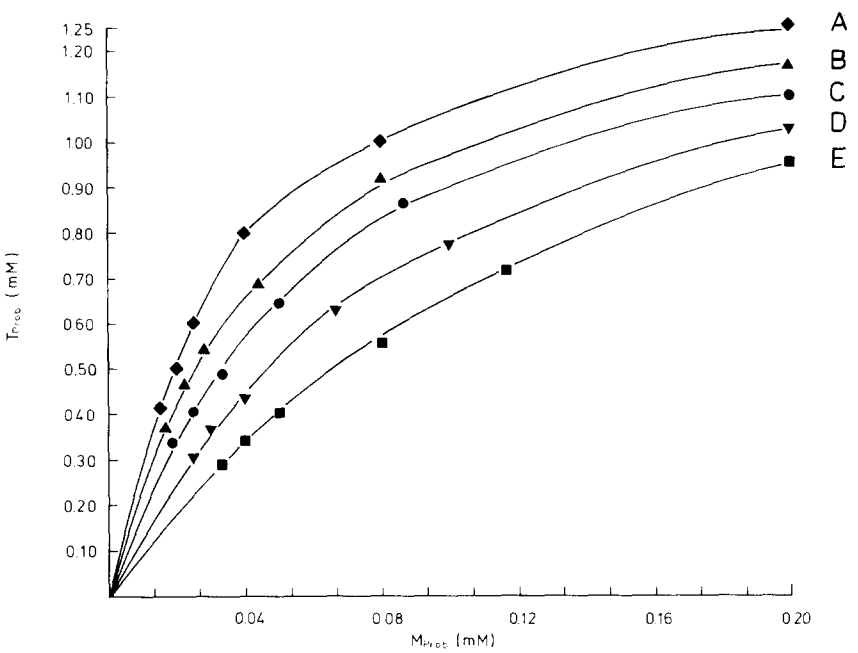

Fig. 2. Effect of *p*-aminohippurate and various phenolsulphonphthalein dyes on the uptake of probenecid in separated renal tubules. Curve A shows the tubular concentration of probenecid without inhibitor. Curves B, C, D, and E illustrate the inhibitory effect of *p*-aminohippurate (0.125 mM), phenol red (0.06 mM), bromophenol blue (0.05 mM), and bromocresol green (0.05 mM), respectively. The figures in parentheses indicate the initial concentration of inhibitor in the medium.

TABLE II

K_m VALUES FOR THE RENAL UPTAKE OF PROBENECID, VARIOUS PHENOLSULPHONPHTHALEIN DYES, *p*-AMINOHIPPURATE, AND THE RESPECTIVE K_I VALUES OF THESE COMPOUNDS

Note that the K_m values for the renal uptake of various phenolsulphonphthalein dyes and *p*-aminohippurate given in the table are taken from our previous work [15—17], whereas the K_I value of probenecid is taken from the recent work of Berner and Kinne [18].

Compound	K_m (mM)	K_I (mM)
Probenecid	0.04	0.05 (ref. 18)
Phenol red	0.15 (refs. 16 and 17)	0.09
Bromophenol blue	0.03 (ref. 17)	0.02
Bromocresol green	0.02 (ref. 17)	0.015
p-Aminohippurate	0.6 (ref. 15)	0.5

probenecid uptake (i.e. the medium concentration which gives half saturation of probenecid uptake) was calculated to be 0.04 mM. K_I values for *p*-aminohippurate and various phenolsulphonphthalein dyes inhibition of probenecid transport may be defined on the basis of K_m,K'_m in the presence of *p*-aminohippurate or phenolsulphonphthalein dyes and the medium concentration (I) of these organic anions, in an analogous way as is done for competitive enzyme inhibition ($K'_m = K_m (1 + I/K_I)$). The results of this analysis are given in Table II from which it appears that the K_I values for *p*-aminohippurate (0.5 mM), phenol red (0.09 mM), bromophenol blue (0.02 mM) and bromocresol green (0.015 mM) agreed very well with the K_m values of these substances reported in the literature [15—19]. This resolution of the experimental data thus demonstrated that the substances in question competitively inhibit the uptake of probenecid in separated renal tubules. The reason for the different results obtained by us and Berndt may be that we used ^{14}C-labelled probenecid which enabled us to study the characteristics of uptake of this compound at relatively low medium concentrations. Berndt has used high concentration of probenecid in his studies, and therefore, was unable to detect the competitive inhibitory effect of *p*-aminohippurate and other organic anions on the uptake and run out of the drug by kidney slices [10,12].

In conclusion, the experimental data on the uptake of probenecid in separated renal tubules, presented in this report, confirm and extend previous results obtained on the accumulation of probenecid in kidney slices [13], which clearly established that probenecid is a true substrate of organic anion transport system. Furthermore, the kinetic analysis of the results shown in Table II provides strong evidence for the specific inhibitory action of probenecid on the renal accumulation of other organic anions like *p*-aminohippurate and phenolsulphonphthalein dyes.

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