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Probenecid inhibition of the renal excretion of dyphylline in chicken, rat and man

M. ACARA*, E. A. CARR JR, AND E. N. TERRY†

Department of Pharmacology and Therapeutics, State University of New York, Buffalo, NY 14214 and †Departments of Surgery and Pharmacology, New York Medical College, New York, NY, USA

The effect of probenecid on the renal excretion of dyphylline was studied in chicken, rat and man. Dyphylline was found to be actively excreted when measured by the Sperber preparation in hens, the isolated perfused kidney of the rat and clearance studies in man. In each study probenecid significantly decreased dyphylline excretion demonstrating that dyphylline occupied the renal organic anion transport system. This same drug interaction, at the level of the renal excretory system, in these three species occurred at comparable concentrations of dyphylline and probenecid.

Dyphylline (dihydroxypropyltheophylline) has been the subject of a number of investigations in which an alternative to theophylline or aminophylline therapy was sought (Maney et al 1946; McColl et al 1956; Hudson et al 1973). The interest in it as a bronchodilator is because it has a low incidence of side effects, particularly in comparison with theophylline. The disadvantage in the use of dyphylline is the frequency of dosing necessary because of its 2 h half-life (Gisclon et al 1979; Simons & Simons 1979). May & Jarboe (1981) have reported that probenecid can extend the half-life of dyphylline in human subjects. Probenecid has been used by many investigators as the classical inhibitor of organic anion transport (Weiner et al 1960).

In addition to demonstrating the prolongation of dyphylline half-life in human plasma by probenecid, this study describes qualitative and quantitative interspecies similarities in organic anion transport by comparing probenecid's inhibition of the renal excretion of dyphylline in chicken, rat and man.

METHODS

Sperber technique in chickens

The avian kidney has a renal portal blood supply which is accessible through a leg vein (Sperber 1948). Infusions into that vein reach the ipsilateral kidney before entering the general circulation. A sphincter valve within the kidney regulates the proportion of flow which will perfuse that kidney. Blood from the general circulation reaches both kidneys equally with the same composition. The chicken has no bladder and after exposing the cloaca, urine may be collected separately from each kidney by placing tubing over each ureter. In order to determine the per cent of infusion reaching the kidney, a marker compound, which is completely excreted by the renal tubules in a single pass through the kidney, is added to the infusion. For these experiments the marker is tetraethylammonium (TEA).

Data from the Sperber technique are expressed as apparent tubular excretion fraction or ATEF. It is calculated as $Exc_{ipsi} - Exc_{contra}/infused \times 100$. The transport efficiency (TE) is the ratio of the ATEF of the substrate to the ATEF of the marker, and gives the proportion of substrate reaching the infused kidney and excreted by it.

[^{14}C]Dyphylline was infused at 0·8 μg min^{-1}. Unlabelled dyphylline was added to the infusion in varying amounts to yield infusion rates from 100 to 600 μg min^{-1}. [^{3}H]TEA was simultaneously infused at 1×10^{-9} mol min^{-1}. Probenecid was added to the dyphylline infusion of 100 g min^{-1} after appropriate control urine collections, at rates from 0·5 to 2·0 mg min^{-1}. After a 10 min equilibration period for each infusion, three 10 min urine collections were obtained.

Aliquots of urine were measured for ^{3}H and ^{14}C by scintillation counting using double label settings. Electrophoresis of the ^{14}C in the urine and in the infusion was performed at 500 V for 60 min in a 0·01 M phosphate buffer, pH 8·1.

Isolated perfused kidney of the rat

These experiments were performed according to Bowman (1975). The perfusate was composed of Krebs-Henseleit buffer at pH 7·4 containing 6 g % Fraction V BSA, 5·5 mM glucose and 0·8 mg mL^{-1}

* Correspondence.

inulin. [^{14}C]Dyphylline was added to the perfusate at a concentration of 2·7 μg mL^{-1} and unlabelled dyphylline at amounts which gave starting perfusate concentrations of 12·7, 27·7 and 52·7 μg mL^{-1}. Total perfusate volume was 75 mL. Flow rate was 50 to 60 mL min^{-1} and pressure was maintained at 100 mm Hg. Ten minute urine collections were obtained and 2 mL of perfusate were taken at the midpoint of each urine collection. All kidneys were perfused for 90 min. Urine data were expressed beginning at 20 min, giving time for the kidney to stabilize. Probenecid, at varying starting concentration from 50 to 250 μg mL^{-1}, was added to each of the dyphylline starting concentrations. Statistical analyses were obtained through the Prophet Computer System.

Clinical studies

Three healthy male subjects between the ages of 25 and 32 were given physical examinations including blood and urine analysis and electrocardiograms. The procedure to be used was approved by the appropriate clinical committee and by the subjects themselves. The subjects fasted from midnight to the end of each study day. On the first study day (day 1) each subject received 1200 mg dyphylline (Dilor) by mouth in tablet form. Blood was drawn before administration of the drug and at serial times thereafter. Urine collections were obtained at 4, 8, 12 and 24 h. On the second non-consecutive study day (day 2), administration of dyphylline followed on five consecutive days during which subjects took 1 g probenecid daily in divided doses. On the final day, dyphylline was administered 30 min after 250 mg probenecid, and blood and urine samples were taken as before. Blood was immediately centrifuged and plasma frozen for subsequent analysis. Urine samples were frozen at the end of the day.

Analyses

Plasma and urine dyphylline concentrations were determined by HPLC. Extracts of plasma or urine were prepared using propanol–chloroform (2 : 8, v/v) with hydroxyethyltheophylline (HET) as internal standard. To 250 μL of plasma was added 50 mg $(NH_4)_2SO_4$ and then precisely 1·0 mL of the extraction solution. The mixture was shaken for 3–4 min and the aqueous top layer and pellet were aspirated. Aliquots of 0·8 mL of the remaining chloroform layer containing dyphylline and HET were transferred to microfuge tubes and evaporated to dryness under a nitrogen stream at 35 °C. The dried sample was reconstituted in 300 μL of mobile phase for injection. Urine samples of 100 μL with 30 mg of ammonium sulphate were prepared for injection. An aliquot of 50 μL was injected on a Bondapak C_{18} column (Waters Assoc.). The mobile phase was 0·1 M acetate buffer, pH 4·5–acetonitrile (9 : 1, v/v). Flow was 2·0 mL min^{-1} with a pressure of 12·4–15·2 MPa.

The best line was fitted to the plasma data points with the least squares linear regression technique. The slope of this line represented the elimination rate constant (ke). The elimination half-life ($t_{\frac{1}{2}}$) was calculated as $t_{\frac{1}{2}} = 0{\cdot}693/ke$. Extrapolation of the determined line gave the concentration at zero time (C_0). The volume of distribution (V_D) was calculated as $V_D = dose/C_0$. The total body clearance (TBC) was calculated as $TBC = V_D \times ke$.

Materials. [^{14}C]Dyphylline (sp.act. = 0·2 mCi mg^{-1}) and [^{3}H]tetraethylammonium (sp.act. = 92 mCi mmol^{-1}) were prepared by New England Nuclear Corp. Boston, Mass. Dyphylline and probenecid for animal experiments were obtained from Byk Gulden, Melville, NY and Sigma Chemical Co., St Louis, MO, respectively. Dyphylline for oral administration was given as Dilor, obtained from Savage Laboratories, Inc. and probenecid as Benemid, obtained from Merck, Sharp and Dohme.

RESULTS

Excretion of dyphylline by the Sperber chicken preparation

When [^{14}C]dyphylline was infused into the renal portal blood supply of the chicken at infusion rates from 100 to 600 μg min^{-1}, the resulting TE of the pooled data was 0·47 ± 0·029 s.e. (Table 1). There was no trend toward an increasing TE with increasing infusion rates, indicating that [^{14}C]dyphylline was not being metabolized by the kidney and that, throughout this concentration range, saturation of the organic anion transport system did not occur. The mean recovery of [^{14}C]dyphylline in Table 1 was 86·4% ± 3·8 s.e., indicating little sequestering of dyphylline in tissue.

Urine, collected during infusions of [^{14}C]-dyphylline, was subjected to electrophoresis and the mobility of the excreted ^{14}C was compared with that of the infused ^{14}C. Both ^{14}C components migrated at the same rate and indicated that [^{14}C]dyphylline was excreted unchanged.

Probenecid inhibition of dyphylline excretion in the chicken

At an infusion rate of 100 μg min^{-1} [^{14}C]dyphylline,

Table 1. Renal transport of [^{14}C]dyphylline in the chicken.

Dyphylline infusion rate ($\mu g\ mL^{-1}$)	ATEF		Transport efficiency	% Recovery	
	[^{3}H]TEA	[^{14}C]Dyphylline	^{14}C	^{3}H	^{14}C
100	59·7	30·7	0·515	82·6	66·5
129	53·3	28·2	0·544	86·6	86·2
221	48·2	21·9	0·452	90·3	83·7
270	48·3	25·7	0·551	82·9	91·0
400	40·8	13·7	0·334	94·0	87·5
540	60·6	31·0	0·509	78·5	91·2
600	54·7	23·5	0·423	105·8	98·4

Data represent mean of 3 × 10 min urine collections in a single chicken. Infusion rate of [^{3}H]TEA was $\times 10^{-10}$ mol min^{-1}.

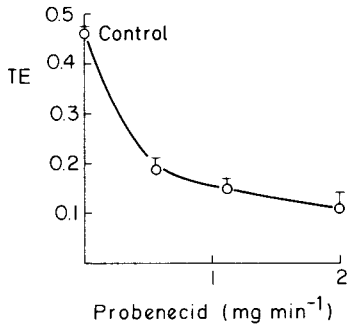

FIG. 1. Inhibition of [^{14}C]dyphylline transport efficiency (TE) by probenecid. Values represent mean ± s.e. N = 3 chickens.

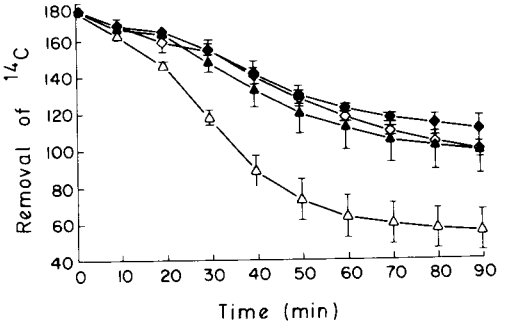

FIG. 2. Probenecid inhibition of removal of ^{14}C (counts $min^{-1}\ mL^{-1} \times 10^{3}$), administered as [^{14}C]dyphylline, from the perfusate recirculating through the rat kidney. △ represents [^{14}C]dyphylline at an initial concentration of 12·7 $\mu g\ mL^{-1}$. Additions of probenecid are represented as ▲ = 50 $\mu g\ mL^{-1}$; ○ = 100 $\mu g\ mL^{-1}$ and ● = 150 $\mu g\ mL^{-1}$. Values are mean ± s.e.

a control TE of 0·46 ± 0·01 was obtained (Fig. 1). Addition of probenecid to the infusion at increasing rates of 0·5, 1·1 and 2·0 mg min^{-1} decreased dyphylline TE by 59, 67 and 75%, respectively. This is evidence that dyphylline occupied the organic anion excretory transport system.

Probenecid inhibition of [^{14}C]dyphylline removal from the perfusate of the rat isolated kidney

In Fig. 2 the concentration of ^{14}C in the perfusate at the starting concentration of 12·7 $\mu g\ mL^{-1}$ was measured and plotted as a function of time with and without the presence of probenecid. Each of the starting concentrations of 12·7, 27·7 and 52·7 $\mu g\ mL^{-1}$ dyphylline showed a similar rate of fall and only the lowest concentration is shown in the Figure. There is a rapid decrease in the concentration of ^{14}C in the perfusate up to approximately 50 min where the concentration levels off. Addition of probenecid at 50, 100, 150 or 250 $\mu g\ mL^{-1}$ to each concentration of [^{14}C]dyphylline produced a significant and considerable decrease in the rate of removal of [^{14}C]dyphylline from the perfusate.

Effect of probenecid on the fractional excretion of [^{14}C]dyphylline by the rat kidney

The clearance of ^{14}C was compared with the clearance of inulin to determine the fractional excretion (FE) of ^{14}C in all experiments. In Fig. 3 the FE for ^{14}C with time is shown for the starting dyphylline concentration of 12·7 $\mu g\ mL^{-1}$. Similar curves were determined for the other two dyphylline concentrations. When [^{14}C]dyphylline alone was present, FE values greater than 1 were found for the 20–60 min period, indicating tubular addition of [^{14}C]-dyphylline to the luminal fluid. Addition of probenecid at 50, 100 and 150 $\mu g\ mL^{-1}$ to the starting perfusate significantly decreased the FE of ^{14}C and the two highest probenecid doses decreased the FE to less than filtration values. A dose-related response may be seen for the effect of probenecid on the FE of [^{14}C]dyphylline at the 30 to 40 min period.

Effect of dyphylline and dyphylline plus probenecid on renal function in the rat isolated, perfused kidney

Only the highest dose of dyphylline resulted in an

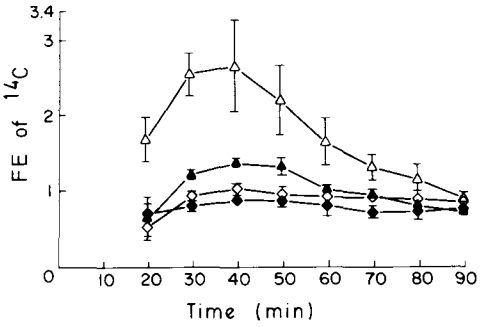

FIG. 3. Inhibition by probenecid of the fractional excretion (FE) of ^{14}C, administered as [^{14}C]dyphylline, by probenecid. Symbols as in Fig. 2.

increased urine volume and the highest dose of probenecid also showed a trend toward increasing urine volumes (data not shown). Protein concentration in urine and the FE of Na, K, Mg and Ca showed no significant differences between dyphylline alone or when it was given in combination with probenecid.

Effect of probenecid on dyphylline half-life in plasma of human subjects

Fig. 4 shows the plasma concentrations of dyphylline obtained in three subjects when dyphylline alone was present and when dyphylline was administered after 4 days of pretreatment with probenecid. The rates of elimination under these two conditions are significantly different. Pretreatment with probenecid significantly decreased the rate of elimination of dyphylline by 150% and extended the $t_{\frac{1}{2}}$ from 2·05 to 5·01 h.

Changes in plasma dyphylline, dyphylline clearance and urine volume produced by probenecid in human subjects

In Table 2, results obtained using the corresponding curves from Fig. 4 are presented. The peak

Table 2. Effect of probenecid on plasma half-life of dyphylline.

	Control	After probenecid
Peak time (h)	0·58 ± 0·08	0·67 ± 0·08
Peak concentration (μg mL^{-1})	29·12 ± 2·80	31·82 ± 2·13
8 h concentration (μg mL^{-1})	1·81 ± 0·33	10·00 ± 0·82
$t_{\frac{1}{2}}$	2·04 ± 0·10	5·01 ± 0·24
V_d(L)	48·68 ± 5·49	39·81 ± 2·85
TBC	225 ± 23	75·67 ± 6·33
% Increase $t_{\frac{1}{2}}$		148 ± 11

Data are presented as mean ± s.e.

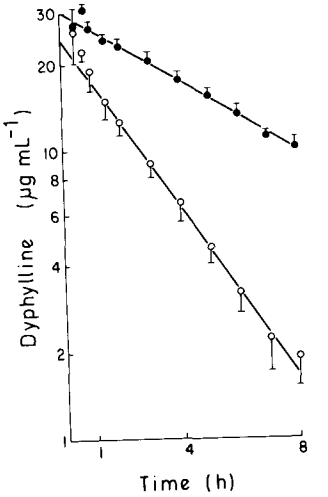

FIG. 4. Plasma elimination rate for dyphylline alone and after pretreatment with probenecid. Data points represent the mean of three subjects. (○) represents dyphylline alone. (●) represents dyphylline in the presence of probenecid.

dyphylline concentration values on both experimental days were reached between 30 and 45 min and were approximately the same, 29·1 and 31·8 μg mL^{-1}, respectively. The 8 h values, however, were very different. When dyphylline alone was present, a low value of 1·80 μg mL^{-1} was obtained. Pretreatment with probenecid increased the 8 h level to 10 μg mL^{-1}. The total body clearance (TBC) was reduced from 224 mL kg^{-1} h to 76 mL kg^{-1} h.

Table 3 shows that probenecid decreased dyphylline clearance while increasing urine volume. The clearance values were decreased approximately three fold in the 0–4 and 4–8 h urine collection periods. In those same periods urine volume was two to three times greater. After 8 h, dyphylline excretion increased due to decreasing probenecid levels

Table 3. Effect of probenecid on dyphylline clearance and urine volume.

	Volume (mL min^{-1})	Clearance (mL min^{-1})	Total amount (mg)
Control (h)			
0–4	1·39 ± 0·48	174·0 ± 27·6	510·9 ± 36·0
4–8	0·90 ± 0·18	309·9 ± 22·2	239·0 ± 14·7
8–12	0·19 ± 0·07		22·0 ± 9·4
12–24	0·78 ± 0·07		32·9 ± 7·3
			804·9 (Total 24 h)
After probenecid			
0–4	2·37 ± 1·22	56·60 ± 10·1	316·1 ± 45·1
4–8	2·71 ± 0·63	83·63 ± 7·27	266·0 ± 44·2
8–12	0·43 ± 0·11		78·7 ± 24·7
12–24	0·78 ± 0·18		130·7 ± 20·6
			800·7 (Total 24 h)

Data are presented as mean ± s.e.

and the per cent of dyphylline recovered over the entire 24 h period with and without probenecid pretreatment was 68 and 66%, respectively.

DISCUSSION

Studies using the Sperber preparation in chickens, demonstrated that dyphylline was actively excreted by the probenecid-sensitive organic anion transport system. Probenecid also decreased the rate of removal of dyphylline from the perfusate recirculating through rat isolated kidneys. Accordingly, pretreatment with probenecid in human subjects led to a prolongation of the plasma half-life of dyphylline in man.

Dyphylline, in a single experiment in the chicken, was shown to be excreted unmetabolized and its recovery was 80 to 90%. Although metabolism of dyphylline in rat or man was not sought in these studies, Zuidema & Merkus (1981) found 84% of an administered dose excreted unchanged in the urine of human subjects in a 24 h period. Thus the main route of elimination of dyphylline is by renal excretion. This is unlike theophylline which is primarily cleared through biotransformation in the liver.

The desire to find a bronchodilator drug which could be used in the therapy of airways obstruction arises because theophylline, currently the most popular drug of this type, can produce serious side effects. Its therapeutic index is narrow and there is a wide variation among individuals in their ability to metabolize the drug. The ability of probenecid to extend the half-life of dyphylline by 2½ times indicates that a combination of these drugs may become therapeutically useful.

The increase in urine volume produced by the administration of probenecid to human subjects was larger than expected. Probenecid was administered in the recommended dosage and there was more than a doubling in urine volume during the initial 8 h period. Dantzler et al (1970) demonstrated that probenecid interfered with the action of vasotocin on snake and frog kidneys. Although no effort was made to determine urine osmolarity or electrolyte concentrations in the present experiments with human subjects, it could be speculated that a water diuresis occurred due to interference by probenecid with antidiuretic hormone activity. In the isolated perfused kidney, where there is little or no circulating ADH, there was only a small increase in urine volume. In the human subject this effect was very pronounced.

Table 4. Comparison of dyphylline and probenecid dosages and plasma levels in chicken, rat and man.

	Dyphylline		Probenecid	
	Concn[a] ($\mu g\ mL^{-1}$)	Dosage ($mg\ kg^{-1}$)	Concn[a] ($\mu g\ mL^{-1}$)	Dosage ($mg\ kg^{-1}$)
Chicken	5–30[b]		14–98	
Rat	13·7–52·7	2·6–9·9[c]	50–250	9·4–46·9
Man	30	17[d]		7·1[d]

[a] Estimated concentration for chicken, peak concentration for rat and man.
[b] Based on infusion into renal blood flow of 20 mL min^{-1} for a 2 kg chicken. Underestimates actual level.
[c] Based on perfusate volume of 75 mL present at 0 time and corrected for weight of rat.
[d] Based on 70 kg man.

Table 4 compares the dosages and concentrations of dyphylline and probenecid administered to chicken, rat and man. Because administration of these drugs to the chicken was as a constant infusion rate, there was no attempt to calculate dosage. The values presented in the Table demonstrate overlap in concentration of plasma dyphylline and probenecid for all three species and similarity in dosage levels for rat and man to achieve the effect of inhibition of dyphylline excretion.

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