Inhibition of p-Aminohippurate Transport by Cholegraphic Agents[1] (35254)

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The radiopaque oral cholecystographic agents are iodinated carboxylic acids with a variety of substituent groups, but all characterized by extreme lipid solubility and strong binding to plasma albumin. They are readily absorbed after oral ingestion and rapidly excreted into the bile. Overall elimination occurs by both the fecal and urinary route, the relative fraction for each depending on the particular compound (1, 2). The urinary excretory product has been identified as a glucuronide for a number of these compounds (3). Iodipamide, the intravenous cholangiographic agent, undergoes rapid biliary excretion, but is not lipid soluble and is not conjugated (4).

These compounds are of pharmacological interest not only because of their primary application in the diagnosis of biliary tract disease, but also because of problems posed by recurrent reports of nephrotoxicity (5). Little is known about the specific mechanisms by which their urinary excretion is regulated. As an intergral part of this problem, it was considered desirable to determine the extent to which they might participate in the renal organic acid transport system. *In vitro* studies on their own uptake by slices of renal cortex are in progress and will be reported elsewhere. However, as an intregral part of the same general problem, it was considered desirable to determine the extent to which they might alter the transport of another organic acid, namely *p*-aminohippurate (PAH).

Methods. The uptake of PAH by slices of rabbit kidney cortex was determined under the following conditions: temperature 25°; oxygen in the gas phase; initial concentrations in the medium: 140 m*M* NaCl, 5 m*M* KCl, 10 m*M* Na acetate, and 7×10^{-5} *M* PAH. Each point was determined as the mean of duplicate incubations. At the conclusion of the incubation, the slices were removed, blotted on filter paper, weighed, and precipitated in trichoroacetic acid. The PAH content in both slice and medium was determined by the Bratton and Marshall method, and the results expressed as slice/medium (*S/M*) concentration ratios. Recoveries of PAH were quantitative and there was no analytical interference from the cholegraphic agents.

Clearance experiments were carried out in mongrel dogs of either sex under pentobarbital anesthesia, using constant speed infusions, with the exogenous creatinine clearance as a measure of glomerular filtration rate (GFR), and with the plasma level of PAH maintained at less than 50 μg/ml. All clearance periods were 20 min in duration. Four control periods were taken in most experiments, three in a few. During the next period (labeled period no. 5 in Fig. 2) an intravenous injection of the sodium salt of the agent under investigation was given slowly over the full 20-min period. Four or five additional clearance periods were then obtained.

We are indebted to the following for generous gifts of drugs: Sterling Winthrop Research Institute, iopanoate and tyropanoate; Squibb Institute for Medical Research, ipodate and sodium iodipamide; Berlin Laboratories, SH-771; and Merck Institute for Therapeutic Research, probenecid. Iophenoxic acid was a gift of the Schering Corporation and was also purified from an old supply of

[1] This study was supported by grants from the NIH (AM-06818 and AM 13020) and the Life Insurance Medical Research Fund.

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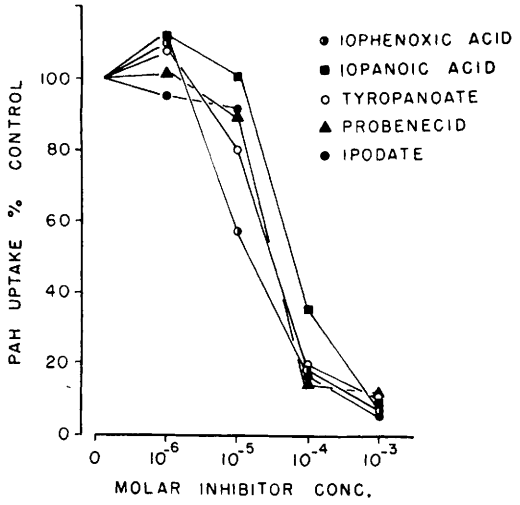

FIG. 1. Inhibition of PAH accumulation by cholegraphic agents. Average of 4 experiments for each compound; all results calculated in reference to individual controls, for which *S/M* PAH ratio was 11.8. At 10^{-3} *M* concentration of inhibitor, PAH *S/M* ratio was 1.09, average of all agents.

oral tablets. ^{125}I labeled compounds were obtained from Abbott Laboratories.

Results and Discussion. As shown in Fig. 1, all of the oral cholecystographic agents inhibited the uptake of PAH in the rabbit slice system, and at high concentrations the degree of inhibition was comparable to the control drug, probenecid. Iophenoxic acid was more potent than the others at 10^{-5} *M* ($p<.05$), but at higher concentrations, there was no significant difference between any of the agents.

Since the cholecystographic agents are strongly protein bound, the effect of added rabbit plasma was examined. Binding of iophenoxic and iopanoic acids to rabbit plasma is approximately the same as to dog or human plasma (unpublished observations). At the concentrations shown in Table I, less than 1% would be free. The addition of plasma completely blocks the inhibitory action of the radiopaque media, except in the case of tyropanoate for which slight inhibition was still apparent.

Renal clearance. As indicated in Fig. 2, the control C_{PAH}/GFR ratios ranged from 2.4 to 2.8, in agreement with values from the literature. Probenecid was used as a control inhibitor of the tubular secretion of PAH, and was given in a dose of 40 mg/kg. This reduced the C_{PAH}/GFR ratio to 1.1, at which it remained stable for at least 80 min. The sodium salts of the radiopaque agents were administered in an identical fashion and in a dose equivalent, on a molecular basis, to the dose of probenecid. As shown in Fig. 2, the C_{PAH}/GFR ratio in period no. 6 (*i.e.*, immediately after the termination of the injection of

TABLE I. PAH Uptake by Rabbit Kidney Cortex Slices.[a]

Effect of plasma on drug inhibition.

Addition of plasma	PAH *S/M* None *S/M*	None *p*	Added *S/M*	Added *p*
Control	12.7		8.7	
Iophenoxic acid	2.8	<.01	7.2	ns
Ipodate	3.6	<.01	7.9	ns
Tyropanoate	3.4	<.01	6.3	<.05
Iopanoic acid	3.4	<.01	8.9	ns

[a] PAH initial concentration 7.5×10^{-5} *M*; all drugs in initial concentration 5×10^{-5} *M*; rabbit plasma diluted with equal parts Krebs–Ringers phosphate; all incubations contained 10 m*M* acetate; each value the mean of 4 experiments. *p* values determined in relation to own control, *i.e.*, without added inhibitor.

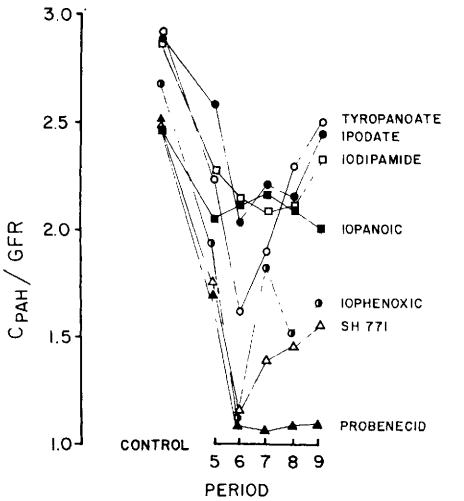

FIG. 2. Depression of PAH/creatinine clearance ratio. Each point is the average of 2 experiments, except for iophenoxic acid and iodipamide for which 3 experiments were done.

the cholecystographic agent) ranged from 1.1 to 2.15. Following this, and during the subsequent 60 min of observation, the C_{PAH}/GFR ratio rose with all agents, returning to within the normal range for 3, but remaining still somewhat depressed for the other 3.

Although the *in vitro* studies had strongly indicated the cholegraphic agents had the potentiality to inhibit PAH accumulation in an isolated slice system, the question arises as to whether their *in vivo* action might be attributed either to a direct action on the cellular aspects of PAH transport, or to an action mediated through changes in renal hemodynamics.

Figure 3 shows that the absolute changes in GFR were rather small. The greatest reduction was seen with iopanoic acid (about 25%). With iodipamide a moderate elevation in GFR was noted. In no experiment was there any striking change in urine volume.

An additional experiment was done with SH-771 to determine renal blood flow by the Fick principle with renal extraction ratios of PAH. The extraction of PAH by the kidney fell from 0.85 to 0.52, and at the same time, renal blood flow (PAH clearance corrected for extraction) fell by 19%. This hemodynamic change is of doubtful significance. In extensive studies on the metabolic aspects of the kidney, Barac-Nieto and Cohen (6) have found no effect of probenecid on renal blood flow at comparable doses.

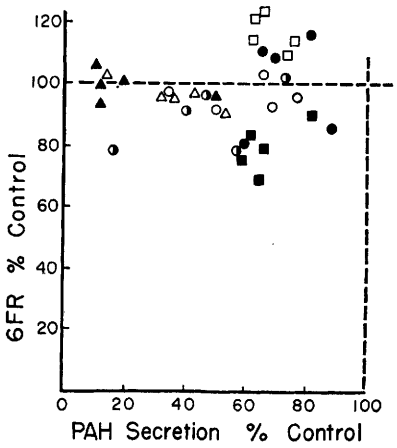

FIG. 3. Summary of changes in clearance. Points correspond to periods 5–9 of Fig. 2. Same symbols as Fig. 2. PAH data recalculated on basis that complete inhibition of secretion would reduce C_{PAH}/GFR ratio to 0.9, uncorrected for plasma binding.

In one of the present experiments, we measured the plasma concentration of iophenoxic acid (Table II). This fell rapidly due to both biliary and urinary excretion. This agrees with other results obtained in an extensive study of this agent (unpublished observations). Presumably, the other gallbladder agents are excreted at an approximately equivalent rate.

Summary and Discussion. These data show that under proper conditions, and at an ap-

TABLE II. Effect of Iophenoxic Acid on PAH Clearance.[a]

Period	(ml/min) GFR	(ml/min) V	P_{PAH} (μg/ml)	C_{PAH} (ml/min)	C_{PAH}/GFR	$P_{Iophenoxic}$ (μg/ml)	$P_{Iophenoxic}$ (% Bound)
Infusion of creatinine and PAH in 10% Mannitol at 1.6 ml/min							
1	34	0.40	25	103	3.0		
2	35	0.33	18	91	2.6		
3	34	0.29	16	94	2.8		
4	31	0.26	15	79	2.5		
Iophenoxic acid 76 mg/kg iv during period 5							
5	35	0.35	13	92	2.6	—	—
6	23	0.33	18	29	1.3	490	97.1
7	23	0.18	19	51	2.2	444	97.2
8	27	0.25	27	46	1.7	376	96.9
9	32	0.48	35	58	1.8	350	96.9

[a] Expt. no. 173; 10.5-kg female dog; pentobarbital anesthesia.

propriate dose, the cholecystographic agents can inhibit the transport of PAH both *in vitro* and *in vivo*. By inference, this suggests that these agents, or their glucuronide conjugates, may participate in the organic acid transport mechanism of the proximal tubule. This has been demonstrated for iophenoxic acid in a separate study (unpublished observations). Definitive experiments on the other oral agents are not available. In the case of iodipamide, its modest inhibition of PAH secretion in the dog can probably not be attributed to its own participation in proximal transort, since Berndt and Mudge (7) could find no evidence for iodipamide secretion in the dog, although it was demonstrable in the rabbit. Using far larger doses, Lindgren (8) has found marked renal hemodynamic responses to this agent.

A major problem posed by these studies is the evaluation of *in vivo* inhibitory potency. Presumably, either total plasma concentration or the concentration of the free (unbound) drug is the appropriate reference point. Considering iophenoxic acid, for which plasma levels are available, in the first post injection period, at the peak of PAH inhibition, the level of total drug in the plasma was 490 μg/ml, of which 2.9% was free. In the case of probenecid, Beyer *et al.* (9) found maximal inhibition of PAH secretion at plasma levels of 200 μg/ml. (In the present experiments the dosage of probenecid was probably supramaximal). From the data of Weiner *et al.* (10), at this plasma level the concentration of free probenecid would be about 60 μg/ml. Even granting the errors intrinsic in the calculation, the data suggest comparable degrees of inhibition of PAH transport at plasma levels of free or unbound drug of 14 μg/ml of iophenoxic acid and 60 μg/ml of probenecid, or, on a molar basis, 2×10^{-5} and 2×10^{-4} *M*, respectively. This evaluation undoubtedly needs readjustment to the extent that unbound iophenoxic acid is a conjugate, rather than the parent drug, and also to the extent that the conjugate might be an active inhibitor of transport. The same considerations may apply to probenecid. However, as a first approximation, the data suggest a rather high inhibitory potency for the iodinated lipid soluble radiographic agents. These calculations from the dog experiments (on the basis of unbound drug) are remarkably close to the *in vitro* results obtained with the rabbit kidney system employing slices of renal cortex.

The authors are indebted to Mrs. Majorie Rossiter and Larry Akerman for their technical assistance.

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Received Sept. 14, 1970. P.S.E.B.M., 1971, Vol. 136.