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Technical Note

Renal Excretion and Metabolism of *p*-Aminohippurate in the Isolated Perfused Rat Kidney

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INTRODUCTION

Para-aminohippurate (PAH) is a prototypic organic anion that is used to probe the mechanisms of renal tubular secretion and as a marker for renal plasma flow (1, 2, and references cited therein). The latter use is possible because of the avid extraction of PAH *in vivo*, such that 91% of this compound is removed from the body in a single pass through the kidneys. However, the ability to discern information on secretory drug transport mechanisms or to obtain accurate estimates of renal plasma flow depends upon a lack of metabolism for PAH in transit through the kidneys. In studying the efficiency of renal extraction of PAH in the isolated perfused rat kidney (IPK), we noticed that a significant portion of the PAH dose was being eliminated as metabolite. This finding plus the relative lack of concern in the literature for potential PAH metabolites (3–6) during experimental studies has prompted this preliminary report.

MATERIALS AND METHODS

Several reports on renal drug transport in the IPK have been published using various combinations of bovine serum albumin + dextran in the perfusate (7–11). As a result, perfusion studies ($N = 2$) were performed in 6.00% bovine serum albumin (BSA) + 0% dextran (D) and in 0% BSA + 4.00% D ($N = 2$). After a 15-min equilibration period, ^{14}C-inulin was introduced as a bolus into the recirculating perfusate; PAH was introduced into the recirculating perfusate as a 150-μg loading dose, followed immediately by an 18.8-μg/min infusion until the conclusion of the experiment. An additional 15 min was then allowed prior to sample collections. PAH (6.25 mg) was dissolved in 50 ml of blank perfusate (no drug or inulin present) and was administered at the constant rate of 0.15 ml/min. Therefore, the replacement of perfusate sample (1.5 ml) during each collection period (10 min) was unnecessary. Still, urine losses were replaced with buffer in order to maintain the original composition of perfusion medium. The details of perfusate composition and the surgical procedure have been reported elsewhere (7).

Functional viability of the kidney was assessed by measuring the fractional excretion of glucose (FEglucose) and the glomerular filtration rate (GFR). The renal clearance of inulin was taken to represent GFR. Renal clearances were calculated for PAH and inulin by dividing the urinary excretion rate of the substance by its perfusate concentration at the midpoint time interval.

Perfusate and urine samples containing PAH and potential metabolites were analyzed by adopting the reversed-phase, high-performance liquid chromatographic (HPLC) assay developed by Prueksaritanont *et al.* (12) with minor modifications. The mobile phase consisted of 3.5% acetonitrile–0.04% phosphoric acid pumped isocratically at a flow rate of 1.5 ml/min for perfusate samples. The mobile phase consisted of 8% acetonitrile–0.04% phosphoric acid pumped isocratically at a flow rate of 1.5 ml/min for urine samples. Under these conditions, PAH and the internal standard, PABA, had retention times of 7.8 and 13.3 min in perfusate, and retention times of 6.3 and 9.6 min in urine, respectively. Using 0.20 ml of perfusate, calibration curves were linear over the perfusate concentration range 0.5–5.0 μg/ml ($Y = 0.460X - 0.002$; $r^2 = 0.993$); using 0.05 ml of urine, calibration curves were linear over the urine concentration range 25–500 μg/ml ($Y = 0.005X + 0.030$; $r^2 = 0.999$). The analysis of *N*-acetyl-PAH was performed in urine samples (0.05 ml) using ultraviolet detection (254 nm) and a mobile phase of 12% acetonitrile–0.04% phosphoric acid pumped at a flow rate of 1.5 ml/min. Under these conditions, *N*-acetyl-PAH and the internal standard, *N*-acetyl-PABA, had retention times of 5.7 and 12 min, respectively. Calibration curves were linear over the urine concentration range 5–100 μg/ml ($Y = 0.030X + 0.009$; $r^2 = 0.999$).

Perfusate and urine samples were assayed for ^{14}C-inulin on a liquid scintillation counter using an external standard method for quench correction. Glucose was assayed colorimetrically using a commercial kit.

Perfusate standards containing PAH (1.8–4.3 μg/ml) were dialyzed for 7 hr and the fraction of unbound drug (fu) was calculated according to the method of Tozer *et al.* (13). The fraction unbound of PAH was 0.667 ± 0.023 in 6.00% BSA + 0% D perfusate and 0.717 ± 0.033 in 0% BSA + 4.00% D perfusate (mean ± SD of four determinations).

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Table I. Physiological Function of the Isolated Perfused Rat Kidney in the Presence of *p*-Aminohippurate

Treatment	Rat No.	Perfusion pressure (mm Hg)	Perfusate flow (ml/min)	GFR (ml/min)	FEglucose (%)	Urine flow (ml/min)	Urine pH
I[a]	1	97.0	37.7	0.667	2.40	0.075	6.17
		(7.4)	(2.1)	(0.092)	(0.52)	(0.028)	(0.09)
	2	87.3	46.3	0.842	4.36	0.116	6.21
		(4.8)	(2.4)	(0.111)	(1.51)	(0.014)	(0.12)
II[b]	3	97.4	23.1	0.727	2.65	0.096	6.33
		(2.8)	(0.4)	(0.038)	(0.82)	(0.011)	(0.05)
	4	99.3	21.8	0.499	4.13	0.130	6.46
		(2.3)	(0.7)	(0.032)	(1.04)	(0.024)	(0.04)

[a] Perfusate composition of 6.00% BSA + 0% D. Each perfusion consists of eight 10-min urine collection periods. Data reported as the mean (±SD) for each perfusion experiment.

[b] Perfusate composition of 0% BSA + 4.00% D. Each perfusion consists of eight 10 min urine collection periods. Data reported as the mean (±SD) for each perfusion experiment.

RESULTS AND DISCUSSION

The physiological function of the isolated perfused rat kidney when PAH was present in both perfusate compositions is shown in Table I. As observed, parameters indicative of kidney function were within the normal range of values for this technique (9–11,14). In particular, functional nephron mass (GFR) and proximal tubular transport (FEglucose) were well maintained.

The renal transport parameters of PAH and its *N*-acetylated metabolite (*N*-acetyl-PAH) are reported in Table II. The total clearance of PAH (CL_T) is comprised of the renal clearance of unchanged PAH (CL_r) plus the *N*-acetyl-PAH that is formed by the kidney (CL_m). As shown in Table II, the *N*-acetyl metabolite accounted for approximately 25% of the total elimination of PAH by the kidney, regardless of whether BSA was present or absent in the perfusate. If one were not aware of this metabolite in the IPK, a substantial underestimation of the efficiency of PAH excretion (ER) and extraction (E) would be made. Interestingly, a 25% conversion of PAH to *N*-acetyl-PAH has been reported in 15 patients with renal impairment (4), while the renal clearance of PAH *in vivo* in the rat has been shown to represent only 75% of its total plasma clearance (15).

p-Aminobenzoic acid (PABA) and *N*-acetyl-p-aminobenzoic acid (*N*-acetyl-PABA) were sought as potential metabolites of PAH in the IPK experiments. This was accomplished by assaying selected perfusate and urine samples for PAH without the addition of internal standard. Still, none of the chromatograms showed a peak in the location previously observed for that of the internal standard, PABA. This indicated that using PABA as the internal standard should not affect the accuracy of the PAH assay. The assay sensitivity of PABA was not tested. However, since the extinction coefficient of PABA (0.017 ml/μg · cm) was higher than that of PAH (0.012 ml/μg · cm), it can be assumed that the metabo-

Table II. Disposition Parameters of *p*-Aminohippurate in the Isolated Perfused Rat Kidney

Treatment	Rat No.	CL_r (ml/min)	CL_m[a] (ml/min)	CL_T[b] (ml/min)	ER[c]	E[d]	fm[e]
I[f]	1	3.86	0.941	4.80	10.8	0.128	0.197
		(0.57)	(0.168)	(0.58)	(1.6)	(0.020)	(0.037)
	2	3.14	1.19	4.33	7.56	0.095	0.288
		(0.92)	(0.13)	(0.82)	(0.79)	(.023)	(0.082)
II[g]	3	2.86	1.06	3.92	7.54	0.170	0.272
		(0.29)	(0.07)	(0.25)	(0.68)	(0.011)	(0.034)
	4	2.22	0.828	3.05	8.54	0.140	0.270
		(0.06)	(0.141)	(0.14)	(0.32)	(0.006)	(0.036)

[a] The formation clearance of the *N*-acetyl metabolite was calculated as the excretion rate of *N*-acetyl-PAH (expressed as PAH weight units) divided by the PAH perfusate concentration.

[b] $CL_T = CL_r + CL_m$.

[c] $ER = CL_T/(fu \cdot GFR)$.

[d] The extraction ratio was calculated as CL_T divided by the perfusate flow rate.

[e] The fraction of PAH metabolized to *N*-acetyl-PAH was calculated as CL_m divided by CL_T.

[f] Perfusate composition of 6.00% BSA + 0% D. Each perfusion consists of eight 10-min urine collection periods. Data reported as the mean (±SD) for each perfusion experiment. The perfusate concentration of PAH ranged from 1.62 to 2.89 μg/ml.

[g] Perfusate composition of 0% BSA + 4.00% D. Each perfusion consists of eight 10-min urine collection periods. Data reported as the mean (±SD) for each perfusion experiment. The perfusate concentration of PAH ranged from 1.61 to 3.24 μg/ml.

lism of PAH to PABA in the IPK was either small (<0.5 μg/ml in perfusate and <25 μg/ml in urine) or absent. In assaying the urine for *N*-acetyl-PAH, the internal standard was omitted in selected samples. Likewise, none of the chromatograms showed a peak in the location previously observed for that of the internal standard, *N*-acetyl-PABA. Since no *N*-acetyl-PABA could be found in these urine samples, this compound was a suitable internal standard for quantitating *N*-acetyl-PAH excretion. It should be noted that the extinction coefficient of *N*-acetyl-PABA (0.050 ml/μg · cm) was the same as *N*-acetyl-PAH. This indicated either that no *N*-acetyl-PABA is formed in the IPK or that negligible amounts (<5 μg/ml) are excreted in the urine.

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