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Renal Tubular Transport of δ-Aminolevulinic Acid in Rat (42297)

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Abstract. δ-Aminolevulinic acid (ALA) interferes with cell membrane and metabolic functions in a variety of tissues. To determine if ALA interacts with renal tubular transport functions, we examined concentrative transport of this heme precursor in rat kidneys. ALA was accumulated against a concentration gradient in rat renal cortical slices. Section freeze-dry autoradiography demonstrated selective accumulation in cells of proximal tubules. Concentrative uptake of ALA was inhibited by KCN, probenecid and *p*-aminohippurate (PAH). ALA inhibited slice uptake of PAH but failed to block slice accumulation of galactose, cycloleucine, lysine, glycine, proline, or α-aminoisobutyric acid and did not alter O_2 utilization. Massive intraperitoneal injection of ALA did not increase 24 hr fractional excretion of amino acids *in vivo*. Concentrative transport of ALA in proximal tubules does not lead to generalized renal tubular transport defects but ALA appears to share the organic acid secretory system in rat kidney. © 1986 Society for Experimental Biology and Medicine.

The heme precursor, δ-aminolevulinic acid (ALA) is accumulated in the blood in both lead poisoning and the hepatic porphyrias and is believed, by some, to mediate the shared neurologic symptoms of these disorders. ALA competes with neuronal receptors for the neurotransmitter, γ-aminobutyric acid (1), and inhibits Na^+, K^+-ATPase (2, 3) and glucose utilization *in vitro* (4). Although the blood brain barrier is relatively impermeable to ALA, ALA gains entry to the cerebrospinal fluid by concentrative transport in the choroid plexus (5). In brain slices, ALA is accumulated against a concentration gradient by a carrier-mediated, energy-dependent transport system (6). ALA is excreted, largely unchanged, within the first 6 hr after intraperitoneal administration to the rat (7).

Increased urinary excretion of ALA in lead poisoning presumably results from elevated blood ALA levels (8), but whether intrarenal mechanisms contribute to increased urinary ALA is unclear. ALA is synthesized in kidney by the inducible enzyme ALA-synthetase (9) and incorporation into heme occurs in the renal cortex (10). Tubular reabsorption of ALA is a carrier-mediated process (11) which could be modified by lead or lead-induced metabolites. Similarly, the Fanconi syndrome seen in acute lead poisoning (12, 13) might be induced by intracellular effects of ALA. Since childhood lead poisoning is associated with marked increases in circulating and urinary ALA, ALA might contribute to the impaired tubular reabsorption in this condition which results in aminoaciduria.

Organic acid transport in the kidney is usually accompanied by cellular accumulation in proximal tubules (14, 15). If ALA is handled like other organic acids in the kidney, concentrative transport in proximal tubule cells could provide local concentrations sufficient to inhibit tubular reabsorption either through a generalized toxic effect or by competitive inhibition. We have, therefore, examined concentrative transport of [^{3}H]ALA in rat renal cortical slices. The intrarenal distribution of [^{3}H]ALA was investigated using a technique of section freeze-dry autoradiography designed for cellular localization of diffusible labeled compounds (15). Participation of ALA in organic acid transport systems, and the effect of ALA on renal cortical slice O_2 consumption, water content, and sugar transport was evaluated *in vitro*. The acute effect of high levels of ALA on sugar and amino acid excretion was also examined *in vivo*.

Materials and Methods. *In vitro experiments.* Female Sprague–Dawley rats, 150–200 g, were anesthetized by the intraperitoneal (ip) administration of Inactin, 40 mg/kg. The kidneys were rapidly excised and placed in chilled Krebs–Ringer bicarbonate buffer (KRB) with 10 m*M* acetate and 95% O_2, 5% CO_2 in the

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gas phase. Slices, approximately 0.3 mm thick, were prepared with a Stadie-Riggs microtome and four slices (weighing approximately 100 mg/flask) were placed in paired, 25 ml Erlenmeyer flasks for each experiment. Incubations were conducted at 25°C for 180 min unless otherwise noted. Slices were heated at 100°C in 1.0 *N* HCl for 10 min and radioactivity was measured in the supernatant using a liquid scintillation counter. Tissue water was determined for each experimental condition by weighing one slice before and after drying at 100°C for 48 hr. Section freeze-dry autoradiography was performed on selected slices as previously described (15, 16). Cellulose acetate chromatography was performed using *n*-butanol, acetic acid, and water in a volume to volume ratio of 25:4:10. ALA was identified by tritium counting and ninhydrin staining.

Renal cortical slice oxygen uptake studies were performed *in vitro* utilizing two slices (weighing approximately 45 mg/chamber) in KRB under the same conditions utilized in the slice uptake studies. Oxygen uptake was measured with Clark electrodes (Yellow Springs Oxygen Monitor, Yellow Springs, Ohio). Four studies were performed under steady-state conditions in which 10 m*M* ALA was added to one of the paired electrode chambers. Slices were subsequently dried at 100°C for 48 hr to obtain dry weight.

In vivo experiments. Three rats were housed in individual metabolic cages for 2 days following which control urines were collected over 24 hr. The rats then received mannitol, 10 mmole in 1 ml saline, ip, and 24 hr urines

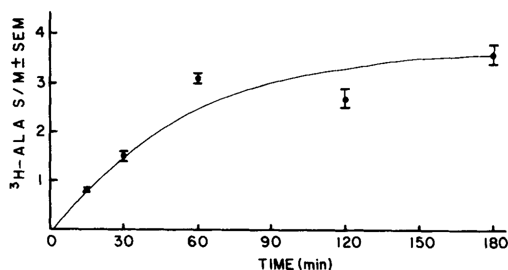

FIG. 1. Accumulation of [^{3}H]ALA (3.5 μM) by rat renal cortical slices in KRB (means ± SEM) *in vitro*. A slice to medium concentration ratio of a $\bar{x}$ of 3.1 ± 0.12 was reached in 60 min and remained at a virtual steady state for 180 min.

TABLE I. INHIBITION OF UPTAKE

Inhibition compound	Concn. (m*M*)	*N*	S/M for [^{3}H]ALA Control	Experimental
PAH	1	5	4.0 ± 0.18	4.1 ± 0.23
PAH	10	10	3.1 ± 0.36	2.4 ± 0.22*
KCN	3	5	4.5 ± 0.66	0.6 ± 0.02*
Probenecid	10	5	3.0 ± 0.32	0.6 ± 0.25*

* $P < 0.05$.

were collected. Following completion of control and mannitol urine collections, the rats received ALA, 10 mmole, ip, and urines were again collected for 24 hr. Assuming distribution of ALA in the total body water, this dose would be expected to achieve plasma ALA levels approaching 200 mg/dl, well above levels reported in humans with acute porphyric attacks or lead toxicity (17). At the end of the experimental periods the animals were anesthetized with Inactin and blood was drawn by heart puncture. Creatinines were determined in plasma and urine samples using a Beckman Creatinine Analyzer 2 (Beckman Instruments, Inc., Fullerton, Calif.). Urine was tested for glucose by the glucose oxidase method using Diastix (Miles Laboratory, Elkhart, Ind.). Amino acid analyses of urine and plasma were performed in the laboratory of Dr. Stanton Segal (18). The significance of the differences between means in cortical slice studies was determined by Student's *t* test for paired data.

Reagents. δ-Aminolevulinic acid hydrochloride [3,5-^{3}H (N)], 2.3 Ci/mmole; proline L-[^{14}C(U)], 273.0 mCi/mmole; lysine, L-[^{14}C(U)], 317.0 mCi/mmole; glycine [^{14}C(U)], 116.0 mCi/mmole; aminocyclopentane-1-carboxylic acid, 1-[carboxyl-^{14}C], 48.5 mCi/mmole, aminoisobutyric acid, α-[*methyl*-^{3}H], 10.0 Ci/mmole and aminohippuric acid, *p*-[glycyl-2-^{3}H], 2.4 Ci/mmole were obtained from New England Nuclear Corporation, Boston, Massachusetts and D-galactose-1-^{3}H, 2.8 Ci/mmole from ICN Chemicals, Irvine, California. Proline, lysine, glycine, and ALA were obtained from Sigma Chemical Company, St. Louis, Missouri; *p*-aminohippuric acid (PAH) from Mann Research Laboratories, New York, New York, and α-aminoisobutyric acid from Calbiochem, Los Angeles,

California. Potassium cyanide was supplied by Fisher Scientific Company and probenecid by Merck Sharp & Dohme Research Lab, Rahway, New Jersey.

Results. Renal cortical slices accumulated [^{3}H]ALA, 3.5 μM, progressively reaching a steady state after 60 min (Fig. 1). The slice-to-medium concentration ratio (S/M) was 3.6 + 0.23 SEM (N = 30) after 180 min. Slice uptake of [^{3}H]ALA, 3.5 μM, was inhibited 22% by 10 mM PAH, 78% by 10 mM probenecid and 84% by 3 mM KCN at 180 min (Table I). Ten millimolar ALA inhibited slice uptake of 50 μM [^{3}H]PAH by 70% but failed to inhibit uptake of 100 μM D-[^{3}H]galactose, [^{14}C]-cycloleucine and [^{3}H]AIB, or 10 μM [^{14}C]proline [^{3}H]glycine and [^{14}C]lysine (Table II). These compounds are either nonmetabolizable organic acids (PAH, AIB, cycloleucine) or are not significantly metabolized by renal cortical slices under our experimental conditions (glycine and lysine (19); proline (20); D-galactose (21, 22)). When [^{3}H]PAH, 50 μM, was incubated with varying concentrations of ALA (0, 1, 3, and 10 mM) the double reciprocal Lineweaver–Burk plot revealed linear regressions consistent with competitive inhibition of PAH by ALA (Fig. 2, Table III).

In four paired studies, 10 mM ALA failed to significantly reduce slice O_2 utilization; renal cortical slice uptake of O_2 was 9.9 ± 0.5 in the presence of ALA and 11.9 ± 1.2 µl/hr/mg dry wt in control slices (N = 4). Section freeze-dry autoradiographs prepared from slices incubated with 3.5 μM [^{3}H]ALA revealed selective accumulation in cells of proximal tubules (Fig. 3). Chromatography showed no difference in migration patterns between preincubation media, postincubation media, and tissue extracts. Concentrative transport of [^{3}H]ALA was evident in S1 and S2 segments of proximal tubules. S3 segments located in the inner cortex were not present in slices prepared for cortical uptake studies. Since [^{3}H]ALA enters the proximal tubule lumen by transport from the antiluminal surface in the slice (15), subsequent transfer from lumen to peritubular surface cannot be discerned in this preparation. The intrarenal distribution of [^{3}H]ALA was distinguishable from that of [^{3}H]PAH by the absence of uphill transport of [^{3}H]ALA into open proximal tubule lumens (15). Autoradiographs of slices incubated with [^{3}H]PAH show higher grain densities associated with open proximal tubule lumens than in association with surrounding epithelial cells (15). *In vivo* administration of ALA, 10 mmole, ip, reduced, rather than increased, fractional excretion of amino acids (Table IV). The mean creatinine clearance of 1.3 ± 0.2 ml/min following mannitol injection increased to a mean of 4.0 ± 0.2 ml/min follow-

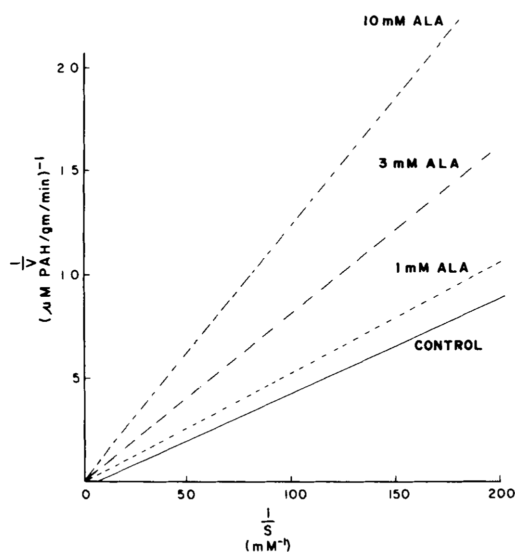

FIG. 2. Lineweaver–Burk plot of the effect of ALA (0, 1, 3, 10 mM) on the uptake of [^{3}H]PAH (0.005, 0.05, 0.1 mM) by rat renal cortical slices. Plot reveals linear regressions consistent with competitive inhibition of [^{3}H]PAH by ALA. Velocity (µmole/g/min) was calculated from the tissue concentration measured after 180 min incubation.

TABLE II. INHIBITION BY 10 mM ALA

	Concn. (μM)	N	S/M Control	S/M Experimental
[^{3}H]PAH	50	10	8.8 ± 0.48	2.5 ± 0.21*
D-[^{3}H]Galactose	100	5	2.1 ± 0.14	2.7 ± 0.44
[^{3}H]AIB	100	10	2.4 ± 0.19	2.1 ± 0.09
[^{14}C]Cycloleucine	100	10	1.6 ± 0.21	1.3 ± 0.10
[^{14}C]Glycine	10	5	5.8 ± 0.90	5.1 ± 0.42
[^{14}C]Lysine	10	5	3.9 ± 0.26	3.7 ± 0.31
[^{14}C]Proline	10	5	2.5 ± 0.39	2.9 ± 0.21

* $P < 0.05$.

TABLE III. LINEAR REGRESSION VALUES FROM LINEWEAVER–BURK ANALYSIS OF ALA INHIBITION OF PAH UPTAKE IN RAT RENAL CORTICAL SLICES

ALA (m*M*)	*N*	Intercept	Slope	*r*
0	68	−0.38	46	0.9
1	20	−0.05	52	0.7
3	10	0.05	81	0.9
10	20	0.11	123	1.0

ing injection of ALA. ALA does not interfere with creatinine determination in the Beckman Creatinine Analyzer 2 so that GFR, creatinine secretion, and/or creatinine production increased following ALA administration. The rats were notably hypoactive for 6 hr and did not defecate for 24 hr following this dose of ALA.

Discussion. These studies show that ALA undergoes concentrative transport in the cells of proximal tubules. Cellular accumulation is inhibited by KCN suggesting direct or indirect active transport with dependence on metabolic energy. Inhibition of slice uptake by probenecid and PAH indicates that ALA shares the organic acid transport system. Inhibition of slice uptake of PAH by ALA is consistent with this view. Although the energy requiring step for tubular reabsorption presumably occurs at the luminal membrane of the proximal tubule, the energy requiring step for secretion occurs at the peritubular side (23). Since cellular accumulation in the slice is the result of transport at the peritubular membrane (15), cellular uptake of ALA is in the direction of tubular secretion in this *in vitro* preparation. The direction of entry of ALA into the cell in the slice is therefore opposite from the direction of entry *in vivo.* Although ALA shares the organic acid secretory system, in contrast to PAH, autoradiographs show no evidence of uphill secretion of ALA from cell to lumen in proximal tubules in the slice preparation.

The relatively high intracellular concentration of ALA achieved in proximal tubules creates a setting in which localized tubular dysfunction might result. ALA has been reported to have toxic effects in neural tissue *in vitro* (4). In addition, this heme precursor was found to inhibit sodium transport in toad skin (24) although no effect on renal salt and water transport was found *in vivo* in dog (25). No evidence of ALA toxicity in rat renal cortical

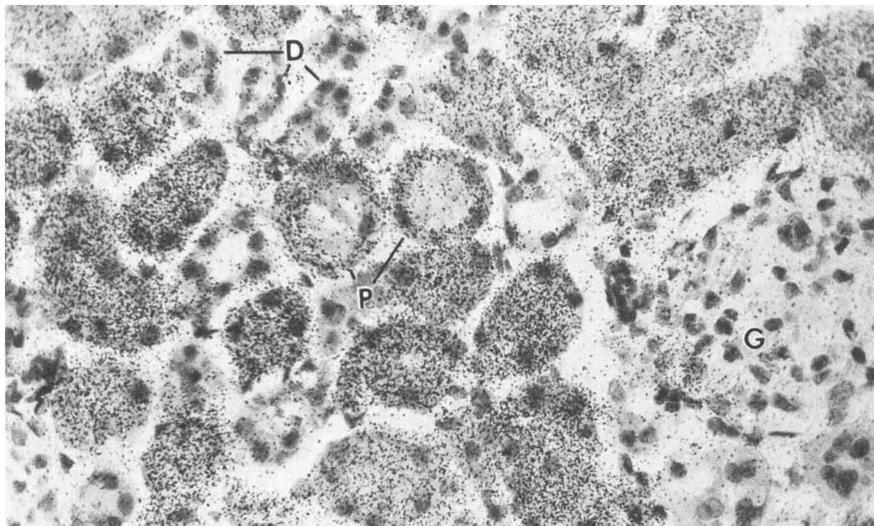

FIG. 3. Section freeze-dry autoradiograph after *in vitro* incubation of rat renal cortical slice with 3.5 μm [^{3}H]ALA. Selective accumulation occurred in cells of S1 and S2 segments of proximal tubules. The intrarenal distribution of [^{3}H]ALA *in vitro* was distinguishable from that of [^{3}H]PAH by the absence of uphill secretion into the lumens of open proximal tubules (P). D = Distal tubule; G = glomerulus; ×400.

TABLE IV. AMINO ACID FRACTIONAL EXCRETION[a]

	Control	Mannitol (%)	ALA
Threonine	1.56	0.76	0.16
Serine	0.19	0.18	0.07
Asparagine	1.44	1.39	0.25
Glutamic acid	1.25	1.96	0.77
Glutamine	0.46	0.24	0.94
Adipic acid	5.42	4.42	0.00[b]
Proline	0.76	0.54	0.13
Glycine	8.64	10.03	2.09
Alanine	3.71	2.21	0.54
Citrulline	2.47	1.93	0.14[b]
α-Amino-*n*-butyric	0.65	0.31	0.02
Valine	0.06	0.04	0.05
Methionine	0.94	0.66	0.22
Isoleucine	0.00	0.00	1.20[c]
Leucine	0.05	0.03	0.03
Tyrosine	0.26	0.44	0.06
Ornithine	0.20	0.12	0.07
Lysine	0.15	0.11	0.13
Histidine	1.83	1.19	0.34
3 Methyl-histidine	33.00	19.00	9.73[b]
Arginine	0.47	0.38	0.10

[a] Mean of three experiments.
[b] One experiment only.
[c] Mean of two experiments.

slices was evident in the present studies; 10 m*M* ALA failed to inhibit slice uptake of 100 μ*M* D-galactose, cycloleucine and AIB, or 10 μ*M* proline, glycine, and lysine. Moreover, ALA did not reduce renal cortical slice oxygen consumption. These findings are consistent with the failure of 25 molar excess amino acids to inhibit tubular reabsorption of ALA *in vivo* in the rabbit (8). A massive intraperitoneal dose (10 mmole) of ALA similarly failed to increase 24 hr amino acid excretion or induce renal glycosuria in rats in the present study.

Although chronic exposure to ALA might produce different results, in the present acute experiments ALA did not appear to interfere with metabolic functions in the proximal tubule and did not impair tubular transport of a number of amino acids or galactose. On the other hand, these studies demonstrate that ALA undergoes concentrative transport in rat proximal tubules and, at least in part, shares the PAH transport system.

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