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Stimulation of renal proximal tubular transport processes by L-proline

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Concentrative uptake of organic solutes into the renal proximal tubule occurs across both the brush border (luminal) and basal-lateral (peritubular) surfaces of the cell. With respect to reabsorptive transport across the brush border (e.g. for sugars [1], amino acids [2], and tricarboxylic acid (TCA) cycle intermediates [3]), it is now abundantly clear that the driving force for transport is the sodium electrochemical potential gradient across the brush border membrane. With respect to secretory transport across the basal-lateral membrane (e.g. for *p*-aminohippurate, PAH), the driving force for transport has not yet been conclusively established although a role for ion gradients has been suggested [4]. Creating the appropriate conditions to support concentrative transport requires cellular metabolism, either to provide energy for a primary transport system (e.g. the sodium pump) or to create the ion gradients (e.g. sodium or protons) which secondarily drive transport. We have shown previously that metabolism of a number of TCA cycle intermediates can stimulate transport of organic solutes across both the peritubular [5] and brush border [6] membranes of rabbit kidney proximal tubule. However, it is not yet clear whether this stimulation of transport occurs only with certain compounds, or can be caused by any compound which, by virtue of being metabolized, increases the renal metabolic rate. Thus, it is of continuing interest to report on additional substances which are found to energize renal proximal tubular transport processes.

Recently, Kuo and Hook [7] reported that L-leucine stimulated PAH uptake by rat and mouse kidney, but not rabbit kidney. These experiments prompted us to look for possible effects on transport processes in rabbit kidney by other naturally occurring amino acids. We confirmed the finding of no effect of L-leucine on transport in rabbit kidney, but discovered that L-proline produced strong stimulation of renal transport processes.

Separated renal tubules were prepared from New Zealand White rabbit renal cortex by a modification [8] of the methods first described by Burg and Orloff [9] and Nagata and Rasmussen [10]. The buffer solution throughout the experiments had the following composition: NaH_2PO_4–Na_2HPO_4 buffer, (pH 7.4), 10 mM; NaCl, 120 mM; KCl, 16.2 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; and $CaCl_2$, 1.0 mM. In addition, uptake solutions contained various concentrations of the amino acids being tested. The compounds whose transport was being examined were present at a total concentration of 0.1 mM (carrier plus ^{14}C-labeled tracer). [^{14}C]-PAH and [^{14}C]α-methyl-D-glucoside were obtained from the New England Nuclear Corp. (Boston, MA), and [^{14}C]-uric acid was obtained from Amersham (Arlington Heights, IL). All other compounds were obtained from the Sigma Chemical Co. (St. Louis, MO).

To begin the transport experiments, 100 μl of the tubule suspension containing approximately 10 mg of tissue was added to 1 ml of uptake medium in a 16 ml polypropylene test tube. The tube was gassed with oxygen for 30 sec, capped tightly, and shaken gently at 22°. At the indicated time, usually 15 min, a 0.5-ml aliquot was removed from the tube and filtered under vacuum through an 8 μm pore size Nuclepore filter. The filter was washed once with 4 ml of ice-cold buffer and placed in a liquid scintillation vial with 10 ml of Aquasol. The time for filtering and washing each sample was under 10 sec. The vials were then counted by liquid scintillation spectrometry. Oxygen consumption was determined with an oxygen monitor fitted with a Clark type electrode as previously described [6].

During initial screening experiments, we examined the effects of 10 mM concentrations of twenty-one naturally occurring L-amino acids on the uptake of PAH into the separated renal tubules. We determined that L-proline had dramatic effects on PAH uptake, and that L-hydroxyproline had a small effect (not further pursued). We then examined the effect of L-proline on renal transport processes in more detail. Shown in Fig. 1 is the concentration dependence of the effects of L-leucine and L-proline on PAH uptake into separated renal tubules. In addition to the usual 15-min uptakes, we also looked at 60-min uptakes to allow comparison with other work [7] in which incubations were as long as 90 min. L-Leucine showed no stimulation of transport, and in fact there was a slight inhibition of PAH transport at higher concentrations. On the other hand, L-proline showed a strong stimulation of PAH uptake with its maximum effect being at 10 mM, with the 15-min uptakes. The effects with 60 min uptakes were similar, but with less stimulation at the higher concentrations.

To determine if the effect of L-proline would be manifest on the uptake of other compounds, we compared the effect of 10 mM L-proline on the uptakes of PAH, uric acid and α-methyl-D-glucoside. The effects of L-proline on the uptake of uric acid and α-methyl-D-glucoside were not as great as the effect of PAH; nevertheless, they were highly significant, with stimulation of uptake of 40 and 63 percent respectively (Table 1). This indicates that the stimulation of uptake is not unique to the PAH transport system, but is apparent with other substrates as well.

We hypothesized that a likely mechanism for the stimulatory effects of L-proline would be by way of effects on cellular metabolism. To test this hypothesis, we examined the effect of several amino acids on tubular oxygen consumption. As shown in Table 2, neither L-alanine nor L-leucine had any significant effect on renal oxygen consumption, whereas L-proline stimulated oxygen consumption by 57 percent. This large stimulation of oxygen consumption by L-proline is of the same order of magnitude as that produced by 10 mM concentrations of TCA cycle intermediates in rabbit separated renal tubules [6].

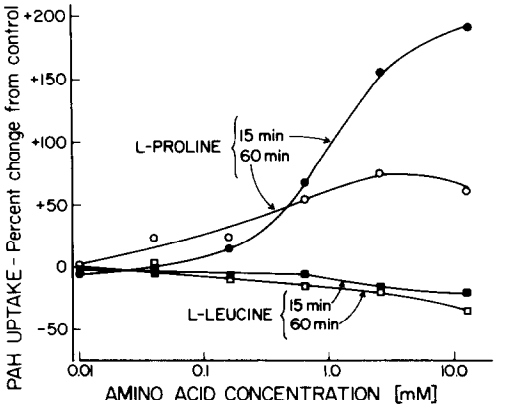

Fig. 1. PAH uptake into separated renal tublues. Uptake was measured after 15 or 60 min of incubation during which various concentrations of L-leucine or L-proline were present. Total PAH concentration (carrier plus tracer) was 0.1 mM. Data are the means of two experiments done in duplicate.

Table 1. Effect of L-proline on the uptake of PAH, uric acid and α-methyl-D-glucoside into separated renal tubules*

Substrate	Uptake [pmoles·(mg protein)$^{-1}$·min^{-1}] Control	10 mM L-Proline	Percent change from control
PAH	101 ± 14	357 ± 35†	+253
Uric acid	86 ± 4	120 ± 13†	+ 40
α-Methyl-D-glucoside	441 ± 39	718 ± 58†	+ 63

* Data are means ± S.E. from three experiments run in duplicate.
† $P < 0.01$.

Table 2. Effects of amino acids (10 mM) on oxygen consumption by separated renal tubules*

Amino acid	Oxygen consumption [nmoles·(mg protein)$^{-1}$·min^{-1}]	Percent change from control
Control	7.86 ± 0.38	
L-Proline	12.37 ± 0.25†	+57
L-Alanine	8.16 ± 0.34	+ 4
L-Leucine	8.43 ± 0.48	+ 7

* Data are means ± S.E. from three experiments.
† $P < 0.01$.

Based on the experiments presented, we propose the following sequence of events to explain the stimulatory effects of L-proline on renal transport processes. First, L-proline must enter the renal cell. This could occur across either the basal-lateral membrane and/or across the brush border membrane. However, Hammerman and Sacktor [11] have shown that L-proline crosses the brush border membrane by a sodium-dependent cotransport process, and this route could be the means of uptake of L-proline in separated renal tubules. We have shown previously that substrate uptake into separated renal tubules can occur across the brush border membrane [6]. Once the L-proline has entered the cell, it fuels the renal metabolic process as evidenced by the increase in oxygen consumption. The increased metabolic energy is available to drive transport processes. The increased uptake of α-methyl-D-glucoside could be secondary to an increase in the magnitude of the sodium gradient across the renal brush border membrane. That is, the increased metabolic energy increases the extrusion of sodium from the cell by the sodium pump on the basal-lateral membrane. This, in turn, increases the driving force for the uptake of the sugar across the brush border membrane. It is known that α-methyl-D-glucoside crosses the brush border membrane by a sodium cotransport process [6].

With respect to the increased uptake of uric acid and PAH, we have less information. There is currently no evidence for the existence of a primary active transport system for PAH or urate in the basal-lateral or brush border membrane. Furthermore, there is no evidence for sodium dependent cotransport of these compounds at either membrane [12, 13]. Recently, Blomstedt and Aronson [14] showed that uptake of PAH and urate across dog renal brush border membranes could be driven by a hydrogen ion gradient across the membranes, suggesting the existence of H^+-anion cotransport or OH^--anion exchange. Thus, the relationship of increased PAH and urate transport to increased cellular metabolism could be by way of an increased proton gradient across the cell produced by the metabolic process. Nevertheless, it is clear that a more definite conclusion must await additional studies on this problem.

Finally, it is becoming evident that stimulation of renal transport of organic solutes is a general phenomenon associated with increasing the cellular metabolic rate [5, 6, 15] and not unique to any particular metabolic fuel or transported substrate. Thus, one should expect to alter the rates of transport of a variety of solutes across the renal plasma membranes by any compound causing sufficient stimulation (or inhibition) of proximal tubular oxidative metabolism.

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Preferential incorporation of sangivamycin into ribonucleic acid in Sarcoma 180 cells *in vitro**

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Sangivamycin is a pyrrolopyrimidine antibiotic with antitumor activity against a variety of experimental tumors†. As an adenosine analog, it undergoes phosphorylation [1] and is incorporated into RNA and DNA of normal tissues [2]. Sangivamycin has a variety of effects on nucleic acid synthesis such as inhibition of nuclear RNA synthesis [3], *de novo* purine synthesis [4], tRNA acylation *in vitro* [5], and *Escherichia coli* RNA polymerase activity *in vitro* [6]. However, none of these studies attempted to correlate the cytotoxicity produced by sangivamycin with a specific biochemical lesion. In a previous study, we reported that sangivamycin produced a pronounced effect on the viability of Sarcoma 180 cells in culture which was highly dependent on the duration of drug exposure [7]. Incorporation of [^{3}H]thymidine or [^{3}H]uridine into nucleic acids was proportionally reduced, although DNA synthesis was inhibited more rapidly than RNA synthesis [7]. Flow cytometry indicated a time-dependent accumulation of cells in the late S and G_2M region of the DNA histogram [7]. In this communication, we report that [^{3}H]sangivamycin is preferentially incorporated into cellular RNA in log and plateau phase Sarcoma 180 cells. Moreover, the incorporation of this drug into total poly(A)RNA shows the same time-dependency as its effects on cell viability and inhibition of nucleic acid synthesis.

Sangivamycin was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD. [^{3}H]Sangivamycin was prepared from [^{3}H]toyocamycin by hydrolysis in 2 N HCl at 100° for 4 hr [8]. Following lyophilization, [^{3}H]sangivamycin was purified to a specific activity of 485 mCi/mole by high performance liquid chromatography using a Partisil SCX column and isocratic elution with 25 mM KH_2PO_4 (pH 3.65)–5% acetonitrile at a flow rate of 1 ml/min. By this procedure, toyocamycin eluted in 5 min and sangivamycin eluted in 9 min.

Sarcoma 180 cells were grown in Earle's medium 199 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin [7]. Replicate flasks of 2 day log phase cells and 4 day early plateau phase cells were incubated with 1×10^{-6} M [^{3}H]sangivamycin (485 mCi/mmole) for 1, 3 and 6 hr. At the end of this period, 2–4 × 10^7 cells from pooled flasks were rinsed three times with Hanks' balanced salt solution, harvested by trypsinization, washed once in phosphate-buffered saline, and frozen immediately in dry ice. Total nucleic acids were extracted with 3 ml of 1% sodium dodecylsulfate–0.1 M Tris–HCl (pH 8.0)–0.01 M EDTA and mixed with 1.5 ml of phenol mixture (phenol–cresol–water), 7:2:2, by vol containing 0.1% 8-hydroxyquinoline) and 1.5 ml of chloroform. The emulsion was separated by centrifugation at 10,000 *g*, and the aqueous phase containing DNA and RNA was precipitated with 3 vol, of 2% potassium acetate in 95% ethanol at −20° overnight. Samples were centrifuged at 10,000 *g* for 20 min, and the precipitate was washed once with 2% potassium acetate in 95% ethanol. The pellet was dissolved in 0.2 ml water and divided into two equal samples. To obtain DNA, a 0.1-ml sample was adjusted to 0.01 M Tris–HCl (pH 7.4)–0.2 M NaCl–0.01 M EDTA and incubated with 20 μg RNase A and 20 units of RNase T_1 for 2 hr at 37°. To obtain RNA, 0.1-ml sample was adjusted to 0.01 M Tris–HCl (pH 7.2)–0.5 M NaCl–0.01 M $MgCl_2$ and incubated with 10 μg DNase for 2 hr at 37°. Each sample was then precipitated with 3 vol. of 2% potassium acetate in 95% ethanol at −20° overnight. To obtain non-poly(A)- and poly(A)RNA, total RNA was fractionated by poly(U)Sepharose chromatography as previously described [9]. All experiments were repeated three times, and the reported results are the means ± S.E. of replicate studies at each time point.

The time-dependent incorporation of 1×10^{-6} M [^{3}H]sangivamycin into total cellular DNA and RNA of log phase and plateau phase Sarcoma 180 cells is shown in Fig. 1. The rate of incorporation of [^{3}H]sangivamycin into RNA and DNA was three to four times greater in log phase cells than in plateau phase cells. Under both conditions of growth, approximately ten times more drug was incorporated into RNA than DNA.

To determine if sangivamycin was preferentially incorporated into a particular class of RNA, total cellular RNA was fractionated into non-poly(A)RNA (rRNA and tRNA) and poly(A)RNA (mRNA) by poly(U)Sepharose chromatography (Fig. 2). Incorporation of drug into non-poly(A)RNA was about six times greater in log phase cells than in plateau phase cells during the initial 3 hr of drug

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