Effects of Inhibitors and Substitutes for Chloride in Lumen on p-Aminohippurate Transport by Isolated Perfused Rabbit Renal Proximal Tubules

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ABSTRACT

The transport step for p-aminohippurate (PAH) from cell to lumen across the luminal membrane of rabbit proximal tubules has not been adequately defined. To examine this process more closely, we determined the effects of possible transport inhibitors and substitutes for chloride on PAH secretion in isolated perfused S2 segments of rabbit proximal tubules. The addition of 4-acetamido-4-‘-isothiocyano-2,2’ disulfonic stilbene (10^-4 M) to the perfusate reversibly inhibited PAH secretion, whereas the addition of probenecid (10^-4 M) to the perfusate reversibly inhibited PAH secretion. PAH secretion was unaffected by thiocyanate replacement of chloride in the luminal perfusate, reversibly inhibited by 15 to 20% by methyl sulfate replacement, and irreversibly inhibited by isethionate replacement. Because the luminal membrane is at least as permeable to thiocyanate as to chloride, less permeable to methyl sulfate, and much less permeable to isethionate, these data suggest that the PAH transport step from cells to lumen does not require chloride in the lumen but does require a highly permeant anion. During inhibition of PAH transport from cells to lumen, PAH uptake across the basolateral membrane was also reduced, suggesting some type of feedback inhibition. The data are compatible with PAH transport across the luminal membrane by an anion exchanger, a potential-driven uniporter, both carriers, or a carrier that can function in both modes.

Transepithelial secretion of p-aminohippurate (PAH) (and other organic anions that share the same system) by renal proximal tubules involves transport into the cells against an electrochemical gradient via a tertiary active transport step at the basolateral membrane followed by movement from the cells into the lumen down an electrochemical gradient via some form of mediated transport process (Dantzler and Wright, 1997). Considerable information is available about the tertiary active transport mechanism at the basolateral membrane, the final step of which involves the transport of PAH (or other organic anion) into the cells against an electrochemical gradient in exchange for a dicarboxylate [physiologically, α-ketoglutarate (αKG)] moving down its electrochemical gradient (Pritchard and Miller, 1993; Dantzler and Wright, 1997). Much less information is available about transport at the luminal membrane (Pritchard and Miller, 1993; Dantzler and Wright, 1997). In fact, although the transport process at the basolateral membrane appears to be essentially the same in all vertebrate species studied, the transport step at the luminal membrane appears to vary between vertebrate classes, between species within a vertebrate class, and even within species (depending on the experimental approach used to study the process) (Dantzler and Wright, 1997).

In all species studied, movement of organic anions from cell to lumen is down an electrochemical gradient and must be mediated in some fashion to account for the relatively high apparent permeability of the luminal membrane to these hydrophilic substances (Dantzler, 1996). Except for this basic concept, nothing else about the transport process is absolutely established. Studies with renal brush-border membrane vesicles (BBMV) from rats and dogs, species whose proximal tubules reabsorb urate, have revealed an anion exchanger that accepts urate and a number of other organic anions including PAH, and inorganic anions including chloride (Aronson, 1989). However, this exchanger appears to be absent from BBMV of mammalian species whose proximal tubules secrete urate (e.g., rabbit and pig) (Aronson, 1989;
Dantzler, 1996; Dantzler and Wright, 1997). Moreover, this exchanger, when evaluated in conjunction with other exchangers in this membrane, especially the Na⁺/H⁺ exchanger, appears most likely to be poised to reabsorb urate, not to secrete PAH (Aronson, 1989). Therefore, it may only be present in species, such as rats, that reabsorb urate. The only direct study of PAH flux from cell to lumen in rat proximal tubules in vivo et situ provides no evidence for this particular anion exchanger; however, it suggests, by exclusion, that PAH enters the lumen via an electroneutral anion exchanger (Kinsella et al., 1979) and rat (Ohoka et al., 1993) renal BBMV, despite the other evidence for an anion exchanger in these species. As a further complication, preliminary studies even suggest that the luminal type I Na⁺/phosphate cotransporter (a weak phosphate cotransporter originally cloned from dog renal tubule) may be able to function as a membrane carrier or channel for organic anions in which movement could be driven by potential (Busch et al., 1995). Finally, with regard to mammalian luminal transporters, studies with bovine (Schmitt and Burckhardt, 1993) and human (Roch-Ramel et al., 1996) BBMV have provided evidence for the presence of a PAH/αKG exchanger in the luminal membrane similar to the one in the basolateral membrane, although how these two transporters might be poised to result in venteral transport of PAH from blood to lumen is not at all clear.

In addition to the controversial aspects of this transport process in mammals, studies with reptilian renal tubules (in which the luminal transport step could involve anion exchange or carrier-mediated, potential-driven diffusion or both) indicate that any inhibition of PAH transport from cells to lumen results in a reduction of PAH uptake into the cells from the basolateral side (Dantzler, 1996; Dantzler and Wright, 1997). This observation suggests that there is some type of feedback coupling between the transport processes at the luminal and basolateral membranes.

Because no studies of the luminal transport process had ever been made in intact rabbit proximal tubules, we undertook to evaluate this process by measuring PAH transport in isolated, perfused S2 segments of rabbit proximal tubules. The results indicate that transport across the luminal membrane could involve either anion exchange or carrier-mediated, potential-driven diffusion or both. They also indicate that, as in snake renal tubules, during inhibition of PAH transport from the cells to the lumen, PAH uptake into the cells at the basolateral membrane is also reduced.

**Materials and Methods**

**Preparation of Isolated Tubules.** New Zealand White rabbits were sacrificed by i.v. injection of pentobarbital sodium. The kidneys were flushed via the renal artery with a solution containing 250 mM sucrose and 10 mM HEPES at pH 7.4. They were then removed gently and placed in chilled (4°C) medium for dissection. The standard solution used for dissecting, bathing, and perfusing tubules contained 110 mM NaCl, 25 mM NaHCO₃, 5.6 mM KCl, 2.0 mM NaH₂PO₄, 1.0 mM MgSO₄, 1.8 mM CaCl₂, mM 10 sodium acetate, 8.3 mM d-glucose, 5.0 mM L-alanine, 0.9 mM glyicine, 1.5 mM lactate, 1 mM malate, and 1 mM sodium citrate. For studies performed with chloride substitutions, both NaCl and KCl were replaced with sodium or potassium salt of isethionate, methyl sulfate, or thiocyanate and CaCl₂ was replaced with CaSO₄. The solution was continuously gassed with a 95% O₂/5% CO₂ mixture and the pH was adjusted to 7.4 with 1 N H₂SO₄ or NaOH as appropriate. The medium bathing perfused tubules also contained 3 g/100 ml of neutral dextran (40,000 ± 3,000 molecular weight) to approximate the plasma protein concentration. The osmolarity of the solutions averaged 290 mOsmol/kg H₂O.

Dissection of tubules from a slice of rabbit kidney was performed, as described by others (Burg et al., 1966), without the aid of enzymatic agents. All dissections were performed at 4°C, but all experiments were performed at 37°C. We used only the S2 segment of the rabbit proximal tubule because it is the primary site of net PAH secretion.

**Perfusion of Tubules.** The in vitro perfusion technique used in the present study was the same as that first described by Burg et al. (1966) and modified for use in our laboratory (Dantzler, 1973, 1974a,b). Briefly, each isolated tubule was transferred into a special temperature-controlled Lucite bathing chamber. Both ends were held in glass micropipettes, and the tubule was perfused through a micropipette with its tip centered in the tubule lumen.

For measurements of the transepithelial secretory fluxes of PAH, [¹⁴C]PAH was added to the bath in a concentration of 25 μM, which is below that necessary to saturate the transport system (Grantham, 1982; Dantzler et al., 1995). No PAH was present in the initial perfusate. Because there is virtually no back flux of PAH from lumen to bath (Grantham, 1982), net transepithelial secretory transport of PAH (J_PAH, mol/min/mm²) was determined from the appearance of [¹⁴C]PAH in the collected tubule perfusate and expressed per unit length and time from the following relationship, as in previous studies (Chatsudthipong and Dantzler, 1992; Dantzler, 1974a,b):

\[
J_{PAH} = \frac{(V_cC_c)(X_bL)}{z_2},
\]

where \(V_c\) is the fluid collection rate (in nl/min), \(C_c\) is the concentration of [¹⁴C]PAH in the collected tubule perfusate and expressed per unit length and time from the following relationship, as in previous studies (Chatsudthipong and Dantzler, 1992; Dantzler, 1974a,b):

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\[
J_{PAH} = \frac{(V_cC_c)(X_bL)}{z_2},
\]
Chemical Co. (St. Louis, MO). All other chemicals were purchased from standard sources and were of the highest purity available.

**Statistical Analysis.** Results are summarized as mean ± S.E.. The n value is the number of experiments (one tubule from a single animal was used for each experiment). In the perfusion experiments, the mean value for the four control periods was compared with the value for each experimental period in the same tubule using analysis of variance, and the significance of the difference between these values was determined with Fisher’s protected least significant difference post hoc test. For the experiments on cell water concentration of PAH, the difference between the control and experimental means was determined with the t test for paired observations. Values were assumed to be significantly different when P < .05.

**Results**

**Effects of SITS in Lumen on \( J_{PAH} \).** It has been suggested in several studies using several approaches and different animal species that the movement of PAH across the luminal membrane could occur by anion exchange (Pritchard and Miller, 1993). However, anion exchange has not been demonstrated for PAH movement across the luminal membrane in rabbit proximal tubules (Aronson, 1989; Martinez et al., 1990; Pritchard and Miller, 1993). To examine this possibility in intact renal tubules, we initially determined the effect of the well known anion exchange inhibitor, SITS (10\(^{-4}\) M) in the lumen on \( J_{PAH} \). We chose this concentration of SITS because it had been found to be an appropriate concentration in earlier studies on PAH uptake by rabbit kidney slices (Hong et al., 1978) and in our previous studies on transepithelial PAH transport by isolated, perfused snake renal tubules (Dantzler and Bentley, 1980). In these experiments, as described in **Material and Methods**, we perfused each tubule initially with control bicarbonate-buffered medium in both bath and perfusate for 20 min to obtain control values for \( J_{PAH} \). After this period, the perfusate was changed to one containing 10\(^{-4}\) M SITS. \( J_{PAH} \) was depressed significantly by about 40% in 10 min (Fig. 1). It remained depressed for three more collection periods with SITS in the lumen. To observe the recovery of \( J_{PAH} \) after SITS treatment, we again changed the perfusate to that free of SITS. \( J_{PAH} \) remained depressed and continued to decrease even more than when SITS was present in the lumen. \( J_{PAH} \) by isolated perfused S2 segments of rabbit tubules normally does not decrease significantly during the time period of these studies, as shown previously (Chatsudthipong and Dantzler, 1992). Therefore, the inhibition of transepithelial PAH transport with 10\(^{-4}\) M SITS in the lumen appeared to be irreversible.

**Effects of Probenecid in Lumen on \( J_{PAH} \).** Our earlier studies on snake renal tubules demonstrated that probenecid, a well known inhibitor of organic anion transport, inhibited net transepithelial transport of PAH by its presence in either bath or lumen (Dantzler and Bentley, 1979). Our subsequent studies (Chatsudthipong and Dantzler, 1991, 1992) indicated that the mechanism for PAH transport into the cells across the basolateral membrane was the same for snake and rabbit renal tubules. Therefore, it seemed possible that the mechanism for PAH exit from cell to lumen in rabbit proximal tubules might be similar to that in snake proximal tubules and, therefore, might be affected by probenecid. To test this possibility, we added 10\(^{-4}\) M probenecid to the perfusate. The protocol for these experiments was the same as in the experiments with SITS in lumen. As shown in Fig. 2, when the perfusate was changed from control buffer to one containing 10\(^{-4}\) M probenecid, \( J_{PAH} \) was significantly depressed by about 60% and remained depressed during the probenecid perfusion. In contrast to the results with SITS, \( J_{PAH} \) gradually but significantly increased when probenecid was removed from the perfusate. The results of the SITS and probenecid experiments suggest that PAH moves from cells to lumen in rabbit proximal tubules via a carrier-mediated process that may involve anion exchange.

![Fig. 1. Effect of 10\(^{-4}\) M SITS in perfusate on \( J_{PAH} \) in tubule perfused and bathed with bicarbonate-buffered medium. Values for \( J_{PAH} \) are shown as a percentage of the mean control value (control being 100%) for each tubule for the first 20 min. Absolute mean rate of net PAH secretory transport for all tubules for the first 20 min is given in fmol min\(^{-1}\) mm\(^{-1}\) (± S.E.). Circles are means of values for all tubules; vertical lines are ± S.E.; n = number of tubules. Arrows indicate when SITS was added to and removed from the perfusate. *Significant difference from control.](image-url)
Effects of Substitution for Chloride in Lumen on $J_{\text{PAH}}$. Because the above data with SITS suggested the possible presence of an anion exchanger for PAH at the luminal membrane of rabbit proximal S2 segments, despite earlier data with BBMV suggesting the absence of such an exchanger (Aronson, 1989), we decided to examine the possibility that chloride in the lumen might be involved in such an exchange. For this purpose, we replaced all the chloride in the luminal perfusate with isethionate, methyl sulfate, or thiocyanate. We chose these replacements because studies with many epithelia, including the pars recta of rabbit proximal tubules and snake distal-proximal tubules, indicate that the luminal membrane should be at least as permeable to thiocyanate as to chloride, less permeable to methyl sulfate, and much less permeable to isethionate (Schafer, et al., 1974, 1975; Wright and Diamond, 1977). The protocol for these experiments was the same as for the studies with SITS and probenecid (see Materials and Methods). As shown in Fig. 3, substitution of isethionate for chloride resulted in a significant depression of $J_{\text{PAH}}$, which reached about 40% after 20 min, a depression similar to that seen with SITS in the lumen (Fig. 1). Also, as in the case with SITS, $J_{\text{PAH}}$ remained depressed when chloride was restored to the tubule lumen (Fig. 3). When chloride was replaced with methyl sulfate, $J_{\text{PAH}}$ also decreased, but only by about 15 to 20% (Fig. 4). In this case, when chloride was restored to the perfusate, $J_{\text{PAH}}$ returned to the control level (Fig. 4). Thus, replacement of luminal chloride with methyl sulfate reversibly inhibited $J_{\text{PAH}}$. In contrast to the chloride replacements with isethionate or methyl sulfate, replacement of chloride in the perfusate with thiocyanate had no significant effect on $J_{\text{PAH}}$ (Fig. 5). Therefore, the movement of PAH from the cells to the lumen was not dependent on the presence of chloride in the lumen but did appear to depend on the presence of a replacement for chloride to which the luminal membrane was highly permeable.

Effects of SITS or Probenecid in Lumen on Concentration of PAH in Cell Water. If the movement of PAH from cells to lumen was inhibited, it might be expected that the concentration in cell water would increase above the one observed during control transport as transport continued at the basolateral membrane. Therefore, we decided to determine the concentration of PAH in the cell water ($[\text{PAH}]_{\text{Cell}}$) under control circumstances and during maximum inhibition of $J_{\text{PAH}}$ with SITS or probenecid in the lumen. Since $[\text{PAH}]_{\text{Cell}}$ cannot be determined under both control and experimental conditions in the same tubule, we determined $[\text{PAH}]_{\text{Cell}}$ in control and experimental tubules from the same kidney studied in parallel. We measured $J_{\text{PAH}}$ for 20 min in both control and experimental tubules to be certain of the experimental effect before we determined $[\text{PAH}]_{\text{Cell}}$. In contrast to expectations, when $J_{\text{PAH}}$ was significantly depressed by SITS in the lumen, $[\text{PAH}]_{\text{Cell}}$ was also significantly depressed (Fig. 6). When $J_{\text{PAH}}$ was significantly depressed by probenecid in the lumen, $[\text{PAH}]_{\text{Cell}}$ was slightly but not significantly less than the control (Fig. 7).

Effect of Methyl Sulfate Substitution for Chloride in Perfusate on Concentration of PAH in Cell Water. Because substitutions for chloride in the lumen would not be expected to have any direct effects on basolateral PAH transport, we also wished to determine the effect of such substitutions on $[\text{PAH}]_{\text{Cell}}$. We chose to study only the effect of methyl sulfate substitution, which reversibly depressed $J_{\text{PAH}}$, because isethionate substitution irreversibly depressed $J_{\text{PAH}}$ and thiocyanate substitution had no effect on $J_{\text{PAH}}$. As shown in Fig. 8, when $J_{\text{PAH}}$ was significantly depressed by methyl sulfate substitution for chloride in the perfusate, $[\text{PAH}]_{\text{Cell}}$ was also significantly depressed.
Discussion

The results of the present study with intact perfused rabbit tubules support the results of all previous studies in indicating that the transport of PAH from the tubule cells to the lumen during net secretion occurs by some form of mediated process. Certainly the inhibitory effects of SITS and probenecid indicate this. The effects of these agents are similar to those observed with intact perfused snake renal tubules (Dantzler and Bentley, 1979, 1980), and the results of these earlier studies indicate that it is very unlikely that these agents could have produced their effects by a direct action at the basolateral membrane. Indeed, given the much higher affinity of the basolateral organic anion transporter for probenecid than for PAH (Dantzler et al., 1995), a direct effect of probenecid somehow reaching the basolateral membrane...
should have been much more profound than that observed. Moreover, studies with rat renal tubules in vivo et situ (Ulrich and Rumrich, 1997) and with OK cells in culture (Takano et al., 1994) also indicate that probenecid can inhibit PAH movement across the luminal membrane.

The present data do not permit a clear distinction between an anion exchanger and a potential-driven uniporter for PAH transport at the luminal membrane in rabbit proximal renal tubules. The inhibitory effect of SITS is certainly compatible with the transport of PAH via an anion exchanger, despite the lack of evidence for an anion exchanger in studies with rabbit BBMV (Aronson, 1989). Although SITS may inhibit

**Fig. 5.** Effect of thiocyanate substitution for chloride on $J_{PAH}$ in tubules perfused and bathed with bicarbonate-buffered medium. See legend of Fig. 1 for complete description of symbols.

**Fig. 6.** Comparison of $J_{PAH}$ values (A) and cell concentrations of PAH, $[PAH]_{cell}$ (B) between control tubules and tubules perfused with solution containing $10^{-3}$ M SITS from the same kidney studied in parallel. Results are means ± S.E. ($n = 5$). *Group means that were significantly different from control values.
some other types of coupled anion transporters (e.g., the basolateral $\text{Na}^{+}$-$\text{HCO}_{3}^{-}$-$\text{CO}_{3}^{2-}$ cotransporter) (Geibel et al., 1989), there is no evidence for this type of coupled PAH cotransporter at the luminal membrane. Moreover, the absence of evidence for an anion exchanger for PAH in rabbit BBMV (Aronson, 1989) is not evidence of the absence of such an exchanger in the intact tubule. An exchanger could have been lost in the preparation of the BBMV. The irreversible inhibition of PAH transport produced in the present study could reflect covalent binding of SITS to an anion exchanger,
lead to an increase in intracellular pH. This in turn could of these anion transporters, depending on the degree, might ing intracellular pH (Aronson, 1989). Inhibition of some or all exist in the luminal membrane. Finally, with regard to the and a potential-driven uniporter, both of which accept PAH, uniporter. It is also possible that a separate anion exchanger tion as either an anion exchanger or as a potential-driven factors for chloride (Aronson, 1989). Moreover, substitution of gluconate for chloride does not affect PAH movement across the luminal membrane of rat proximal tubules in vivo (Ull- rich and Rumrich, 1997). However, the observation that PAH transport continued at the control rate only when chloride was replaced by a substitute to which the luminal membrane was highly permeable also suggests that the potential across the membrane might be important. The less permeable the luminal membrane to the anion replacement, the more neg- ative the tubule lumen will become (Dantzler and Bentley, 1981) and the smaller will be the electrical gradient favoring the movement of PAH into the lumen via a potential-driven carrier. Thus, the present data on the effects of chloride substitutions are also compatible with carrier-mediated, po- tential-driven PAH transport across the luminal membrane in intact rabbit proximal renal tubules, as suggested by stud- ies with rabbit BBMV (Martinez et al., 1990). It is possible that, in the intact tubule, the luminal transporter can func- tion as either an anion exchanger or as a potential-driven uniporter. It is also possible that a separate anion exchanger and a potential-driven uniporter, both of which accept PAH, exist in the luminal membrane. Finally, with regard to the substitutions for chloride, the failure of \( J_{\text{PAH}} \) to recover after isethionate substitution and the slow recovery after methyl sulfate substitution may reflect some other effect of these substitutions on the luminal transporter.

In the present study, as in our previous ones on snake renal tubules (Dantzler and Bentley, 1979, 1980, 1981), when PAH transport into the lumen was inhibited, PAH uptake into the cells across the basolateral membrane was also re- duced. Although this observation suggests that some form of feedback exists between transport of PAH into the lumen and basolateral transport of PAH into the cells, the mechanism involved in such a possible feedback is not clear. It is possible that the inhibition of PAH uptake at the basolateral mem- brane resulted from an effect of the inhibitors that was quite separate from their effect on PAH transport itself. Both SITS and probenecid can interfere with a number of other anion exchangers in the luminal membrane and substitutions of isethionate or methyl sulfate for chloride might do the same for those involving chloride (Aronson, 1989). A number of these transporters, when considered in conjunction with the luminal Na\(^+\)/H\(^+\) exchanger, appear to play a role in regulat- ing intracellular pH (Aronson, 1989). Inhibition of some or all of these anion transporters, depending on the degree, might lead to an increase in intracellular pH. This in turn could lead to a decrease in the intracellular concentration of \( \alpha KG \) (Boyd and Goldstein, 1979; Lemieux et al., 1980). Since Pritch- ard (1995) has shown that the intracellular concentration of \( \alpha KG \) can control basolateral PAH uptake via the \( \alpha KG/PAH \) exchanger (high concentration stimulating uptake; low con- centration reducing it), this decrease in intracellular \( \alpha KG \) concentration could account for the observed decrease in PAH uptake at this time. Whether or not such changes in intracellular pH and intracellular \( \alpha KG \) occur to a significant extent or in an appropriate time frame to account for the current observations is not known at present.

In summary, the current study indicates that PAH transport across the luminal membrane of S2 segments of rabbit proximal tubules may involve an anion exchanger, a potential- driven uniporter, both carriers, or a carrier that can function in both modes. They also indicate that, during inhib- ition of PAH transport from the cells to the lumen, PAH uptake into the cells across the basolateral membrane is reduced.

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References


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