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Stopped-Flow Microperfusion Studies of Urate Absorption from the Rat Proximal Tubule (40913)

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Abstract. The absorptive flux of [2-^{14}C]urate in the rat proximal tubule was examined using stopped-flow and continuous-flow microperfusion techniques. In the stopped-flow studies, the luminal perfusion was a steady-state equilibrium solution and the luminal contact time was 60 sec. The ratio of disintegrations per minute (dpm) of urate in the reaspirated sample to the initial solution (C/I) was 0.42 ± 0.02 in control experiments. The addition of either phloridzin or *p*-chloromercuribenzoate to the perfusion solution resulted in significantly higher C/I ratios of 0.52 ± 0.03 ($P < 0.05$) and 0.61 ± 0.06 ($P < 0.01$), respectively. Substitution of choline for sodium in the perfusion solution or the addition of probenecid did not significantly change the C/I ratio. In continuous-flow microperfusion studies, the fractional absorption of urate from an isotonic saline solution averaged 10.2 ± 0.8%/mm tubule and was significantly inhibited by the addition of probenecid to the perfusion solution. Urate absorption from a steady-state perfusion solution averaged 4.7 ± 1.0%/mm tubule ($P < 0.01$ compared to the saline solution) and was not affected by the addition of probenecid. These studies indicate that the presence of either phloridzin or *p*-chloromercuribenzoate in the solution bathing the luminal side of the rat proximal tubule cells inhibits urate absorption. Neither the substitution of choline for sodium in the perfusion solution nor the addition of probenecid to the perfusion solution had any significant effect. The failure to demonstrate an effect of probenecid in the stopped-flow studies may have been due to the requirement to use a non-water absorbing solution. When water absorption is permitted to occur, the addition of probenecid to the perfusion solution inhibited urate absorption.

Previous microperfusion studies from this and other laboratories have presented evidence that urate absorption from the rat proximal tubule occurs, at least in part, by a carrier-mediated mechanism (1–4). The characteristics of this carrier, however, have not been clearly defined. Recently, Boumendil-Podevin *et al.* have reported results of studies examining urate uptake by the isolated brush border of rabbit tubules (5). In these studies, urate uptake was linearly related to the urate concentration of the media with no evidence for saturation of the uptake reaction, even at supraphysiologic concentrations of urate in the media. Inferential evidence that urate uptake from these vesicles was not totally passive derived from experiments in which the temperature or pH of the media was altered or when *p*-chloromercuribenzoate, a sulfhydryl bond inhibitor, was added. Finally, in these rabbit vesicle studies, the presence or absence of a sodium gradient across the vesicle membrane did not affect the rate of urate uptake. These rabbit vesicle studies represent the first published data which examine in detail the role of the luminal membrane in urate absorption using these techniques. As such, they are of interest. Since net urate transport differs significantly between the rabbit and the rat, it is not certain that these results are applicable to the latter species. The present stopped-flow microperfusion studies were performed, therefore, to determine if some of the conclusions derived from the *in vitro* rabbit vesicle studies are also true in the proximal tubule of the rat.

Methods. Male Sprague–Dawley rats were anesthetized with nembutal (50 mg/kg body wt), injected intraperitoneally, and prepared for micropuncture as previously described from this laboratory (2, 3). During the experiments, the animals received an intravenous solution of isotonic saline at a rate of 1.2 ml/hr. Stopped-flow mi-

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croperfusion was performed in the proximal convoluted tubule by filling the lumen with a column of mineral oil after which the oil column was split by a droplet of the test solution. The average volume of test solution injected was 2 nl. After a variable period of contact, the droplet was reaspirated. Continuous-flow microperfusion was performed using the Hampel microperfusion apparatus as previously described (2).

The perfusion solution for the stopped-flow studies and for some of the continuous flow studies was an equilibrium solution containing sodium chloride (110 meq/liter) and mannitol, adjusted to a final osmolality of 280 mOsm/kg water and a pH of 7.4. Where examined, choline chloride was substituted for sodium chloride. Radioactive urate (60 mCi/mmole) (Amersham Searle, Arlington Heights, Ill.) was added to a final concentration of 1.9 mg%. In addition, [*methoxy*-^{3}H]inulin (New England Nuclear, Boston, Mass.) was added to provide a marker for contamination and to permit correction for any small water fluxes due to osmolality differences. A stopped-flow sample was considered technically satisfactory if both proximal and distal oil blocks remained stationary during the perfusion and no visible leaks were observed. Samples were included for analysis if the ratio of disintegrations per minute of inulin in the initial to collected perfusate ranged between 0.90 and 1.10. In some of the continuous-flow microperfusion studies, the perfusion solution was isotonic sodium chloride containing radioactive urate and inulin. Where examined, phloridzin, probenecid, or *p*-chloromercuribenzoate in concentrations of 10^{-4} *M* were added to the perfusion solution.

Radioactivity of samples was determined in Biofluor (New England Nuclear, Boston, Mass.) using a Tri-Carb liquid scintillation counter (Packard Instruments, Downers Grove, Ill.). Samples were counted for 100 min each. Counts per minute were converted to disintegrations per min after correction for crossover counts, quenching, and efficiency of counting each isotope. The standard deviation of counting was ±2%. Although the total number of counts was low in the stopped-flow studies, they were at least 3 SD's above background counts. Thus, the isotope counts in the samples were statistically significantly above background counts in all samples ($P < 0.015$). Statistical significance was determined using the *t* test for unpaired data.

Results. The ratio of initial to collected disintegrations per minute of inulin averaged 0.98 ± 0.02 for all the tubules examined, with no significant differences observed in any of the subgroups. For each sample, the urate ratios were corrected for the water flux due to the small asymmetries in the osmolalities between the perfusion solution and the individual animal. The relationship between the ratio of disintegrations per minute of urate in the collected to initial perfusion solution for an equilibrium solution containing 1.9 mg% of urate and the contact time is shown in Fig. 1. As noted, there is a time-dependent decrease in the ratio of isotope counts. All the remaining stopped-flow studies were performed with contact times of 60 sec.

The ratio of disintegrations per minute of urate in collected/initial perfusion solution from the control solution was 0.42 ± 0.02 (Table I). The addition of probenecid in a concentration of 10^{-4} *M* did not significantly affect the ratio of counts. Since we previously had reported that the addition of probenecid to a microperfusion solution that permits water absorption inhibited the fractional absorption of urate, additional studies were performed (1, 2). Continuous-flow microperfusion studies were

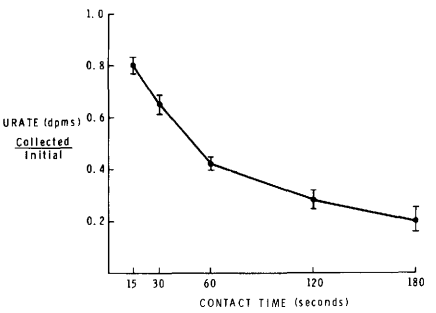

FIG. 1. The relationship between the ratio of disintegrations per minute of [2-^{14}C]urate in the collected to initial perfusion solutions and contact time. The initial urate concentration was 1.9 mg%. Each point is the mean ± SEM of 4 to 10 determinations.

TABLE I. RATIO OF DISINTEGRATIONS PER MINUTE OF URATE IN COLLECTED VERSUS INITIAL PERFUSION SOLUTION[a]

Solution	*n*	Urate (dpm) Collected/Initial	*P*
Control	10	0.42 ± 0.02	—
Probenecid (10^{-4} *M*)	11	0.43 ± 0.07	NS
Phloridzin (10^{-4} *M*)	5	0.52 ± 0.03	<0.05
Choline chloride	11	0.45 ± 0.04	NS
p-Chloromercuribenzoate (10^{-4} *M*)	10	0.61 ± 0.06	<0.01

[a] Values represent mean ± SEM. Urate concentration in the initial perfusion solution was 1.9 mg%. Intraluminal contact time was 60 sec. *n* = number of observations. *P* values are compared to controls. NS = not significant. All values were corrected for water flux as determined by the collected to initial dpm of inulin.

performed in 10 animals. The perfusion solution was isotonic saline (with or without probenecid, 10^{-4} *M*), or the equilibrium solution (with or without probenecid, 10^{-4} *M*). The urate concentration was 1.9 mg% and the perfusion rate was 20 nl/min. Two to three microperfusions were performed with one solution not containing probenecid and an additional two to three microperfusions performed in previously unpunctured tubules with the same solution containing the drug. The order was reversed in the other animals. For each animal the results of perfusions with each of the solutions were averaged. The results are expressed as the mean of means ± SEM and statistical comparison was determined by the *t* test for paired data (Table II). Using the equilibrium solution, fractional urate absorption averaged 4.7 ± 1.0%/mm tubule when the drug was not present, and 4.8 ± 0.9%/mm tubule when probenecid was included (P = NS). With the absorbing saline solution, without probenecid, urate absorption averaged 10.2 ± 0.8%/mm tubule ($P < 0.01$ compared to the results obtained with the equilibrium solution not containing probenecid) and decreased significantly to 7.4 ± 1.1 ($P < 0.01$) when probenecid was added. Water absorption averaged 2.41 ± 0.35 nl/min/mm and 2.53 ± 0.47 nl/min/mm tubule with the solutions containing or not containing probenecid, respectively (P = NS).

Table I summarizes results of studies using an equilibrium solution containing 1.9 mg% of urate with a contact time of 60 sec. As noted above, probenecid did not significantly alter the ratio of isotope counts. Substitution of choline chloride for sodium chloride also did not alter the ratio. Phloridzin and *p*-chloromercuribenzoate (10^{-4} *M*), however, significantly increased the ratio of counts, indicating inhibition of urate absorption.

Discussion. Studies in the rat reported from this and other laboratories have attempted to examine the characteristics of urate absorption from the proximal tubule (1–4). It has not been possible to demonstrate saturation of the absorptive process in the rat, even when the concentrations of urate in the tubular lumen were increased to supraphysiologic concentrations (3, 6). The

TABLE II. EFFECTS OF PROBENECID ON FRACTIONAL URATE ABSORPTION (%/mm TUBULE)[a]

	Equilibrium solution	Isotonic saline solution	*P*
	(n = 5)	(*n* = 5)	
Without probenecid	4.7 ± 1.0	10.2 ± 0.8	<0.01
With probenecid	4.8 ± 0.9	7.4 ± 1.1	NS
P	NS	<0.01	

[a] Values represent mean of means ± SEM. NS = not significant. *n* = number of animals studied.

evidence that urate uptake is carrier-mediated derives from studies in this species which have indicated that probenecid, pyrazinamide, and some diuretics inhibit urate absorption (7, 8, 14). The studies in the isolated vesicles prepared from the luminal membranes in the rabbit, as summarized in the introduction, provide a more direct assessment of the role of luminal membrane in urate absorption (5). As in the rat, in the vesicles it has not been possible to demonstrate saturation of the urate uptake reaction even at very high media concentrations (1, 3, 4). The present study attempts to determine if some of the other conclusions derived from the rabbit studies are also valid in the rat.

The stopped-flow technique, using a nonreabsorbing steady-state perfusion solution, was used to obviate the possible influence of variations in flow rate and water absorption on urate transport (1). The loss of isotope from the lumen was taken to reflect urate absorption from the lumen, as discussed in previous communications from this laboratory (2, 3). Preliminary evidence from this laboratory, using the continuous microperfusion technique at low rates of flow, suggests that the specific activity of urate does not change along the perfused nephron segment (10). Recent continuous-flow microperfusion studies from this laboratory had indicated that the presence of glucose or phloridzin in the tubular lumen inhibited urate absorption (2). As noted in Table I, the addition of phloridzin resulted in an increased ratio of collected to initial disintegrations per minute of urate. Probenecid, however, was without an effect. This result was surprising in view of recent evidence from this laboratory that the presence of probenecid in the tubular lumen inhibited urate absorption (1, 2). It is of interest that Lang *et al.* also were unable to demonstrate an effect of probenecid when added to a steady-state equilibrium solution perfusing the proximal tubule of the rat (11). In the rabbit vesicles, probenecid was also without a discernible effect (5).

To clarify these observations and resolve the discrepancy between the results of the present studies and those of our prior studies, an additional series of experiments were performed. Using an equilibrium solution which did not permit net water absorption, probenecid had no effect on the fractional absorption of urate. When isotonic saline was used as the perfusion solution, fractional urate absorption was significantly higher than that observed with the equilibrium solution. These findings may be due to the higher mean intraluminal urate concentration in the studies using the water-absorbing solution as compared to the steady-state perfusion solution. A higher intraluminal urate concentration, in turn, would favor urate absorption by either a facilitated mechanism or by creation of a more favorable concentration gradient for urate movement through a passive permeation pathway. When probenecid was added to the isotonic saline solution, urate absorption was significantly decreased in each animal. The average decrease for all animals was 27%, comparable to the effect previously reported (1, 2). Further studies will be required to fully elucidate the mechanism of these findings.

The substitution of choline for sodium in the perfusion solution did not affect urate absorption. In view of the lack of effect of a sodium gradient on urate uptake in isolated vesicles, the results in the rat support the conclusion that urate absorption is not intimately linked to that of sodium (5, 6). Also in confirmation of the results in rabbit vesicles, the addition of *p*-chloromercuribenzoate inhibited urate absorption, suggesting that the carrier is sensitive to sulfhydryl bond inhibitors.

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1. Senekjian, H. O., Knight, T. F., and Weinman, E. J., Kidney Int. 17, 155 (1980).
2. Knight, T. F., Senekjian, H. O., Sansom, S. C., and Weinman, E. J., Amer. J. Physiol. **236**, F526 (1979) or Amer. J. Physiol. Renal Fluid Electrolyte Physiol. 5, F526 (1979).
3. Weinman, E. J., Steplock, D., Suki, W. N., and Eknoyan, G., Amer. J. Physiol. **231**, 509 (1976).
4. Kramp, R. A., Lassiter, W. E., and Gottschalk, C. W., J. Clin. Invest. **50**, 35 (1971).

5. Boumendil-Podevin, E. F., Podevin, R. A., and Prior, C., Amer. J. Physiol. **236**, F519 (1979) or Amer. J. Physiol. Renal Fluid Electrolyte Physiol. 5, F519 (1979).
6. Roch-Ramel, F., Diezi-Chomèty, F., deRougemont, D., Tellier, M., Widmer, J., and Peters, G., Amer. J. Physiol. **230**, 768 (1976).
7. Frankfurt, S. J., and Weinman, E. J., Proc. Soc. Exp. Biol. Med. **159**, 16 (1978).
8. Frankfurt, S. J., and Weinman, E. J., Proc. Soc. Exp. Biol. Med. **155**, 554 (1977).
9. Weinman, E. J., Knight, T. F., McKenzie, R., and Eknoyan, G., Kidney Int. **10**, 295 (1976).
10. Weinman, E. J., Sansom, S., Steplock, D., Knight, T. F., and Senekjian, H. O., Clin. Res. **27**, 767A (1979).
11. Lang, F. *in* "Amino Acid Transport and Uric Acid Transport, Symposium Innsbruck, June 1975" (S. Silbernagl, F. Lang and R. Greger, eds.), p. 217. Thieme, Stuttgart (1976).

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