PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 554-557 (1977)

The Effect of Probenecid on Urate Transport in the Rat Kidney (39849)

SEYMOUR J. FRANKFURT AND EDWARD J. WEINMAN[1]

Department of Medicine, Veterans Administration Hospital, Houston, Texas 77211, and Baylor College of Medicine, Houston, Texas 77030

p-(Dipropylsulfamyl)benzoic acid (probenecid) has been utilized in clinical practice and in experimental studies to decrease the tubular reabsorption of uric acid (1). Probenecid is also known to inhibit the secretion of organic acids in the kidney (2). Despite the extensive use of this agent, its separate effects on the urate reabsorptive and secretory processes have not been examined by direct techniques. The current investigations, therefore, were designed to examine the effects of probenecid on net urate transport, urate reabsorption, and urate secretion using clearance, microinjection, and precession techniques in the rat.

Methods. Male Sprague-Dawley rats anesthetized with Inactin (Promonta, Hamburg, Germany), 100 mg/kg body wt intraperitoneally, were used for all studies. Probenecid was dissolved in a solution of dilute NaOH to a final concentration of 10-20 mg/ml and was administered in a dose of 100 mg/kg body wt/hr intravenously. In control animals and in control periods of the clearance experiments, diluent alone was infused.

Clearance studies. After a tracheotomy, cannulae were placed in two jugular veins, in a femoral artery, and in the urinary bladder. Body temperature was maintained at 37°. Five percent mannitol in isotonic saline was infused at a rate of 12 ml/hr to match the protocol of the microinjection studies. Through the other venous line, normal saline containing [*methoxy*-^{3}H]inulin (25 μCi/ml) was infused at a rate of 1.2 ml/hr. After 60-90 min of equilibration, two control urine samples, 20 min each, were collected. One milliliter of blood was obtained from the femoral artery at the midpoint of each period and was replaced with the same volume of blood from a donor rat. Following collection of control samples, probenecid (100 mg/kg body wt/hr) was infused. After an additional 60 min, repeat blood and urine samples were obtained.

Microinjection studies. Animals were prepared for study as above, except that inulin was not infused. Animals were prepared for micropuncture as previously described (3-5). The ureter of the left kidney was cannulated with PE-50 tubing to permit separate urine collections from each kidney. The urine flow rate of the micropunctured kidney was at least 85% of that from the contralateral kidney. Intratubular microinjections were performed with a solution containing [2-^{14}C]urate (50 μCi/ml) and [*methoxy*-^{3}H]inulin (100 μCi/ml), adjusted to a pH of 7.4 with a solution of $NaHCO_3$ (30 mequiv/liter). Three samples of equal volume (12-20 nl) were prepared, one of which was used for the microinjection, while the other two were counted directly for total radioactivity. Microinjections were performed into early or late proximal tubule sites over a 60- to 90-sec interval, and total urine collections were obtained sequentially in 1-min intervals from both right and left kidneys over a 10-min period. The procedures for microinjection, the localization of injection sites, and the calculations of the recovery rates were identical to those of Kramp *et al.* (6) and have been previously described in detail from this laboratory (3-5).

Secretory studies. Animals were prepared for study as in the microinjection experiments. One hundred nanoliters of the [2-^{14}C]urate and [*methoxy*-^{3}H]inulin solutions were placed upon the surface of the decapsulated left kidney as a droplet. Urine was collected in 15-sec samples from the left kidney only. No attempt was made to quantitate total recoveries. Urate secretion was considered to be present when the ratio of [2-^{14}C]urate counts to [*methoxy*-^{3}H]inulin

[1] To whom all correspondence should be addressed at Veterans Administration Hospital, Houston, Texas 77211.

ISSN 0037-9727

counts in the first urine sample to contain inulin, divided by the ratio of counts in the droplet solution, was greater than 1.

Radioactivity of blood, urine, microinjection, and droplet samples was determined in Biofluor (New England Nuclear, Boston, Mass.) in a liquid scintillation counter (Tri-Carb, Packard Instruments, Downers Grove, Ill.) with appropriate corrections for crossover of ^{14}C counts appearing in the ^{3}H channel and for quench. Clearances and fractional excretion rates were calculated from standard formulas. Sodium concentrations were determined by flame photometry. Uric acid in blood and urine was measured by a uricase method in a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.) (3). All data are expressed as mean ± SEM. Statistical significance was determined by either Student's *t* test or the *t* test for paired data where appropriate.

Results. Clearance studies. In order to control for the time course of these studies, five animals were studied under the same experimental conditions, except that probenecid was not given in the experimental periods. There were no significant changes in the GFR, clearance of urate, or plasma concentration of urate. As seen in Table I, the administration of probenecid in a dose of 100 mg/kg body wt/hr resulted in no significant change in the GFR. There was, however, a significant increase in the clearance of urate from 238 ± 32 in controls to 567 ± 57 μl/min/g kidney wt ($P < 0.02$) and in the fractional excretion of urate from 23.7 ± 1.9 to 55.2 ± 5.0% ($P < 0.02$). There was a statistically significant increase in the plasma urate concentration.

Microinjection studies. Only samples in which the inulin recoveries were 95% or greater were included for analysis. No ^{14}C counts were recovered from the right kidney. Delayed excretion ranged from 0 to 6% in control and experimental animals with no significant differences between control and experimental animals. The results, expressed as total recoveries, are summarized in Table II. In control animals, urate recoveries averaged 73 ± 2% following microinjections into early proximal tubule sites and 83 ± 1% following microinjections into late portions of the proximal convoluted tubule. Probenecid administration increased the urinary recovery of microinjected urate from the early proximal tubule to 89 ± 2% ($P < 0.001$ compared to controls) and to 94 ± 2% after late proximal tubule microinjections ($P < 0.001$ compared to controls).

Secretory studies. In control animals, the $^{14}C/^{3}H$ urine/droplet ratio of counts in the first urine sample to contain inulin averaged 1.70 ± 0.15, indicating urate secretion (Table III). Following administration of probenecid, the ratio of counts was significantly decreased to 0.90 ± 0.06 ($P < 0.01$).

TABLE II. MICROINJECTION STUDIES[a]

	Microinjection site	
	Early proximal tubule	Late proximal tubule
Control	73 ± 2% (12)	83 ± 1% (9)
Probenecid	89 ± 2%[b] (22)	94 ± 2%[b] (13)

[a] Values expressed as the percentages of total [2-^{14}C]urate recovered (mean ± SEM). Numbers in parentheses indicate number of intratubular microinjections.

[b] $P < 0.001$ when compared to control values.

TABLE III. SECRETORY STUDIES[a]

	$^{14}C/^{3}H$ Urine/droplet (ratio of counts)
Control	1.70 ± 0.15 (11)
Probenecid	0.90 ± 0.06 (13)

[a] $P < 0.01$. Numbers in parentheses indicate the number of droplet studies performed.

TABLE I. THE EFFECTS OF PROBENECID ON THE CLEARANCE OF URIC ACID[a]

	C	E	*P*
C_{inulin} (μl/min/g kidney wt)	1002 ± 91	1010 ± 79	NS
C_{urate} (μl/min/g kidney wt)	238 ± 32	567 ± 57	<0.02
FE_{urate} (%)	23.7 ± 1.9	55.2 ± 5.0	<0.02
Serum uric acid (mg/100 ml)	1.03 ± 0.05	1.54 ± 0.13	<0.02

[a] Values are expressed as mean ± SEM. FE_{urate}, fractional excretion of uric acid; C, control periods; E, experimental periods. Number of animals = 8.

Discussion. The ability of some drugs to inhibit the tubular transport of organic acids has been widely utilized in clinical practice. Within the class of such drugs, probenecid is most extensively employed. Initially introduced because of its ability to inhibit the tubular secretion of penicillin and thereby maintain higher blood levels of this antibiotic, it is currently used as a uricosuric agent in the treatment of some disorders of uric acid metabolism (1, 7). Despite its use in hyperuricemic disorders, its direct effects on urate transport have not been studied in detail by microanalytic techniques. In conjunction with prior studies in this laboratory on the effects of certain drugs on the urate secretory and reabsorptive mechanisms, the current study examines the effect of probenecid on these transport processes (3–5, 8, 9).

The administration of probenecid in a dose of 100 mg/kg body wt/hr to mildly diuretic rats results in an increase in the fractional excretion of urate. Of note is the rise in the serum urate concentration which, while of small magnitude, is statistically significant. Although urate loading results in a rise in urate excretion (10), in nonurate loaded animals, the rise in plasma urate concentration observed in the current studies is not of sufficient magnitude to explain the increase in urate clearance (Weinman and Frankfurt, unpublished observations). The increase in urate clearance, therefore, implies an alteration in urate reabsorption. The mechanism for the increase in plasma urate concentration is unknown and opposite to that observed in man. In contrast to the human liver, rat liver contains uricase which metabolizes urate to allantoin. It is possible that, in the rat, probenecid affects the activity of the hepatic uricase enzyme system or, more likely, inhibits the uptake of urate into hepatic cells.

The intratubular microinjection technique and a modification of the precession technique of Chinard and Enns (11) were utilized to examine the effects of probenecid on the bidirectional transport of urate. The validity of these techniques to yield qualitative estimates of unidirectional fluxes of urate has been discussed in this and other laboratories (4–6). As seen in Table II, the urinary recoveries of urate microinjected directly into early or late portions of the proximal convoluted tubule are increased in animals receiving probenecid. This finding indicates a decrease in urate reabsorption in the proximal convoluted tubule and at sites distal to the accessible portion of the proximal tubule. Probenecid also inhibits the secretion of urate from the interstitial fluid into the tubular lumen as evidenced by a decrease in the ratio of $^{14}C/^{3}H$ counts in the first urine sample to contain inulin as compared to control animals. Thus, probenecid inhibits both the tubular secretion and reabsorption of urate, the latter effect predominating. The net effect is a marked increase in the urinary excretion of urate.

Prior studies of the effect of probenecid in the rat by Boudry have indicated that probenecid results in an increased excretion of urate, but only when given at higher concentrations than in the present studies (10). The differences between these studies may be the result of differences in the strains of rats utilized or of differences in the experimental models. Kramp and co-workers (6) have previously examined the tubular absorption of urate using techniques identical to those used in the current investigations and have obtained similar results, that is, a marked inhibition of reabsorption by probenicid. In that study, however, urate secretion and net urate transport were not examined.

The pharmacology of probenecid and its effects on urate excretion in the intact animal have been extensively studied and reviewed (12). The current investigations provide direct evidence on the effect of this agent on the urate reabsorptive and secretory mechanisms. They are in accord with conclusions derived from prior clearance studies and confirm that this drug inhibits both secretion and reabsorption of uric acid.

The authors gratefully acknowledge the technical assistance of Ms. Lois Hawk, Ms. Deborah Steplock, and Steven Sansom and the secretarial assistance of Ms. Polly Dunham. Dr. Weinman is a Clinical Investigator of the Veterans Administration. Dr. Frankfurt is a Fellow in Nephrology of Baylor College of Medicine and an Associate Investigator of the Veterans Administration.

A portion of this work was presented at the national meeting of the American Federation for Clinical Research held in Washington, D. C., April 30-May 1, 1977, and appeared in abstract form in Clinical Research **25,** 41A.

1. Gutman, A. B., and Yu, T. F., Trans. Ass. Amer. Phys. **64,** 279 (1951).
2. Beyer, K. H., Russo, H. F., Tillson, E. K., Miller, A. K., Verwey, W. F., and Gass, S. R., Amer. J. Physiol. **166,** 625 (1951).
3. Weinman, E. J., Suki, W. N., and Eknoyan, G., J. Clin. Invest. **55,** 283 (1975).
4. Weinman, E. J., Steplock, D., Suki, W. N., and Eknoyan, G., Amer. J. Physiol. **231,** 509 (1976).
5. Weinman, E. J., Knight, T. F., McKenzie, R., and Eknoyan, G., Kidney Int. **10,** 295 (1976).
6. Kramp, R. A., Lassiter, W. E., and Gottschalk, C. W., J. Clin. Invest. **50,** 35 (1971).
7. Miller, C. S., U. S. Patent No. 2,608,507 (1952).
8. Frankfurt, S. J., and Weinman, E. J., Clin. Res. **25,** 41A (1977).
9. Kramp, R. A., and Lenoir, R. H., Amer. J. Physiol. **229,** 1654 (1975).
10. Boudry, J. F., Pflügers Arch. **328,** 279 (1971).
11. Chinard, F. P., and Enns, T., Amer. J. Physiol. **182,** 247 (1955).
12. Gutman, A. B., Advan. Pharmacol. **4,** 91 (1966).

Received March 14, 1977. P.S.E.B.M. 1977, Vol. 155.