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RENAL SECRETION OF PURINE NUCLEOSIDES AND THEIR ANALOGS IN MICE*

J. ARLY NELSON,† JOHN F. KUTTESCH, JR.‡ and BARBARA H. HERBERT

Pharmacology Laboratory, Department of Experimental Pediatrics, The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, Houston, TX 77030, U.S.A.

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Abstract—Previous results have indicated that 2′-deoxyadenosine (dAdo) and 2′-deoxytubercidin (dTub) are secreted by the mouse kidney. Secretion of dTub appeared to occur via the organic cation carrier [J. F. Kuttesch, Jr. *et al.*, *Biochem. Pharmac.* **31**, 3387 (1982)]. In the current study, the structural specificity of the secretory system for dTub was probed by evaluating the renal clearance of several sugar-modified dTub analogs. The following sugar-modified derivatives also underwent apparent secretion: 3′-deoxy, arabinosyl, and xylosyl. These results suggest a lack of structural specificity of the secretory system for dTub. Tubercidin was apparently reabsorbed, analogous to the observation in mice that adenosine clearance is less than that of inulin. In related experiments, a transport maximum for dAdo could not be demonstrated due to the marked pharmacologic activity of dAdo. Cimetidine was found to selectively inhibit the organic cation secretory system since it blocked the renal secretion of tetraethylammonium but not that of *p*-amminohippurate in mice. Correspondingly, cimetidine prevented the renal secretion of dTub; however, cimetidine did not inhibit the renal secretion of dAdo nor the renal reabsorption of Ado. These results suggest that renal secretion of dTub occurs via the organic cation carrier. The mechanisms for the renal secretion of dAdo and for the renal reabsorption of Ado may be unique and independent of the organic cation system.

The renal handling of purines is of potential and realized pharmacologic interest. That is, some drugs are purines themselves, and their pharmacokinetics are related frequently to mechanisms for renal elimination. Furthermore, effects of a large number of purine and non-purine drugs may be modified by alterations in endogenous purine pools which occur secondary to changes in renal function. Examples of these generalizations range from the obvious and realized (i.e. probenecid treatment of hyperuricemia) to the not-so-obvious (i.e. caffeine antagonism of adenosine receptors) [1]. Also, the renal mechanisms are of physiologic interest since normal purine homeostasis is probably maintained, at least in part, by the kidney.

Recent studies in a child lacking ADA§ and in adults treated with the potent ADA inhibitor, DCF, indicated that apparent secretion of dAdo and reabsorption of Ado occur in the human kidney [2]. The renal handling of these purine nucleosides in mice treated with DCF was qualitatively similar to the human [2]. Secretion of dAdo in mice is probably real since the excess purine nucleoside present in urine over the filtered fraction was not due to "ion-trapping" in the acid urine nor to synthesis by the kidney tissue [2]. Subsequently, we observed that dTub, a nonmetabolized analog of dAdo, was also secreted by mouse kidney [3].

This report concerns our efforts to understand further the mechanism(s) for the renal secretion of dAdo and dTub. The results indicate that the secretory process for dTub is probably identical with the organic cation secretory system known to secrete TEA. A preliminary report of this work has been presented [4].

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† Address correspondence to: J. Arly Nelson, The University of Texas, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave., Houston, TX 77030, U.S.A.

‡ Predoctoral fellow of the McLaughlin Fellowship Fund, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX.

§ Abbreviations: ADA, adenosine aminohydrolase, EC 3.5.4.4; DCF, 2′-deoxycoformycin (NSC 218321); dAdo, 2′-deoxyadenosine; Ado, adenosine; dTub, 4-amino-7-(2′-deoxy - β - D-*erythro* - pentofuranosyl)pyrrolo[2,3 - *d*]-pyrimidine; Tub, tubercidin; 3′-dTub, 4-amino-7-(3′-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*] - pyrimidine; AraTub, 4-amino-7-(β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]pyrimidine; XylTub, 4-amino-7-(β-D-xylofuranosyl)pyrrolo[2,3-*d*]pyrimidine; TEA, tetraethylammonium bromide; and HPLC, high performance liquid chromatography.

MATERIALS AND METHODS

Materials. DCF was supplied by the Drug Synthesis and Development Branch, National Cancer Institute, Bethesda, MD. Cimetidine was a gift from Smith Kline & French Laboratories, Philadelphia, PA. Tub was purchased from P-L Biochemicals, Milwaukee, WI; however, the sugar-modified Tub analogs were synthesized by Dr. Morris J. Robins as previously described [5–7]. [^{3}H]dTub was prepared by ^{3}H-exchange labeling by Moravek Biochemicals, Brea, CA. Inulin[^{14}C]carboxylic acid (10 μCi/μmole) was purchased from the Amersham Corp., Arlington Heights, IL. [1-^{14}C]TEA (4.4 μCi/μmole) was a product of the New England Nuclear Corp., Boston, MA. Other purine nucleo-

sides were obtained from P-L Biochemicals, and other chemicals were of the highest purity available from commercial sources.

Methods. Renal clearance determinations were made in male mice using the procedure described by Konikowski *et al.* [8] and as illustrated in a previous report from our laboratory [2]. In this procedure, urine is permitted to accumulate in the bladder *in situ* during a defined time interval following urethral ligation. Radioactive inulin is used to estimate glomerular filtration rate, and clearance values for the nucleosides are compared to the inulin clearance determined simultaneously in the same animal. Samples of plasma were obtained at the midpoint of the urine collection interval, or a midpoint plasma value was estimated from measurements made in samples collected at the end of the experiment and at the time of urethral ligation [2]. Plasma and urine were diluted in phosphate-buffered saline (0.01 M phosphate, pH 7.4, 0.9% NaCl). Measurements of Ado, dAdo and inosine were made using HPLC as previously described [2]. For measurements of the Tub analogs, the diluted plasma and urine samples were incubated for 1 hr with ADA (1 unit/sample) to remove endogenous Ado and dAdo. Protein was then removed by centrifugal ultrafiltration using Centriflo CF-25 membrane cones (25,000 mol. wt cutoff, Amicon Corp., Lexington, MA). Fluorescent derivatives were then prepared using chloroacetaldehyde as previously described [9]. With the exception of 3′-dTub, HPLC analysis was performed using a linear gradient from 10 mM KH_2PO_4, pH 5.5, to 40% methanol formed in 30 min. The flow rate was 2 ml/min using a μBondapak (Waters Associates, Milford, MA) reversed-phase column. For measurements of 3′-dTub, a strong cation exchange column (Partisil SCX; Whatman, Inc., Clifton, NJ) was used. After a 5-min delay, a linear gradient from 10 mM $NH_4H_2PO_4$, pH 2.6, to 500 mM $NH_4H_2PO_4$ was formed in 10 min. At a flow rate of 2 ml/min, the fluorescent derivative of 3′-dTub eluted in about 18 min. Detection of eluting material employed fluorescent detection as previously described [9]. Recovery of the Tub compounds from plasma was greater than 80% in this procedure.

RESULTS

Data in Table 1 compare the renal handling of three purine nucleosides (Ado, dAdo and inosine) in mice. Without DCF treatment, dAdo levels were below the limit of detection (<0.10 nmole/ml) in plasma under the conditions of the assay. The clearance of Ado was significantly less than that of inulin (clearance ratio = 0.41), whereas that of inosine did not differ significantly from that of inulin. After DCF administration, plasma levels of Ado and dAdo increased, whereas that of inosine declined (Table 1). The time-course for changes in the plasma levels of the adenine derivatives has been reported [2]. The inosine level fell within 10 min after DCF administration from the control value of 6.9 to a value near 2 μM. This level remained relatively constant throughout a 60-min interval following DCF administration. Erythrocyte ADA activity was inhibited by more than 95% during this interval after DCF administration, and erythrocyte levels of ATP and GTP were not changed markedly [10]. Treatment with DCF did not alter the clearance of Ado or inosine relative to that of inulin (Table 1). The renal clearance of dAdo could be measured accurately after DCF administration, and renal secretion is suggested by the significantly greater clearance value of dAdo versus inulin.

Since secretion of substances by the kidney is an active, carrier-mediated process, saturation of the carrier by the substrate is an experimental means to confirm the mechanism involved. We attempted to saturate a putative carrier for dAdo by administering the nucleoside to DCF-treated mice (Table 2). It was possible to attain plasma levels of dAdo in excess of 600 nmoles/ml without lethality to the mice in the time interval used for renal clearance measurements. In spite of this high plasma level of dAdo, however, the clearance of dAdo relative to that of inulin was not reduced significantly (Table 2). There was a progressive decrease in the renal clearance of inulin (i.e. from 0.271 to 0.002 ml/min in Table 2) as the plasma level of dAdo was increased. The apparent increase of Ado clearance observed during what amounts to renal failure may be due to severe hypoxia in the renal tissue, resulting in the breakdown of adenine nucleotides to release Ado. Ado, rather than inosine or hypoxanthine, may accumulate in the urine because the animals were pretreated with DCF to inhibit ADA.

Since we were unable to demonstrate a transport maximum for dAdo without significant pharmaco-

Table 1. Renal clearance of endogenous purine nucleosides

Treatment*	Plasma concentration (nmoles/ml) Ado	dAdo	Ino	Clearance ratio (CL_x/CL_{Inulin}) Ado	dAdo	Ino
Control	8.24 ± 2.68	<0.10	6.90 ± 1.48	0.41 ± 0.14		0.88 ± 0.16
DCF†	14.0 ± 2.6	1.11 ± 0.10	2.38 ± 0.32	0.42 ± 0.11	1.71 ± 0.16	1.16 ± 0.30

*Male AKR mice were given inulin [^{14}C]carboxylic acid (0.05 μCi/g) subcutaneously. The urethra of each animal was ligated 20 min after inulin administration. Blood was collected from the retro-orbital sinus, and the animals were killed 50 min after inulin administration. The bladder contents were washed into a vessel with 10 ml of phosphate-buffered saline. The purine nucleosides were determined in plasma and urine by HPLC as described in the text. Plasma and urine inulin concentrations were determined by liquid scintillation spectrometry. The results shown are the mean ± S.E. values for at least seven animals per group. Inulin clearance values in the two groups were 0.161 ± 0.037 and 0.193 ± 0.034 ml/min.

† Animals in this group were treated with DCF (5 mg/kg) 10 min prior to inulin administration.

Table 2. Attempt to demonstrate a transport maximum for dAdo secretion in mice

dAdo dose* (nmoles/g)	N	Plasma concentration† (nmoles/ml) Ado	dAdo	Inulin	Renal clearance (ml/min) Ado	dAdo	Inulin	Clearance ratio (Cl_x/CL_{inulin}) Ado	dAdo
100	8	11.0 ± 0.1	11.9 ± 1.2	4.00 ± 0.11	0.064 ± 0.008	0.537 ± 0.066	0.271 ± 0.038	0.28 ± 0.04	1.92 ± 0.08
500	3	10.0 ± 0.4	69.2 ± 0.3	5.45 ± 0.34	0.030 ± 0.005	0.317 ± 0.120	0.123 ± 0.017	0.61 ± 0.32	2.53 ± 0.98
1000	3	11.3 ± 0.9	248 ± 3	14.5 ± 0.2	0.009 ± 0.001‡	0.064 ± 0.025‡	0.020 ± 0.006‡	0.28 ± 0.14	3.00 ± 0.59
1500	3	7.9 ± 0.8	642 ± 62	16.1 ± 0.9	0.042 ± 0.018	0.005 ± 0.002‡	0.002 ± 0.001‡	24.3 ± 7.4‡	2.50 ± 1.04

* Renal clearances were measured in male AKR mice as described in Table 1. dAdo (100–1500 nmoles/g) was administered subcutaneously 10 min after DCF treatment. Additionally, groups of four mice were treated similarly as in the renal clearance experiments except that blood samples were collected 30 min after DCF treatment. The "midpoint" plasma concentrations of dAdo were calculated from the mean plasma value of the samples obtained at 30 and 60 min after DCF. Ado and dAdo were assayed in neutralized, acid-soluble extracts of plasma and urine using HPLC. N = the number of determinations. Control values are given in Table 1.

† Estimated "midpoint" value as described above and in the text.

‡ Significantly different from control, $P < 0.05$.

logic effect on renal function (Table 2), we attempted an alternate means to evaluate possible carrier-mediated transport of a purine deoxynucleoside analog, dTub. dTub is structurally related to dAdo, and it is secreted by the mouse kidney (Table 3; [3]). Unlike dAdo, dTub is not a substrate for ADA and it is not metabolized significantly by mouse kidney [3]. To test the structural specificity for dTub secretion in mice, a series of sugar-modified analogs of dTub were used. The structures of the analogs are given in Fig. 1. With the exception of Tub, net secretion of these compounds was observed in mice (Table 3). In the case of Tub, apparent reabsorption is indicated by its renal plasma clearance value being less than that of inulin.

Previous results using dTub suggested that dTub is a substrate for the organic cation secretory system in mouse kidney [3]. That is, dTub inhibited TEA uptake by mouse kidney slices and TEA inhibited dTub uptake. The effects of dAdo and Ado on dTub uptake were complex, and these compounds failed to inhibit TEA uptake [10]. Cimetidine has been reported to be an inhibitor of TEA secretion in rats [11]. We observed that cimetidine (300 mg/kg, i.p.) blocked TEA, but not PAH, secretion in mice (Table 4). Thus, cimetidine serves the purpose of being an *in vivo* antagonist of the secretion of organic cations.

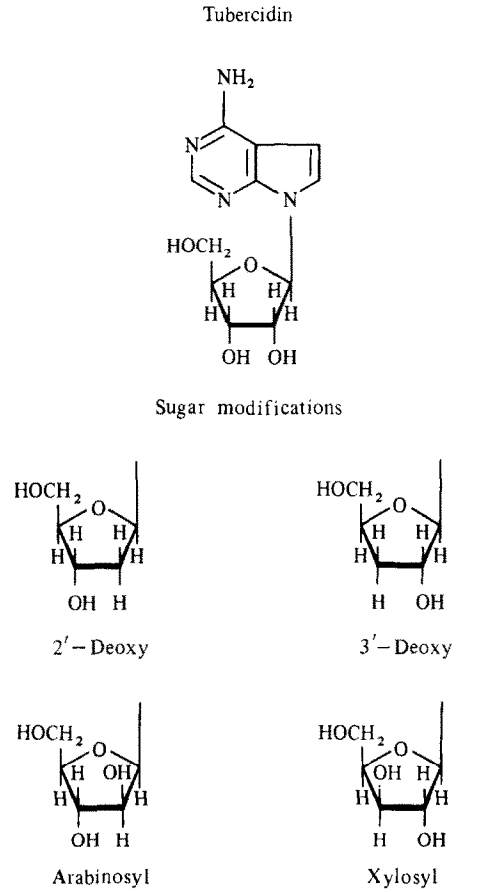

Fig. 1. Structures of tubercidin and its sugar-modified analogs.

Table 3. Renal clearance of Tub and its analogs in mice

Compound studied*	Dose (mg/kg)	N	Plasma level (nmoles/ml)	Cl_x/CL_{Inulin}†
dTub‡	1	9	1.49 ± 0.34	3.24 ± 0.39
	10	4	12.0 ± 1.2	2.71 ± 0.50
3'-dTub	1	3	0.39 ± 0.10	3.45 ± 0.34
	10	4	3.04 ± 0.53	4.97 ± 0.55
Tub	10	4	0.64 ± 0.11	0.018 ± 0.006
AraTub	10	4	5.86 ± 0.62	2.54 ± 0.06
XylTub	10	5	3.53 ± 0.49	2.02 ± 0.16

* The renal plasma clearances of Tub and its sugar-modified analogs were determined in male AKR mice as described in Materials and Methods. The clearance values are compared to the simultaneously measured clearance of [^{14}C]inulin in the same animal. Plasma and urine samples were diluted in phosphate-buffered saline, incubated with ADA, and subjected to centrifugal membrane filtration to remove proteins prior to analysis by HPLC [2, 9].

† Inulin clearance values were greater than 0.1 ml/min in all experiments.

‡ Data from Table 1 of Ref. 3.

Cimetidine prevented the renal secretion of [^{3}H]dTub, but cimetidine was without effect on the renal handling of dAdo or Ado (Table 4).

DISCUSSION

Marked differences in the renal handling of endogenous purine nucleosides are implied from measurements of the renal clearances of Ado, dAdo, and inosine in mice (Table 1). Relative to the inulin clearance, clearance values were not significantly different (inosine), were significantly greater (dAdo), and were significantly less (Ado). These results are suggestive of simple filtration (inosine), filtration and secretion (dAdo), and filtration and reabsorption (Ado). We were unable to saturate a putative carrier for dAdo in mouse kidney (Table 2). Failure to demonstrate a transport maximum (Table 2) for dAdo should not necessarily be construed to indicate that active secretion of this compound does not occur. As seen herein, systemic toxicity has often been a problem in demonstrating transport maxima and for antagonism of organic cation secretion *in vivo* [12]. The progressive decrease in inulin clearance (glomerular filtration rate) as the dose of dAdo was increased (Table 2) may be a reflection of the ability of dAdo to cause renal vasoconstriction. Unlike other vascular beds in which vasodilation is often observed, adenine derivatives have been shown to be vasoconstrictive in the kidneys of several species [13–15]. It seems plausible that some of the toxicities (including renal) observed in the clinical studies of DCF may relate to these and other actions of purine nucleosides that become elevated during ADA inhibition [16–18].

Previous observations concerning dTub secretion by mouse kidney indicated that renal secretion of this dAdo analog may occur by the organic cation system [3]. If true, this carrier would not be anticipated to demonstrate a marked degree of structural specificity since a wide range of basic, organic compounds has been shown to undergo active renal secretion, apparently via the same carrier-mediated mechanism [12]. The observation that sugar-modified Tub analogs (dTub, 3'dTub, xylTub, and AraTub; Fig. 1) are all secreted by the mouse kidney

Table 4. Effect of cimetidine on the renal clearance of purine nucleosides

Substance*	Plasma level (nmoles/ml) Control	Plasma level (nmoles/ml) Cimetidine†	Clearance ratio (CL_x/CL_{Inulin}) Control	Clearance ratio (CL_x/CL_{Inulin}) Cimetidine†
[^{3}H]dTub (10 mg/kg)	13.1 ± 0.5	27.6 ± 0.6	3.12 ± 0.25	0.82 ± 0.06
dAdo‡	0.65 ± 0.11	0.44 ± 0.13	2.74 ± 0.60	5.69 ± 1.16
Ado‡	2.46 ± 0.39	3.82 ± 0.72	0.16 ± 0.07	0.14 ± 0.03
[^{14}C]TEA	1.92 ± 0.04	5.08 ± 0.52	5.44 ± 0.37	0.89 ± 0.03
[^{14}C]PAH	0.34 ± 0.06	0.52 ± 0.08	6.27 ± 0.85	7.70 ± 1.80

* The renal clearances were determined in ICR outbred mice as described in Materials and Methods. Exogenous [^{3}H]dTub, [^{14}C]TEA and [^{14}C]PAH were given s.c. 20 min prior to urethral ligation. Either [^{3}H]inulin or [^{14}C]inulin was given simultaneously for measurement of inulin clearance. The inulin clearance exceeded 0.1 ml/min in all experiments. Mean values ± S.E. obtained from four to seven mice per group are given.

† Cimetidine (300 mg/kg, i.p.) was given 10 min prior to urethral ligation.

‡ Measurement of endogenous dAdo and Ado was by HPLC with fluorescence detection [9]. DCF (5 mg/kg) was given 45 min prior to urethral ligation in these experiments.

(Table 4) is consistent with the hypothesis that secretion occurs via a non-selective process. Of the Tub analogs used, Tub itself is the only one metabolized to a significant extent, and it is also the only member of this group to demonstrate marked cytotoxicity toward cells in tissue culture ([19] and data not shown). Thus, it seems possible or even likely, that reabsorption of Ado (Table 1) or Tub (Table 4) is only apparent due to extensive metabolism of these compounds by adenosine kinase of the renal tubular cells. These data, however, do not prove that active reabsorption of Ado and/or Tub does not occur. Although the Tub analogs tested herein may not serve as suitable models for the renal handling of dAdo and Ado, the similar qualitative observations of net secretion of the deoxyribonucleosides (dAdo and dTub) and apparent net reabsorption of the ribonucleosides (Ado and Tub) are striking.

Data regarding mechanisms for the renal handling of dTub are rather clear-cut. That is, dTub meets the requisite criteria expected of a substrate for the organic cation secretory system. The uptake of dTub by mouse kidney slices is an active, saturable process [3]; dTub inhibits TEA, but not PAH, uptake by kidney slices [3]; TEA, but not PAH, inhibits the uptake of dTub [3]; dTub is secreted by the kidney *in vivo*, apparently by a structurally nonspecific mechanism (Tables 3 and 4); and the renal secretion of dTub and TEA, but not PAH, was blocked by cimetidine (Table 4). Since dAdo fails to meet many of these criteria (i.e. cimetidine did not inhibit dAdo secretion, Table 4), we conclude that the mechanism for dAdo secretion may be different from that of dTub.

REFERENCES

1. J. W. Daly, R. F. Bruns and S. H. Snyder, *Life Sci.* **28**, 2083 (1981).
2. J. F. Kuttesch, Jr. and J. A. Nelson, *Cancer Chemother. Pharmac.* **8**, 221 (1982).
3. J. F. Kuttesch, Jr., M. J. Robins and J. A. Nelson, *Biochem. Pharmac.* **31**, 3387 (1982).
4. J. A. Nelson, J. F. Kuttesch, Jr., B. Herbert and M. J. Robins, *Fedn Proc.* **41**, 1558 (1982).
5. M. J. Robins and W. H. Muhs, *J. chem. Soc. Chem. Commun.* 269 (1976).
6. M. J. Robins, Y. Fouron and W. H. Muhs, *Can. J. Chem.* **55**, 1260 (1977).
7. M. J. Robins, J. R. McCarthy, Jr., R. A. Jones and R. Mengel, *Can. J. Chem.* **51**, 1313 (1973).
8. T. Konikowski, T. P. Haynie and L. E. Farr, *Proc. Soc. exp. Biol. Med.* **135**, 320 (1970).
9. J. F. Kuttesch, Jr., F. C. Schmalstieg and J. A. Nelson, *J. Liquid Chromat.* **1**, 97 (1978).
10. J. F. Kuttesch, Jr., *Ph.D. Dissertation.* University of Texas Medical Branch, Galveston, TX (1981).
11. I. M. Weiner and L. Roth, *J. Pharmac. exp. Ther.* **216**, 516 (1981).
12. B. R. Rennick, *Am. J. Physiol.* **240**, F83 (1981).
13. K. Thurau, *Am. J. Med.* **36**, 698 (1964).
14. B. R. Nechay, *J. Pharmac. exp. Ther.* **153**, 329 (1966).
15. T. Bhanalaph, A. Mittelman, J. L. Ambrus and G. P. Murphy, *J. Med.* **4**, 178 (1973).
16. P. P. Major, R. P. Agarwal and D. W. Kufe, *Blood* **58**, 91 (1981).
17. P. P. Major, R. P. Agarwal and D. W. Kufe, *Cancer Chemother. Pharmac.* **5**, 193 (1981).
18. R. S. Benjamin, W. Plunkett, M. L. Keating, L. G. Feun, V. Hug, J. A. Nelson, G. P. Bodey and E. J. Freireich, *Proc. Am. Soc. clin. Oncol.* **21**, 337 (1980).
19. C. E. Cass, M. Selner, T. H. Tan, W. H. Muhs and M. J. Robins, *Cancer Treat. Rep.* **66**, 317 (1982).