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RENAL TRANSPORT OF 2′-DEOXYTUBERCIDIN IN MICE*

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Abstract—Previous results [J. F. Kuttesch, Jr. and J. A. Nelson, *Cancer Chemother. Pharmac.* **8**, 221 (1982)] from this laboratory indicate that mechanisms exist for renal secretion of 2′-deoxyadenosine and possibly for reabsorption of adenosine in humans and in mice. Since significant metabolism of these purine nucleosides occurs even in the presence of adenosine deaminase inhibitors, the renal handling of a compound which is not significantly metabolized by the deaminase or by kinases was studied. Unlike 2′-deoxyadenosine itself, the 2′-deoxyadenosine analog, [4-amino-7-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-pyrrolo-(2,3-*d*)pyrimidine; 2′-deoxytubercidin], is not significantly metabolized by mammalian tissues. In mice, the renal plasma clearance of 2′-deoxytubercidin exceeded that of inulin by about 3-fold. Also, mouse kidney slices concentratively accumulated 2′-deoxytubercidin by a saturable and metabolically dependent process. The uptake by mouse kidney slices was inhibited by classical substrates for the organic cation secretory system (tetraethylammonium, choline and N^1-methylnicotinamide) but was not markedly inhibited by classical substrates for the organic anion secretory system (*p*-aminohippurate, phenol red and probenecid). Since 2′-deoxytubercidin inhibited the active, concentrative uptake of [^{14}C]tetraethylammonium but failed to inhibit the uptake of *p*-[^{14}C]aminohippurate by mouse kidney slices, it is concluded that 2′-deoxytubercidin may be secreted by the organic cation system. Additional studies are required, however, to unequivocally establish the relationships between 2′-deoxytubercidin, 2′-deoxyadenosine and tetraethylammonium renal secretory mechanisms.

Measurements of adenine compounds in a child lacking adenosine deaminase and in patients treated with deoxycoformycin, a potent inhibitor of the enzyme, suggested that the human kidney secretes 2′-deoxyadenosine and possibly reabsorbs adenosine [1]. Similar conclusions were reached when the renal handling of these purine nucleosides was studied in mice. Marked structural specificity of a putative transport system(s) for these nucleosides is implied since 2′-deoxyadenosine differs from adenosine only in the absence of the 2′-hydroxy group in the ribofuranosyl moiety (Fig. 1). This nucleoside is quite toxic in a number of cell culture systems [2] and may be the causal agent in severe combined immunodeficiency disease associated with a genetic deficiency of adenosine deaminase [3]. The role of the secretory system would perhaps be more apparent in the absence or inhibition of adenosine deaminase.

The results of preliminary experiments designed to study a transport system for 2′-deoxyadenosine using mouse kidney slices *in vitro* were equivocal due to metabolism of the compound even in the presence of high concentrations of adenosine deaminase inhibitors. Consequently, we resorted to the use of a 2′-deoxyadenosine analog, dTub¶ (Fig. 1),

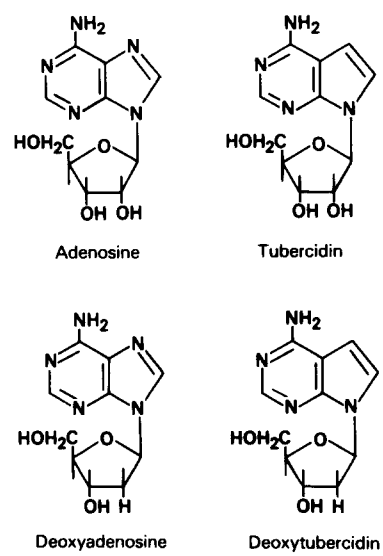

Fig. 1. Structures of adenosine, 2′-deoxyadenosine, tubercidin and 2′-deoxytubercidin.

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¶ Abbreviations: dTub (2′-deoxytubercidin), 4-amino-7-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-pyrrolo-(2,3-*d*)-pyrimidine; PAH, *p*-aminohippuric acid; TEA, tetraethylammonium bromide; HPLC, high performance liquid chromatography; PPO, 2,5-diphenyloxazole; and POPOP, 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene.

to study the renal secretory mechanism. As reported herein, dTub appears to be secreted by the mouse kidney *in vivo*; it was not extensively metabolized by mouse kidney slices *in vitro*; and it was concentratively accumulated by mouse kidney slices. The results suggest that dTub is a significantly stronger base than 2′-deoxyadenosine (pK_a 5.3 vs 3.8) and that dTub is a substrate for the mammalian renal organic cation secretory system. A preliminary report of this work has appeared [4].

METHODS

Renal plasma clearance of dTub in mice. The renal clearance of [^{3}H]dTub and of [^{14}C]inulin were measured in male AKR mice as previously illustrated and described [1]. The method involves collection of urine in the bladder *in situ* after urethral ligation and measurements of plasma levels of the compounds of interest during intervals in which the levels are relatively constant and predictable [5]. The plasma binding of dTub was determined by centrifugal ultra filtration as previously described [1].

Transport of substances by mouse kidney slices. Kidneys were removed from adult AKR mice (Jackson Laboratories, Bar Harbor, ME) and placed in cold 0.9% NaCl. The capsules were removed, and slices of approximately 1 mm thickness were prepared from whole kidneys using a disc gel slicing apparatus (model 190, Bio-Rad Laboratories, Richmond, CA). Three or four slices (~100 mg) were preincubated for 15 min in 3 ml of acetate Ringer solution (134 mM NaCl; 5 mM KCl; 1.5 mM $CaCl_2$; 1 mM $MgSO_4$; 10 mM sodium acetate; 5 mM dextrose; 1 mM NaH_2PO_4 and 10 mM Tris/HCl buffer, pH 7.4) in a shaking water bath at 25° under an atmosphere of 100% oxygen. The substance to be studied was then added, and the slices were, generally, incubated for an additional 30 min. The slices were then blotted on filter paper and weighed using a Mettler H51 Analytical Balance (Mettler Instruments Corp., Highstown, NJ). For determination of radioactivity, slices were solubilized in 1 ml of NCS Tissue Solubilizer (Amersham Corp., Arlington Heights, IL) by incubation at 55° overnight. Toluene + PPO–POPOP (Spectrafluor, Amersham Corp.) was then added (19 ml), and radioactivity was measured using a Beckman LS7500 Liquid Scintillation System. Aliquots of medium were treated in the same manner. When nonradioactive dTub was used, the slices were homogenized using a Polytron homogenizer (Brinkman Instruments, Westbury, NY) in 2 ml of 0.4 N perchloric acid at 4°. After centrifugation, the acid-soluble extracts were neutralized with 10 N KOH, and the cold, insoluble potassium perchlorate was removed by centrifugation. Recovery of dTub was virtually complete under these conditions. The extracellular space of the mouse kidney slices was 38 ± 1% (mean value ± S.E., N = 17) of the wet weight determined under the incubation conditions described above using 0.05 μCi/ml of [^{3}H]inulin. The values reported herein are not corrected for extracellular space.

HPLC methods. dTub was measured in the neutralized, acid extracts of kidney tissue and medium using HPLC as follows. A linear gradient from 10 mM KH_2PO_4, pH 5.5, to 10 mM KH_2PO_4 plus 40% methanol was formed in 30 min at a flow rate of 2 ml/min. The instrument used for this purpose was a Laboratory Data Control LC 7800 System equipped with a Chromatography Control Module II (CCM) with two Constametric pumps. The CCM plotted and integrated the areas under the u.v. absorbing peaks measured at 254 nm or at 270 nm (UV III Monitor and Spectro-II Monitor, Laboratory Data Control, Riviera Beach, FL). Identity of dTub was confirmed by comparison of the retention volumes and 270:254 peak area ratios with authentic solutions. This HPLC system was also used to establish the purity and stability of [^{3}H]dTub. Measurements of ATP and other nucleotides in the extracts of mouse kidney were performed as previously described [6] using a Partisil-SAX column (Whatman, Inc., Clifton, NJ).

Determination of the pK_a *of dTub.* The pK_a of dTub was determined spectrophotometrically [7]. Solutions of dTub (50 μM) were prepared with pH values ranging from 1 to 13. Dilute solutions of HCl (0.001 N to 0.1 N) were used to attain pH values of 1 to 3; sodium acetate buffers were used to attain pH values of 4 to 9; and dilute solutions of NaOH were used to attain pH values greater than 9.0. The u.v. absorbance of dTub under these conditions was monitored from 220 to 360 nm using a Beckman model 25 Scanning Spectrophotometer. The pK_a estimate was made from measurements of absorbance at 230 nm, a wavelength at which the maximum pH-dependent deflection occurred.

Materials. dTub was synthesized as described by Robins and Muhs [8]. Tritium-exchange labeling of a 100-mg sample of dTub was performed by Moravek Biochemicals, Brea, CA. The [^{3}H]dTub was at least 98% pure and the radiolabel was stable upon incubation for 3 days at physiological pH and temperature. Other radiolabeled compounds and their sources were as follows: [^{3}H]inulin (3 Ci/mmole) and inulin[^{14}C]carboxylic acid (6.8 mCi/mmole), Amersham Corp.; 1-[^{14}C]tetraethylammonium bromide (4.4 mCi/mmole) and *p*-[glycyl-1-^{14}C]aminohippuric acid (41.3 mCi/mmole), New England Nuclear Corp., Boston, MA. Choline chloride, iodoacetamide, N^1-methylnicotinamide, PAH and TEA bromide were obtained from the Sigma Chemical Co., St. Louis, MO. Carbonyl cyanide *p*-trifluoroethoxyphenylhydrazone was supplied by the E. I. du Pont de Nemours Co., Wilmington, DE. Probenecid was obtained from Merck, Sharp & Dohme, West Point, PA. Other reagents were obtained from the Fisher Chemical Corp., Fairlawn, NJ.

RESULTS

Renal clearance of [^{3}H]dTub in mice. dTub appeared to be secreted by the mouse kidney since its clearance was approximately 3-fold that of inulin (Table 1). dTub was not significantly metabolized during the period of clearance measurements, and it was not significantly bound to mouse plasma proteins, i.e. 9 ± 2% bound at a concentration of 1 μM (mean value ± S.E., N = 3). Thus, at least 90% of the dTub in mouse plasma was available for glom-

Table 1. Renal clearance of [^{3}H]2′-deoxytubercidin and inulin[^{14}C]carboxylic acid in mice*

Dose of dTub (mg/kg)	N	Plasma concentration† (nmoles/ml)		Renal excretion (nmoles/min)		Renal clearance (ml/min)		Clearance ratio (CL_{dTub}/CL_{inulin})
		dTub	Inulin	dTub	Inulin	dTub	Inulin	
1	9	1.49 ± 0.34	12.68 ± 1.60	1.38 ± 0.30	4.96 ± 1.57	1.01 ± 0.35‡	0.36 ± 0.20	3.24 ± 0.39
10	4	12.02 ± 1.22	10.80 ± 2.07	10.54 ± 2.06	4.38 ± 2.06	0.94 ± 0.28‡	0.37 ± 0.11	2.71 ± 0.50

* Male AKR mice were given [^{3}H]2′-deoxytubercidin (1.0 μCi/g) (dTub) and inulin[^{14}C]carboxylic acid (0.05 μCi/g; 4.04 mCi/mmole) (inulin) subcutaneously. The urethra was ligated 20 min after dTub and inulin administration. Blood samples (25 μl) were collected from the tail vein immediately after ligation, 15 min after ligation, and 30 min after ligation. A microhematocrit (2 μl) was also collected at these intervals. The animal was killed 30 min after ligation and the bladder was then washed with 10 ml of phosphate-buffered saline. Whole blood and urine levels of dTub and inulin were determined using liquid scintillation spectrometry. Plasma values for dTub and inulin were calculated from the whole blood values and the hematocrit. In separate experiments, inulin was shown to be excluded from the erythrocyte whereas dTub appeared to be equally distributed in plasma and erythrocytes. Values are the mean ± S.E.; N = number of determinations.

† Estimated "midpoint" value determined from data obtained in each animal (0, 15 and 30 min after ligation).

‡ Significantly greater than the simultaneously measured inulin clearance, $P < 0.05$, Student's paired t-test.

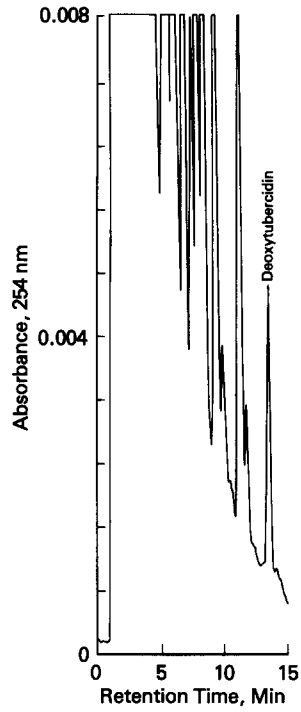

Fig. 2. High performance liquid chromatographic analysis of 2′-deoxytubercidin in extracts of mouse kidney slices. A 200-μl portion of the neutralized acid-soluble tissue extract was applied to a Waters Associates μBondapak C_{18} column and was eluted at a flow rate of 2 ml/min using a 30-min linear gradient from 10 mM KH_2PO_4, pH 5.5, to 10 mM KH_2PO_4 + 40% methanol, pH 5.5. Eluting compounds were monitored at 254 nm. A representative separation of 0.6 nmole of 2′-deoxytubercidin in an extract is shown. No peak corresponding to the retention volume of 2′-deoxytubercidin was present in control extracts.

erular filtration in these experiments. In separate experiments, dTub was found to rapidly-distribute equally into whole blood whereas inulin was restricted to the extracellular space. Thus, the plasma values in Table 1 are whole blood measurements of [^{3}H]dTub concentrations whereas inulin plasma levels were estimated from [^{14}C]inulin whole blood measurements and corrected to plasma volume. The secretion of dTub was apparent whether the plasma level was 1.5 or 12 μM (Table 1).

dTub transport by mouse kidney slices in vitro. Prior to acquiring radiolabeled dTub, we used an HPLC method to measure the drug in extracts of mouse kidney as described in Methods. The representative HPLC separation of dTub shown in Fig. 2 contained 0.6 nmole of dTub in a 200-μl portion of the neutralized, acid extract of mouse kidney slices. A standard curve for measurement of dTub at 254 nm and at 270 nm was established by adding known amounts of dTub immediately prior to acid extraction (data not shown). When [^{3}H]dTub became available to us, we confirmed that the measurement of dTub uptake by mouse kidney slices using the HPLC method agreed with that using the radiolabeled compound.

Incubation of [^{3}H]dTub with mouse kidney slices was not followed by appreciable metabolism of the

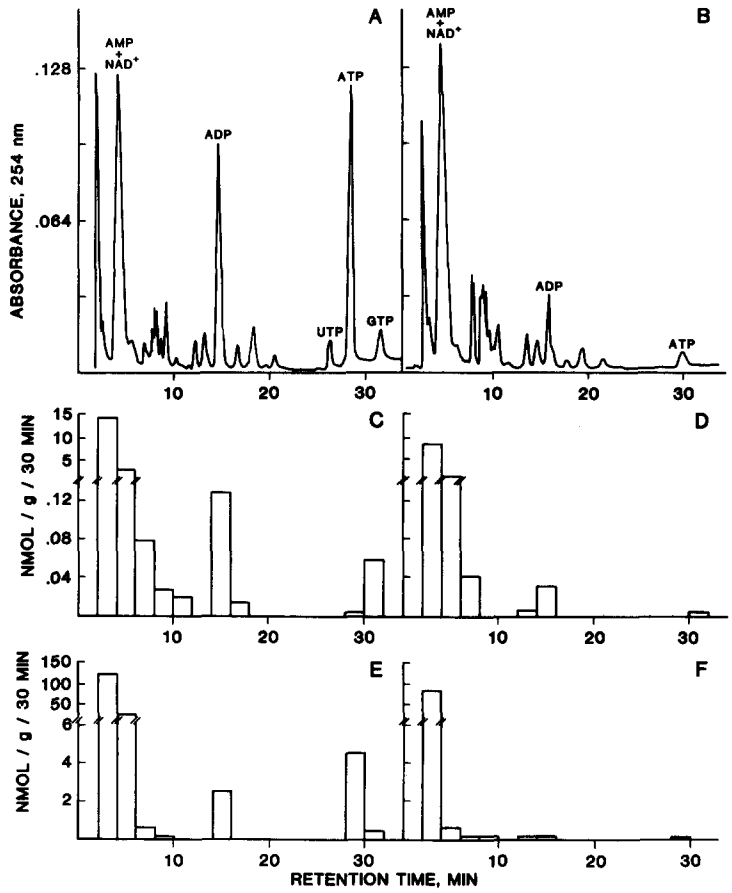

Fig. 3. Lack of significant metabolism of [^{3}H]2′-deoxytubercidin by mouse kidney slices. Mouse kidney slices were preincubated in the absence (panels A, C and E) or presence (panels B, D and F) of 10 mM sodium azide prior to, and during, incubation with [^{3}H]2′-deoxytubercidin. The kidney slices were incubated in medium containing 10 μM (100 mCi/mmole, panels C and D) or 100 μM (10 mCi/mmole, panels E and F) [^{3}H]2′-deoxytubercidin for 30 min. Kidney slices were pooled from triplicate determinations prior to homogenization and extraction in 0.4 N perchloric acid. Neutralized, acid-soluble extracts of the mouse kidney slices were analyzed by HPLC using a Partisil-SAX column as described by Nelson *et al.* [6]. Fractions (2 min) of eluting material were collected, and radioactivity was assayed by liquid scintillation spectrometry (panels C, D, E, and F). Panels A and B illustrate the u.v. chromatographic profile of extracts which were equivalent to 10 mg tissue. These data are representative of two separate experiments. Note that the ordinate is expanded after 4–6 min in panels C, D, E and F.

compound (Fig. 3). The chromatograms on the left of Fig. 3 were obtained in control tissue extracts whereas the chromatograms on the right of Fig. 3 were obtained from extracts of tissue incubated with 10 mM sodium azide. Incubation with azide markedly reduced the endogenous nucleoside di- and triphosphate pools (A vs B in Fig. 3). There was apparent formation of small amounts of dTub nucleotides in the mouse kidney slices (i.e. C and E in Fig. 3), amounts which formed to a lesser extent in the presence of azide (i.e. D vs C and F vs E in Fig. 3). Note however, that the ordinate is expanded for substances which eluted with retention times greater than 4–6 min in chromatograms C, D, E and F. Thus, the bulk of the radioactivity > 96% was unretained material (eluting in 2–6 min). Separation of these tissue extracts on the reverse phase column, as illustrated in Fig. 2, confirmed that this material was unchanged [^{3}H]dTub (data not shown). Thus, dTub was metabolized to a very small extent (< 4%) by the mouse kidney slices *in vitro*.

dTub was concentratively accumulated (i.e. the tissue to medium ratio was greater than 1) by mouse kidney slices (Table 2). Slices of brain, heart, liver and spleen did not demonstrate this ability. The time-course for the accumulation of dTub by mouse kidney slices in the presence or absence of 10 mM sodium azide is shown in Fig. 4. At all times, there was an apparent azide-sensitive component of uptake. Also, after 10 min of incubation, the concentration of dTub in the slices exceeded that in the medium, eventually reaching a level approximately 2- to 3-fold that of the medium. For subsequent experiments, an incubation period of 30 min was used.

When the concentration of dTub was varied from

Table 2. Uptake of 2′-deoxytubercidin by slices from various mouse tissues*

Tissue	Tissue/medium ratio
Kidney	2.16 ± 0.12
Brain	1.11 ± 0.08
Heart	0.85 ± 0.05
Liver	0.65 ± 0.10
Spleen	1.24 ± 0.03

* Kidney slices were prepared and incubated under conditions described in Methods. Uptake of 50 μM dTub was measured after a 30-min incubation by analysis of neutralized, acid-soluble extracts of tissue and medium using HPLC. Values are the mean ± S.E. of six determinations, each performed in duplicate.

5 to 400 μM in the medium, the uptake measured at 30 min was as depicted in Fig. 5. Again, there were at least two components of uptake, an azide-sensitive and an azide-insensitive component. The azide-insensitive uptake (azide-treated curve in Fig. 5) was essentially nonconcentrative since the medium and tissue concentrations were almost equal. Furthermore, the azide-insensitive uptake did not appear to be saturable. On the other hand, the azide-sensitive component of uptake was saturated at a level of about 200 μM dTub in the medium (Fig. 6). Approximately one-half of the maximum uptake occurred at a level of about 50 μM.

The uptake of dTub by mouse kidney slices was inhibited by aerobic and anaerobic metabolic inhibitors (Table 3). Additionally, the uptake appeared to be temperature and oxygen dependent since uptake in a nitrogen atmosphere or at 4° was less than that in the presence of oxygen and at 25° (Table 3). As expected, these various treatments were very effective in reducing the ATP content of the slices. ATP was determined as illustrated in Fig. 3. Thus, there was a metabolic or energy-dependent component of dTub uptake by mouse kidney slices.

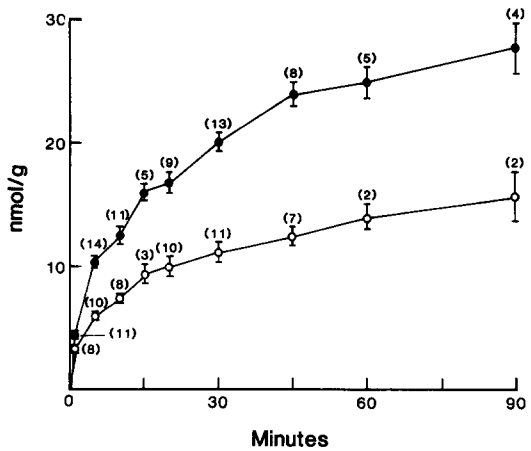

Fig. 4. Time-course of 2′-deoxytubercidin uptake by mouse kidney slices. Uptake of 10 μM dTub (or [^{3}H]dTub) by mouse kidney slices was measured in the absence (●——●) or in the presence (○——○) of 10 mM sodium azide. Nonradioactive dTub was assayed in neutralized, acid-soluble extracts of kidney slices using the HPLC method illustrated in Fig. 2 or [^{3}H]dTub was assayed in solubilized tissue samples using liquid scintillation spectrometry. Each point is the mean value ± S.E. for the number of experiments shown.

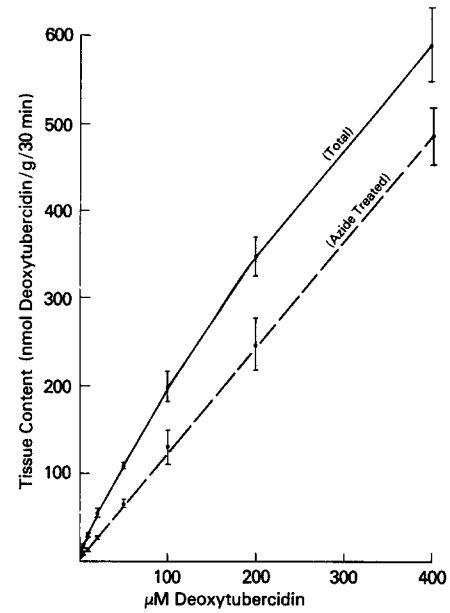

Fig. 5. Uptake of 2′-deoxytubercidin by mouse kidney slices. The slices were incubated for 30 min in acetate Ringer medium containing dTub at concentrations ranging from 5 to 400 μM in the absence (total curve) or presence (azide-treated curve) of 10 mM sodium azide. Acid-soluble extracts were prepared and analyzed as illustrated in Fig. 2. Each point is the mean ± S.E. for a least four determinations.

To determine the relationship between dTub uptake by mouse kidney slices and uptake by the classical organic cation and organic anion secretory systems, the experiments summarized in Table 4 were performed. Organic cations (choline Cl, N^1-methylnicotinamide and TEA) and organic anions (PAH, phenol red and probenecid) were tested for their abilities to inhibit [^{3}H]dTub, [^{14}C]TEA or [^{14}C]PAH uptake. The tissue to medium ratios for

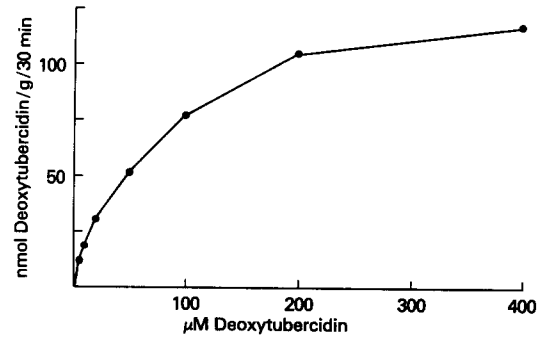

Fig. 6. Azide-sensitive uptake of 2′-deoxytubercidin by mouse kidney slices. The sodium azide-sensitive uptake was calculated by subtracting the uptake in the presence of 10 mM sodium azide from the total uptake shown in Fig. 5.

Table 3. Effect of metabolic inhibitors, N_2 atmosphere, and temperature on the uptake of 2′-deoxytubercidin by mouse kidney slices*

	Tissue/medium ratio	ATP (nmoles/g)
Control	2.31 ± 0.13	419 ± 72
Sodium azide		
1 mM	1.54 ± 0.25†	
10 mM	1.11 ± 0.06‡	80 ± 20‡
Sodium cyanide		
1 mM	1.67 ± 0.08†	
10 mM	0.99 ± 0.04‡	93 ± 16†
Iodoacetamide		
1 mM	1.36 ± 0.08‡	
10 mM	1.00 ± 0.04‡	40 ± 9‡
Carbonyl cyanide *p*-trifluoro-methoxyphenyl hydrazone		
10 μM	1.22 ± 0.06‡	
100 μM	0.95 ± 0.04‡	83 ± 18†
2′Deoxyglucose		
10 mM	1.46 ± 0.05‡	32 ± 32†
N_2 atmosphere	1.33 ± 0.22‡	145 ± 36†
4°	1.12 ± 0.15‡	146 ± 37†

* Kidney slices were prepared and incubated as described in Methods. Metabolic inhibitors were added at the beginning of the preincubation period. Uptake of [^{3}H]dTub was measured after a 30-min incubation period. Values are the mean ± S.E. for at least four determinations, each in duplicate.

† Significantly less than control, $P < 0.05$, Student's unpaired *t*-test.

‡ Significantly less than control, $P < 0.005$.

these compounds were significantly greater than 1, and sodium azide reduced the uptake for all three agents. DTub was effective as an inhibitor of [^{3}H]dTub and of [^{14}C]TEA uptake; however, dTub did not inhibit the uptake of [^{14}C]PAH. TEA and the other organic cations were active against [^{3}H]dTub as well as [^{14}C]TEA uptake, but these compounds were inactive as inhibitors of [^{14}C]PAH uptake. The organic anions were more potent inhibitors of [^{14}C]PAH accumulation than they were of accumulative of [^{3}H]dTub or of [^{14}C]TEA. In summary, the effects of the compounds shown in Table 4 on [^{3}H]dTub and [^{14}C]TEA uptake were qualitatively similar, and dTub and TEA each inhibited the transport of the other substance. Taken together, these results suggest that dTub is a substrate for the organic cation secretory system of mouse kidney. Since substrates for this process are relatively strong bases (pK_a values greater than 7, i.e. [9]) the pK_a of dTub was determined as illustrated in Fig. 7. The one-half maximum absorbance change at 230 nm occurred at a pH of 5.3 (inset of Fig. 7).

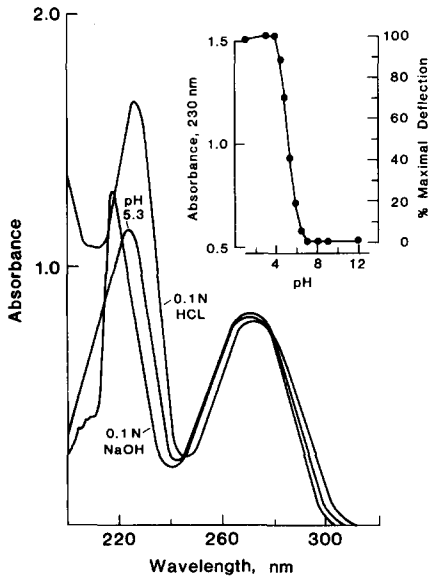

Fig. 7. Spectrophotometric determination of the pK_a of 2′-deoxytubercidin.

DISCUSSION

The observation that 2′-deoxyadenosine is secreted by human and mouse kidney whereas adenosine appears to be reabsorbed [1] is of physiological interest for several reasons. Although purine nucleosides are transported across the cell membranes of eukaryotic cells, this transport is described as a facilitated diffusion process rather than an active or concentrative one [10]. Furthermore, the nucleoside carrier is relatively nonspecific in its structural requirements. Thus, the transepithelial transport of these purine nucleosides by mammalian kidney represents a unique opportunity to study the mechanism for an active process which may have strict structural requirements.

Classically, the organic anion and organic cation secretory systems have been studied in considerable detail [11], and it is possible to study the transport of prototype chemicals such as TEA and PAH using renal tissue slices. When we attempted to use mouse kidney slices to monitor the uptake of 2′-deoxyadenosine or of adenosine, metabolism of the compounds rendered interpretation of the results complex. Therefore, we elected to use a non-metabolized

Table 4. Effect of organic cations and organic anions on the uptake of [^{3}H]2′-deoxytubercidin, 1-[^{14}C]tetraethylammonium bromide and *p*-[glycyl-l-^{14}C]aminohippurate by mouse kidney slices*

	[^{3}H]2′-Deoxytubercidin	[^{14}C]Tetraethylammonium bromide	*p*-[^{14}C]Aminohippurate
Control	1.86 ± 0.05	9.06 ± 0.45	2.80 ± 0.19
Sodium azide, 10 mM	1.10 ± 0.03†	1.45 ± 0.21†	0.91 ± 0.04†
2′-Deoxytubercidin			
10 μM	1.76 ± 0.15	8.02 ± 0.48	2.90 ± 0.44
100 μM	1.73 ± 0.04	6.56 ± 0.33†	2.52 ± 0.31
1,000 μM	0.94 ± 0.03†	3.61 ± 0.38†	2.54 ± 0.24
Choline chloride			
10 μM	1.83 ± 0.13	9.08 ± 0.32	3.18 ± 0.19
100 μM	1.57 ± 0.11†	6.66 ± 0.35†	3.14 ± 0.24
1,000 μM	1.48 ± 0.02†	3.20 ± 0.12†	3.19 ± 0.24
10,000 μM	1.26 ± 0.02†	1.10 ± 0.03†	3.48 ± 0.38
N^1-Methylnicotinamide chloride			
10 μM	1.80 ± 0.11	9.76 ± 0.48	3.27 ± 0.12
100 μM	1.71 ± 0.06‡	8.00 ± 0.36	2.88 ± 0.13
1,000 μM	1.48 ± 0.12†	4.20 ± 0.43†	2.82 ± 0.20
10,000 μM	1.15 ± 0.05†	1.57 ± 0.11†	3.20 ± 0.35
Tetraethylammonium bromide			
10 μM	1.85 ± 0.10	9.57 ± 0.31	3.08 ± 0.24
100 μM	1.50 ± 0.08†	4.73 ± 0.18†	2.42 ± 0.18
1,000 μM	1.27 ± 0.03†	1.69 ± 0.10†	2.45 ± 0.14
10,000 μM	1.26 ± 0.04†	0.68 ± 0.01†	2.33 ± 0.11
p-Aminohippurate			
10 μM	1.84 ± 0.13	10.55 ± 0.71	2.39 ± 0.13
100 μM	1.82 ± 0.12	8.39 ± 0.49	1.38 ± 0.08†
1,000 μM	1.91 ± 0.13	8.30 ± 0.50	0.67 ± 0.04†
10,000 μM	1.73 ± 0.09	8.14 ± 0.50	0.49 ± 0.05†
Phenol red			
10 μM	1.80 ± 0.06	9.75 ± 0.58	2.37 ± 0.12
100 μM	1.69 ± 0.06	9.52 ± 0.44	1.55 ± 0.10†
1,000 μM	1.73 ± 0.09	9.52 ± 0.39	0.80 ± 0.02†
10,000 μM	1.26 ± 0.09†	6.72 ± 0.50‡	0.51 ± 0.03†
Probenecid			
10 μM	1.86 ± 0.14	7.48 ± 0.14	1.61 ± 0.09†
100 μM	1.68 ± 0.11	7.82 ± 0.29	0.91 ± 0.04†
1,000 μM	1.45 ± 0.08†	5.54 ± 0.27†	0.57 ± 0.01†

* Kidney slices were prepared and incubated as described in Methods. After a 15-min preincubation, [^{3}H]2′-deoxytubercidin, 1-[^{14}C]tetraethylammonium bromide or *p*-[glycyl-l-^{14}C]aminohippurate was added to the medium at a final concentration of 10 μM. Uptake was measured after a 30-min incubation. 2′-Deoxytubercidin, choline chloride, N^1-methylnicotinamide, tetraethylammonium bromide, *p*-aminohippurate, phenol red and probenecid were added simultaneously with the radiolabeled substrate. Sodium azide was added at the beginning of the preincubation period. Values are reported as tissue/medium ratios (means ± S.E.) for at least three animals.

† Significantly less than control ($P < 0.005$), Student's unpaired *t*-test.

‡ Significantly less than control ($P < 0.05$).

analog, dTub, as a possible model compound to study the mechanism of the renal secretion of 2′-deoxyadenosine. dTub is neither a substrate nor an inhibitor of adenosine deaminase (data not shown); it was not extensively metabolized by mouse kidney slices (Fig. 3); and it is relatively nontoxic to mammalian cells grown in tissue culture [12]. Since dTub was secreted by mouse kidney (Table 1), our interest in the substance as a possible model compound gained impetus. Using mouse kidney slices *in vitro*, it was possible to demonstrate an energy-dependent (Table 3), saturable (Fig. 6), and organ-selective, concentrative uptake (Table 2) of dTub. This process appears to have occurred via the organic cation mechanism since classical substrates for the organic cation carrier (choline, TEA and N^1-methylnicotinamide) inhibited dTub accumulation whereas classical substrates for the organic anion system were relatively inactive (Table 4). Conversely, dTub inhibited the uptake of [^{14}C]TEA but did not inhibit the uptake of [^{14}C]PAH. These observations suggested that dTub may be a rather strong base since substrates for the organic cation carrier are generally strong bases [9]. dTub had a pK_a of 5.3 (Fig. 7) which makes it a comparatively stronger base than 2′-deoxyadenosine (pK_a of 3.8 [13]). This also implies that a pK_a value greater than 7 is not an absolute requirement for a compound to have appreciable affinity for the TEA transport system.

What then, are the relationships between the dTub, the 2′-deoxyadenosine and the TEA renal secretory mechanism(s)? Clearly, additional studies are needed to resolve this issue and to determine whether or not adenosine is reabsorbed. Meanwhile, the current report establishes with some degree of certainty that dTub is secreted by the mouse kidney

in vivo and that it has significant affinity for the organic cation system *in vitro*.

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