Biochimica et Biophysica Acta, 1149 (1993) 202–208

BBAMEM 76004

Substrate selectivity, potential sensitivity and stoichiometry of Na^+-nucleoside transport in brush border membrane vesicles from human kidney

Marcelo M. Gutierrez and Kathleen M. Giacomini

Schools of Pharmacy and Medicine, University of California, San Francisco, CA (USA)

(Received 6 August 1992)
(Revised manuscript received 18 February 1993)

Key words: Sodium ion transport; Nucleoside transport; Nucleoside; Brush-border membrane; (Human kidney)

Recently, we demonstrated the presence of a Na^+-nucleoside cotransport mechanism that transports both purine and pyrimidine nucleosides in human renal brush-border membrane vesicles (BBMV) (Gutierrez et al. (1992) Biochim. Biophys. Acta 1105, 1–9). The objective of this study was to further elucidate the characteristics of this cotransport system in terms of electrical potential sensitivity, stoichiometry and substrate selectivity with respect to nucleoside analogs. In BBMV from human kidney, Na^+-thymidine uptake was stimulated by an inside negative potential difference created by K^+ and valinomycin. A hyperbolic relationship between initial rate of uridine uptake and Na^+ concentration was obtained suggesting a Na^+-nucleoside coupling stoichiometry of 1:1. Our previous study had demonstrated that the pyrimidines, thymidine, cytidine, and uridine and the purines, adenosine, 2′-deoxyadenosine, and guanosine, but not inosine and formycin B, were substrates of this system. To further define the substrate selectivity of the transporter, the interaction of the drugs, 2-chloroadenosine (2-ClAdo), 5-fluorouridine (5-FUrd) and 5-iodo-2′-deoxyuridine (5-IdUrd), nucleoside analogs that are modified on the base moiety was studied. The three compounds inhibited Na^+-thymidine uptake in the vesicles via a competitive mechanism. The IC_{50} values for 2-ClAdo, 5-FUrd and 5-IdUrd were 75, 49, and 16 μM, respectively. In addition, 5-IdUrd *trans*-stimulated the initial uptake of thymidine into the vesicles suggesting that the two compounds share the same transporter. Collectively, these data suggest that Na^+-nucleoside transport in the human renal brush-border membrane is an electrogenic process and that the kidney may play a role in the disposition and targeting of clinically important nucleoside analogs.

Introduction

Purine and pyrimidine nucleoside analogs are currently being developed and used as antineoplastic, antiviral and antiparasitic agents. For example, 2-chloro-2′-deoxyadenosine has demonstrated clinical activity against lymphoid malignancies and hairy cell leukemia [1] by inhibiting DNA synthesis [2]. AZT (azidothymidine) and ddI (dideoxyinosine), nucleoside analogs which inhibit viral DNA formation and RNA expression, are used in the clinical treatment of AIDS [3]. Iododeoxyuridine (5-IdUrd) has been shown to be an effective radiosensitizer in adult sarcomas [4–6]. In addition, fluoropyrimidines are currently the most widely used drugs in the treatment of various malignant neoplasms [7].

Correspondence to: K.M. Giacomini, Schools of Pharmacy and Medicine, Box 0446, University of California, San Francisco, CA 94143, USA.

Understanding the mechanisms by which the kidney handles nucleosides and nucleoside analogs is essential in rational drug therapy, since the kidney may play an important role in the pharmacology, toxicology and disposition of many of these compounds [7–10]. For example, nephrotoxicity is one of the limiting toxicities of some nucleoside analogs and has been observed in deoxycoformycin and tubercidin therapy in animals [8,9]. Fluoropyrimidine toxicities are aggravated in patients with renal impairment [10], suggesting that the kidney is involved in the disposition of fluoropyrimidines. In addition, adenosine, a neurotransmitter involved in the regulation of renal blood flow is cytotoxic [11].

Nucleosides appear to be transported in brush-border membrane vesicles (BBMV) prepared from rat, rabbit and bovine kidney by both Na^+-dependent and Na^+-independent processes [12–19]. The Na^+-independent process is inhibitable partly by NBMPR, an inhibitor of a ubiquitous, facilitated nucleoside transport system termed es [20]. In bovine BBMV, Na^+-de-

pendent nucleoside transport involves two distinct systems, termed N1 and N2 [18]. The systems, which both require Na^+ as the driving force and have a 1:1 Na^+/nucleoside-coupling ratio, differ in terms of their substrate specificity. In general, N1 appears to be selective for purine nucleosides including guanosine and formycin B, whereas N2 appears to be selective for pyrimidine nucleosides, including thymidine and cytidine. However, there is some substrate overlap. For example, the purine nucleoside adenosine and the pyrimidine nucleoside uridine are transported by both systems.

Recently, we studied the mechanisms of transport of nucleosides in BBMV prepared from human kidney [21]. These mechanisms appear to be somewhat different from those observed in bovine [18] and rabbit [19] renal BBMV. In the human renal brush-border membrane, the Na^+-dependent transport mechanism involves a single Na^+-nucleoside cotransporter (N4) that interacts with uridine, thymidine, cytidine, adenosine and guanosine, but not formycin B and inosine. Separate N1 and N2 transport systems are not apparent.

The overall goal of this study was to further elucidate the mechanisms of Na^+-nucleoside cotransport in the brush-border membrane of the human kidney in terms of Na^+-nucleoside stoichiometry and electrogenicity. In addition, the interaction of three nucleoside analogs with the Na^+-nucleoside cotransporter was investigated. Our results are consistent with an electrogenic Na^+-nucleoside transport mechanism with a 1:1 coupling ratio. The drugs, 2-ClAdo, 5-FUrd and 5-IdUrd, inhibited Na^+-thymidine transport in a competitive fashion and 5-IdUrd *trans*-stimulated Na^+-thymidine uptake. These results suggest that the cotransporter may play a role in the renal handling and disposition of these compounds.

Materials and Methods

Human kidney tissue. Human kidneys were donated to our laboratory for research purposes (Table I) by the Organ/Tissue Transplant Services at the University of California, San Francisco. These kidneys were unsuitable for transplant and were perfused according to transplant protocol prior to use. BBMV were prepared immediately from the outer cortex as described previously [21]. In some cases, the cortex was divided into small portions (15–30 g), frozen in liquid nitrogen, and stored at −70°C. Our previous study suggested that similar results were obtained in vesicles prepared from either fresh or frozen kidney [21].

Preparation of brush-border membrane vesicles. BBMV were prepared by divalent (Mg^{2+}) cation precipitation [22], as modified in our laboratory [21,23–26]. For studies involving frozen human kidney, BBMV were prepared from tissue that had been thawed at room temperature.

TABLE I

Characteristics of Kidney Donors

Donor	Age	Sex	Associated medical problems	Drugs received prior to procurement of kidney
1	40	F	Hypertension, diabetes	verapamil, ampicillin, insulin, heparin
2	53	M	Hypertension, Intracerebral bleed	clonidine, dyazide
3	62	F	Hypertension, Intracerebral bleed	dopamine, lasix
4	62	M	Cardiac arrest	ASA, Trafium
5	21	M	Intracerebral bleed	dopamine, cephalosporin
6	64	M	Coronary artery disease	diltiazem, lasix, pitressin

Protein concentration was measured using the Bio-Rad Protein Assay Kit™ (Bio-Rad, Richmond, CA, USA). Bovine serum albumin was the standard. The activity of maltase, an enzyme associated with the brush-border membrane, was enriched 10-fold in the BBMV preparation as compared to the activity in the corresponding homogenate [26]. Na^+/K^+-ATPase activity which is associated with the basolateral membrane was not enriched [26].

Transport studies. The uptake of [^{3}H]uridine and [^{3}H]thymidine at 22°C was measured by an inhibitor-stop filtration technique as described previously [14]. For potential sensitivity studies, the vesicles were incubated with 2 μM valinomycin for 1 h before the transport study. For stoichiometry studies, the uptake of [^{3}H]uridine was measured at 5 s in the presence of increasing sodium concentrations (range 0 to 200 mM). For IC_{50} studies, increasing concentrations of three nucleoside analogs (Fig. 3) were present in the reaction mixture, and the uptake of [^{3}H]thymidine (2 μM) was measured at 5 s.

Data analysis. The Michaelis-Menten kinetic parameters (K_m and V_{max}) for thymidine transport were obtained as described previously [21]. The IC_{50} or the concentration of drug which causes a 50% reduction in the uptake of thymidine was determined. The IC_{50} was estimated by a sigmoidal inhibition model using FIT FUNCTION on the National Institute of Health PROPHET system, an iterative nonlinear least squares regression program computer system. Data were fit to the following equation:

$$V = V_o/(1+(I/IC_{50})^n) \quad (1)$$

where V is the uptake of thymidine in the presence of the inhibitor, V_0 is the uptake of thymidine in the absence of any inhibitor, I is the inhibitor concentration and n is the slope. Assuming a competitive mechanism of interaction, the K_i was determined by the following equation:

$$K_i = IC_{50}/(1 + C/K_m) \quad (2)$$

where C represents the concentration of thymidine used in inhibitor studies, K_m represents the K_m of thymidine uptake determined in preliminary Michaelis-Menten studies (data not shown). Data points were determined in triplicate. Experiments were repeated using at least two different vesicle preparations. Data are expressed as mean ± S.D. from a representative experiment or as the mean ± S.E. from three replicate experiments. Data were analyzed by analysis of variance and the Student-Newman-Keuls test.

Chemicals. [*methyl*-^{3}H]Thymidine (74 Ci/mmol) and uridine [5-^{3}H] (27.1 Ci/mmol) were purchased from NEN Research Products (Wilmington, DE, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA) and were the highest grade available.

Results

Time-course of thymidine uptake

In the presence of an inwardly-directed Na^+-gradient, thymidine transiently accumulated against its concentration gradient in human renal brush-border membrane vesicles (Fig. 1A). Accumulation of thymidine reached a maximum at 60 s and then decreased subsequently. In the absence of a Na^+-gradient, no overshoot was observed, and thymidine accumulated to the same equilibrium value.

Potential sensitivity studies

The role of both the membrane potential and the presence of a sodium gradient on the Na^+-dependent transport of thymidine was studied (Fig. 1B). A very slight 'overshoot phenomenon' was observed in the presence of an inside-negative potential difference alone generated by incubating the vesicles with 2 μM valinomycin and potassium ($[K^+]_{in} > [K^+]_{out}$). Na^+ concentrations were equilibrated across the vesicular membrane. In the presence of an inwardly-directed Na^+-gradient with the membrane potential clamped by valinomycin and K^+ ($[K^+]_{in} = [K^+]_{out}$), a substantial overshoot was observed, indicating that a Na^+ gradient alone can support the transport of thymidine against its concentration gradient. The combined effects of a K^+ diffusion potential ($[K^+]_{in} > [K^+]_{out}$) and an inwardly-directed Na^+-gradient on the uptake of thymidine resulted in a significantly higher overshoot phenomenon than with the Na^+-gradient alone, suggesting that the Na^+-dependent transport of thymidine is an electrogenic process involving the transport of a positive charge per transport cycle [27].

Na^+-nucleoside stoichiometry

The Na^+-uridine stoichiometry of the human renal BBMV was investigated using the activation method [28]. The initial rates (5 s) of [^{3}H]uridine uptake were determined as a function of increasing extravesicular Na^+ concentrations. A hyperbolic relationship between uridine uptake and Na^+ concentration, suggesting a Na^+:uridine stoichiometry of 1:1 was obtained (Fig. 2). The data were fit to the Hill equation [29]:

$$\text{flux} = V_{max} \cdot [Na^+]^n / (K^n_{Na^+} + [Na^+]^n) \quad (3)$$

where K_{Na^+} is the Na^+ concentration which gives 50% of the maximal velocity, V_{max}, and n is the Hill coefficient. The Hill coefficient (mean ± S.E.) obtained in

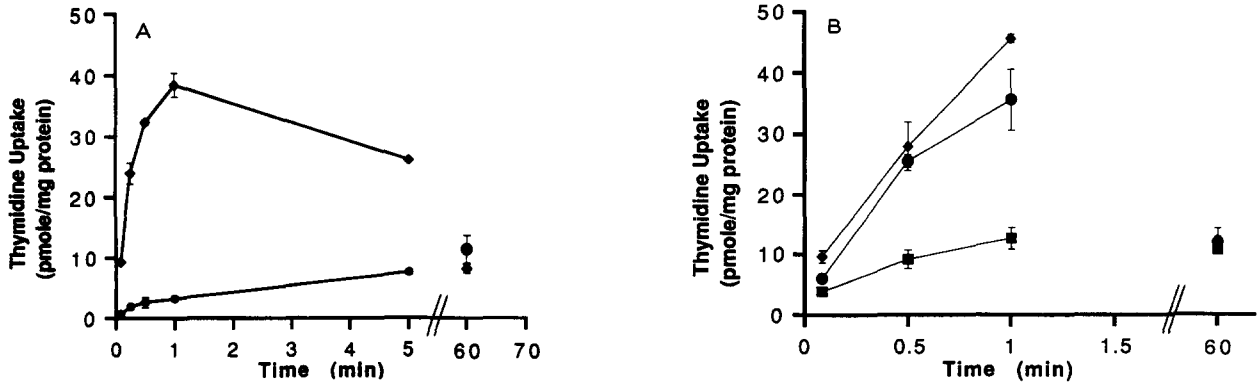

Fig. 1. (A) [^{3}H]Thymidine (5 μM) uptake into human renal BBMV as a function of time. The data represent the uptake of thymidine in the presence (♦) or absence (●) of an initial inwardly-directed Na^+-gradient (150 mM). Data are from a representative experiment (mean ± S.D.) in fresh human kidney BBMV preparation. (B) Time-course of Na^+-thymidine uptake in the presence of both an inwardly-directed Na^+-gradient and a K^+ diffusion potential (♦), an Na^+ gradient alone (●) and a K^+ diffusion potential alone (■). Data are representative of two experiments (mean ± S.D.) from BBMV prepared from frozen human kidneys.

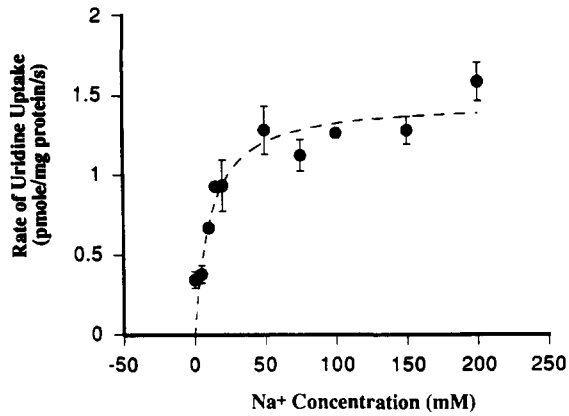

Fig. 2. Rate of uridine uptake in human renal BBMV at 5 s as a function of increasing extravesicular Na^+ concentration. The Hill coefficient obtained in this experiment was 1.06. Data are from one of three representative experiments (mean ± S.D.).

three experiments was 0.97 ± 0.13, a value which is not significantly different from 1.0. These data are consistent with a single Na^+-binding site on the transport protein. In addition, a K_{Na^+} (mean ± S.E.) of 23.5 ± 9.1 mM and a V_{max} of 6.1 ± 3.6 pmol/mg protein per s were obtained.

Substrate specificity of nucleoside analogs

The interaction with the Na^+-nucleoside cotransporter of several nucleoside analogs structurally modified on the base was investigated. A representative plot of thymidine uptake as a function of increasing drug concentration (range 0–100 μM) for each compound is shown in Fig. 3. These compounds were variable in their potencies, as indicated by their IC_{50} and K_i values for competitive inhibition (Table II). For each compound, the slope factor, n, did not differ significantly from 1.0 (Table II), consistent with a competitive interaction. We further investigated the nature of the interaction between 5-IdUrd (the most potent inhibitor of the three compounds) and thymidine by carrying out two Michaelis-Menten experiments. The K_m (mean ± S.E.) of thymidine was 27.4 ± 10.8 μM and was 54.7 ± 1.8 μM in the presence of 10 μM 5-IdUrd. The V_{max} was 5.21 ±3.6 pmol/mg protein per s and 7.19 ± 3.4 pmol/mg protein per s, in the absence and presence of inhibitor, respectively. These data, together with the inhibition data demonstrating a slope n, not significantly different from 1, support the notion of a competitive interaction between this analog and thymidine.

To determine whether 5-IdUrd was translocated by the Na^+-thymidine cotransport mechanism, a counterflux study using the two compounds was performed (Fig. 4). In these studies, the Na^+ concentration was the same across the vesicular membrane. The uptake of [^{3}H]thymidine at early time points (5, 15 and 30 s) was significantly enhanced (*trans*-stimulation) in the vesicles that had been preloaded with 5-IdUrd when

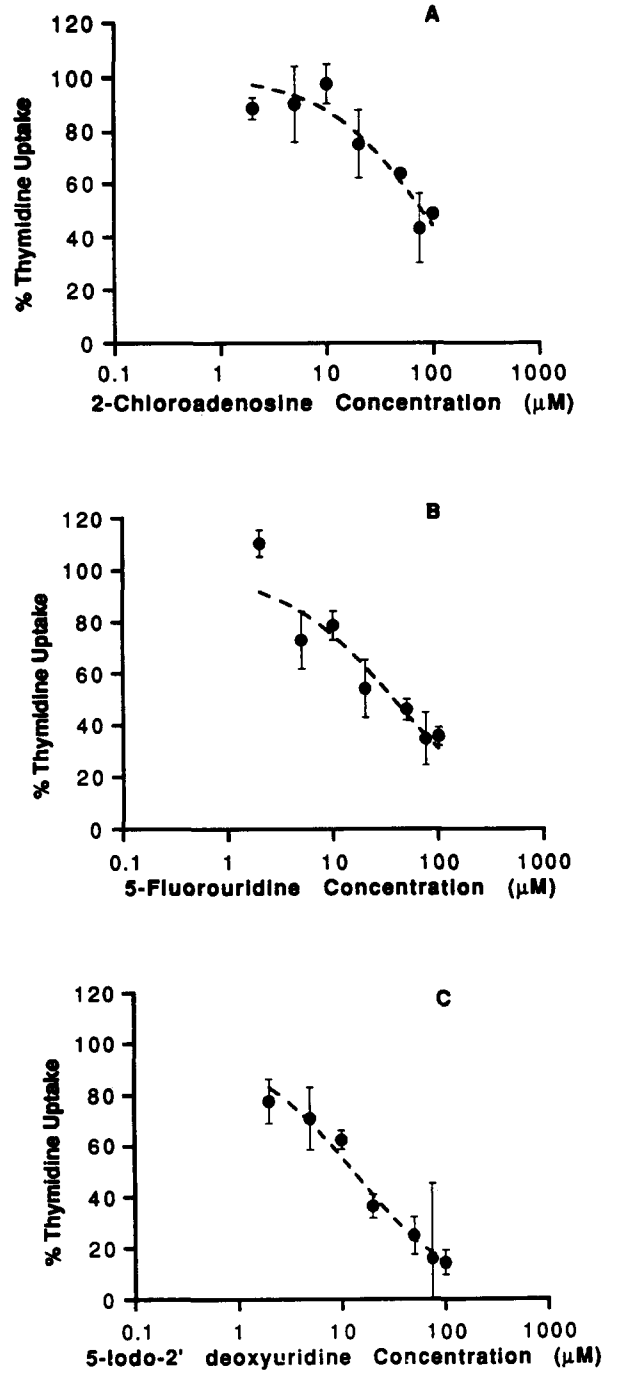

Fig. 3. The inhibition of thymidine uptake at 5 s by (A) 2-chloroadenosine, (B) 5-fluorouridine and (C) 5-iodo-2′-deoxyuridine in human renal BBMV. Data are from one representative experiment and are expressed as the percent (mean ± S.D.) of maximum thymidine uptake in the presence of Na^+. The curves were generated by computer fit as described in the text.

TABLE II

IC_{50} and K_i values of nucleoside drugs

Drug	IC_{50} (μM0	K_i (μM)
2-Chloroadenosine	75 ± 4	63 ± 4
5-Fluorouridine	49 ± 11	41 ± 10
5-Iodo-2′-deoxyuridine	16 ± 4	14 ± 3

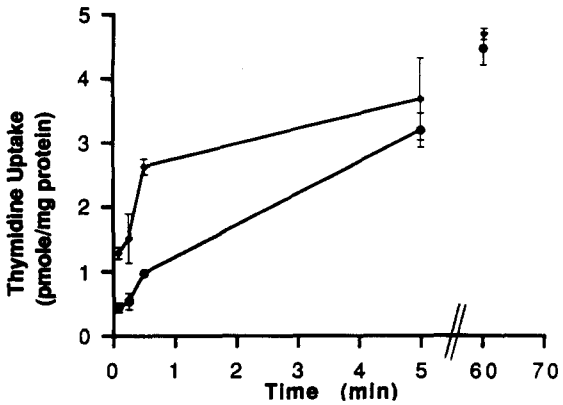

Fig. 4. The uptake of [^{3}H]thymidine (2 μM) in human renal BBMV in which Na^+ was equilibrated across the vesicular membrane ($[Na^+]_i = [Na^+]_o$). ●, Data obtained in unloaded vesicles and ◇, data obtained in vesicles initially incubated with 50 μM of 5-iodo-2′ deoxyuridine for 2 h. Data are from one representative experiment (mean ± S.D.) obtained from frozen human kidneys.

compared to the unloaded vesicles. These data suggest that the two compounds share the same transport mechanism.

Discussion

Secondarily-active Na^+-dependent nucleoside transport systems have been described in intestinal [30] and renal [12–19,21] BBMV, choroid plexus [31,32] and intestinal [20,33] epithelial cells, spleen cells [34–37], leukemia cells [37,38], hepatic cells [39] and macrophages and fibroblasts [37]. Recent studies have suggested that there are at least two major types of Na^+-nucleoside cotransport systems [18,20]. The systems, which both require Na^+ and have 1 : 1 Na^+/nucleoside-coupling ratios, differ in terms of their substrate specificities with N1 generally purine selective and N2 generally pyrimidine selective. Adenosine and uridine are transported by both systems. More recently, we presented evidence that in rabbit choroid plexus epithelium, there is a third type (N3) of Na^+-nucleoside cotransporter which has a 2:1 Na^+/nucleoside-coupling ratio and is more broadly selective [32]. Both purine and pyrimidine nucleosides appear to be transported by this system. Thus, it is becoming increasingly clear that there may be multiple types of Na^+-nucleoside cotransport systems that may exhibit inter-species, as well as inter-tissue differences in terms of their biological functions, kinetics and characteristics. To completely understand the importance of Na^+-dependent nucleoside transport systems, their mechanisms, molecular properties, distribution among cells and tissues and their contribution to nucleoside homeostasis need to be elucidated.

Recently, we presented the first evidence of a Na^+-dependent nucleoside transport system in the human renal brush-border membrane [21]. The system (N4) appears to be similar to the N2-pyrimidine-selective transport system, except that guanosine is also a substrate. (Previously, guanosine had been found to be transported exclusively by the purine selective transport systems [18,20]). The goal of this study was to further elucidate the mechanisms of nucleoside transport in the human kidney to aid in our understanding of the role of the human kidney in nucleoside homeostasis. A second goal was to characterize the interaction of clinically important nucleoside analogs with the cotransporter.

We directly examined the Na^+-nucleoside coupling stoichiometry by measuring [^{3}H]uridine transport into human renal BBMV as a function of Na^+ concentration. A hyperbolic relationship between Na^+-dependent uridine flux and Na^+ concentration conforming to a 1:1 coupling ratio was observed. This is consistent with the Na^+-uridine coupling stoichiometry observed in BBMV from rabbit [19], rat [13,14] and bovine [18] kidney and from rabbit intestine [30]. The stoichiometry differs from the ratio of 2:1 obtained for Na^+/nucleoside transport in ATP-depleted choroid plexus tissue slices from rabbit [32].

During one complete cycle of transport, the rate of net transport will be influenced by the electrical potential across the membrane if there is a net transfer of charge across the membrane [27]. Presumably, in the transport of Na^+ and thymidine, the transporter interacts with both substrates on the outside of the membrane, reorients across the brush-border membrane, and delivers both substrates into the cell. The transporter then reorients back to its original conformation before completing the cycle. Therefore, a net positive charge will be transferred into the cell after one cycle.

We directly examined the effects of both the Na^+ gradient and the membrane potential on Na^+-thymidine transport. The highest magnitude of overshoot was achieved when an inwardly-directed Na^+-gradient and a K^+ diffusion potential ($[K^+]_{in} > [K^+]_{out}$) were imposed (Fig. 1B). However, a Na^+ gradient alone was able to stimulate thymidine uptake above equilibrium. This is consistent with the results obtained in bovine renal BBMV [19]. Furthermore, a K^+-diffusion potential alone was able to support a slight, uphill transport of thymidine. Although the magnitude of overshoot was small, the uptake values were significant from those observed when no Na^+ gradient and no K^+-diffusion potential were imposed (results not shown). These data suggest that Na^+-thymidine transport in human renal BBMV is an electrogenic process.

The structural specificity of the human renal brush-border membrane Na^+-nucleoside cotransporter was further investigated by studying the uptake of [^{3}H]thymidine in the presence of varying concentra-

tions of nucleoside analogs. Previously, we reported that the drugs AZT, ddC and Ara-C, all structurally modified on the ribose ring did not interact with the human Na^+-nucleoside cotransporter at 100 μM concentrations [40]. In this study, we examined the effects of nucleoside analogs modified on the base. All three compounds studied were potent inhibitors of Na^+-thymidine transport (Fig. 3) and the inhibition appeared to be competitive in nature (Table II). The radiosensitizer, 5-IdUrd, was the most potent inhibitor with an IC_{50} value of 16.1 μM (Table II). The data demonstrating that 5-IdUrd *trans*-stimulates thymidine transport suggests that 5-IdUrd and thymidine are translocated by the same transporter (Fig. 4). In addition to counterflux, inhibition of efflux of the radiolabeled nucleoside by 5-IdUrd may contribute to the enhancement observed. However, since the enhancement was observed at very early time points, this contribution may not be very substantial.

The IC_{50} of the nucleoside analog, 5-IdUrd, in inhibiting Na^+-thymidine transport is in the range of the concentrations required to produce pharmacologic effects [41] whereas the IC_{50} of 5-FUrd is considerably greater than pharmacologic concentrations [42]. These data suggest that at therapeutic concentrations, these compounds would be expected to interact with the Na^+-nucleoside cotransporter in the human kidney. Therefore, this transporter, located on the renal brush-border membrane, may function physiologically in the reabsorption of nucleoside analogs. It is important to note that drug-drug interactions or drug-endogenous nucleoside interactions may occur at this transporter. Moreover, the transporter may intensify renal toxicities by mediating the accumulation of potentially nephrotoxic nucleosides and analogs in the proximal tubule cells. The interaction with this transporter may be an important consideration in the design of nucleoside analogs targeted to or away from the kidney.

In conclusion, the results suggest that the Na^+-driven transport of thymidine into human renal BBMV is an electrogenic process. The Na^+/nucleoside coupling ratio appears to be 1. In contrast to nucleoside analogs modified on the ribose ring, analogs modified on the base moiety interact quite potently with the transporter. Further studies are warranted to understand the role of the co-transporter in the biological disposition and targeting of these compounds to the kidney.

Acknowledgements

This work was supported by grants from the National Institutes of Health (GM 36780 and GM 42230). We would like to acknowledge the help of the Organ/Tissue Transplant Services at the University of California, San Francisco for the donation of human kidneys.

References

1 Estey, E.H., Kurzrock, R., Kantarjian, H.M., O'Brien, S.M., McCredie, K.B., Beran, M., Koller, C., Keating, M.J., Hirsch-Ginsberg, C., Huh, Y.O., Stass, S. and Freireich, E.J. (1992) Blood 79, 882–887.
2 Carson, D.A., Wasson, D.B., Kaye, J., Ullman, B., Martin, D.W., Robins, K.W. and Montgomery, J. (1980) Proc. Natl. Acad. Sci. USA 77, 6865–6869.
3 Yarchoan, R., Mitsutya, H., Myers, C.E. and Broder, S. (1989) New Eng. J. Med. 11, 726–738.
4 Belanger, K., Klecker, R.W., Rowland, J., Kinsella, T.J., and Collins, J.M. (1986) Cancer Res. 46, 6509–6512.
5 Goffman, T.E., Dachowski, L.J., Bobo, H., Oldfield, E.H., Steinberg, S.M., Cook, J., Mitchell, J.B., Katz, D., Smith, R. and Glatstein, E. (1992) J. Clin. Oncol. 10, 264–268.
6 Kinsella, T.J. and Glatstein, E. (1987) Cancer 59, 908–915.
7 Weckbecker, G. (1991) Pharmacol. Ther. 50, 367–424.
8 Grever, M.R., Siaw, M.F.E., Jacob, W.F., Neidhart, J.A., Miser, J.S., Coleman, M.S., Hutton, J.J. and Balcerzak, S.P. (1981) Blood 57, 406–417.
9 Bisel, H.F., Ansfield, F.J., Mason, J.H. and Wilson, W.L. (1970) Cancer Res. 30, 76–78.
10 Van Oosterom, A.T., Ten Bokkel Huinink, W.W., Van der Burg, M.E.L., Vermorken, J.B., Willemse, P.H.B. and Neijt, J.P. (1991) Eur. J. Cancer 27, 747–749.
11 Fox, I.H. and Kelley, W.N. (1978) Annu. Rev. Biochem. 47, 655–686.
12 Franco, R., Centelles, J.J. and Kinne, R.K. (1990) Biochim. Biophys. Acta 1024, 241–248.
13 Lee, C.W., Cheeseman, C.I. and Jarvis, S.M. (1990) Am. J. Physiol. 258, F1203–F1210.
14 Lee, C.W., Cheeseman, C.I. and Jarvis, S.M. (1988) Biochim. Biophys. Acta 942, 139–149.
15 Le Hir, M. (1990) Renal Physiol. Biochem. 13, 154–161.
16 Le Hir, M. and Dubach, U. (1985) Pflügers Arch. 404, 238–243.
17 Le Hir, M. and Dubach, U.C. (1985) Eur. J. Clin. Invest. 15, 121–127.
18 Williams, T.C. and Jarvis, S.M. (1991) Biochem. J. 274, 27–33.
19 Williams, T.C., Doherty, A.J., Griffith, D.A. and Jarvis, S.M. (1989) Biochem. J. 264, 223–231.
20 Vijayalakshmi, D. and Belt, J.A. (1988) J. Biol. Chem. 263, 19419–19423.
21 Gutierrez, M.M., Brett, C.M., Ott, R.J., Hui, A.C. and Giacomini, K.M. (1992), Biochim. Biophys. Acta 1105, 1–9.
22 Booth, A.G. and Kenny, A.J. (1974) Biochem J. 142, 575–581.
23 Hsyu, P.-H. and Giacomini, K.M. (1987) J. Biol. Chem. 262, 3964–3968.
24 Hsyu, P.-H., Gisclon, L.G. Hui, A.C. and Giacomini, K.M. (1988) Am. J. Physiol. 254, F56–F61.
25 Hsyu, P.-H. and Giacomini, K.M. (1987) Am. J. Physiol. 252, F1065–F1072.
26 Ott, R.J., Hui, A.C., Yuan, G. and Giacomini, K.M. (1991) Am. J. Physiol. 261, F433–F451.
27 Stein, W.D. (1990) in Channels, Carriers and Pumps, pp. 174–176, Academic Press, San Diego.
28 Turner, R.J. and Moran, A. (1982) J. Membr. Biol. 67, 73–80.
29 Segel, I.H. (1976) in Biochemical Calculations, pp. 309–312, Wiley, New York.
30 Jarvis, S.M. (1989) Biochim. Biophys. Acta 979, 132–138.
31 Spector, R. (1982) Arch. Biochem. Biophys. 216, 693–703.
32 Wu, X., Yuan, G., Brett, C.M., Hui, A.C. and Giacomini, K.M. (1992) J. Biol. Chem. 267, 8813–8818.
33 Schwenk, M., Hegazy, E. and Lopez des Pino, V. (1984) Biochim. Biophys. Acta 805, 370–374.
34 Darnowski, J.W., Holdridge, C. and Handschumacher, R.E. (1987) Cancer Res. 47, 2614–2619.

35 Plagemann, P.G.W. and Woffendin, C. (1989) Biochim. Biophys. Acta 981, 315–325.
36 Plagemann, P.G.W., Aran, J.M. and Woffendin, C. (1990) Biochim. Biophys. Acta 1022, 93–102.
37 Plagemann, P.G.W. and Aran, J.M. (1990) Biochim. Biophys. Acta 1025, 32–42.
38 Crawford, C.R., Ng, C.Y., Noel, L.D. and Belt, J.A. (1990) J. Biol. Chem. 265, 9732–9736.
39 Holstege, A., Gengenbacher, H.M., Jehle, L. and Hoppmann, J. (1991) Hepatology 14, 373–380.
40 Brett, C.M., Washington, C.M., Ott, R.J., Gutierrez, M.M. and Giacomini, K.M. (1993) Pharm. Res. 10, 423–426.
41 Fischer, P.H., Vazquez-Padua, M.A., Reznikoff, C.A. and Ratschan, W.J. (1986) Cancer Res. 46, 4522–4526.
42 Danenberg, K.D., Becker, D., Mulkins, M.A. and Danenberg, P.V. (1984) Pharm. Res. 3, 110–115.