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Substrate Specificity of Organic Cation/H^+ Exchange in Avian Renal Brush-Border Membranes

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ABSTRACT

The substrate specificity of the avian renal organic cation exchanger was examined in isolated renal brush-border membrane vesicles. Endobiotic and xenobiotic organic cations (OCs) were tested at a concentration of 100 μM for *cis*-inhibition of ^{14}C-tetraethylammonium (TEA)/H^+ exchange and at 1 mM for *trans*-stimulation of ^{14}C-TEA efflux. The xenobiotic cations amiloride, cimetidine, mepiperphenidol, procainamide, quinidine, quinine, and ranitidine *cis*-inhibited TEA uptake $\geq$ 80%; isoproterenol and unlabeled TEA inhibited uptake at least 30%. In contrast, the endogenous cations acetylcholine, choline, and guanidine did not inhibit TEA uptake; however, epinephrine, N^1-methylnicotinamide, serotonin, and thiamine inhibited uptake as much as 60%. Each endogenous cation, except thiamine, *trans*-stimulated TEA efflux, and xenobiotic cations, excluding isoproterenol and TEA, *trans*-inhibited TEA efflux. The data suggest that the avian renal tubule luminal OC exchanger has greater affinity for xenobiotic cations than for endobiotic cations, but greater transport capacity for endobiotics than for xenobiotics.

The role of the "cation or hydrogen exchange mechanism" in the renal excretion of xenobiotic OCs was first proposed by Baer and colleagues (Baer *et al*., 1956). This hypothesis is based on observations that induction of aciduria results in an increased excretion of xenobiotic cations such as mecamylamine, nicotine and quinine in humans and dogs (Baer *et al*., 1956; Haag and Larson, 1942; Haag *et al*., 1943). Later *in vitro* studies on luminal membrane vesicles isolated from renal cortical tissue and on isolated perfused renal proximal tubules directly demonstrated OC transport by a proton exchange mechanism (for reviews, see Pritchard and Miller, 1993; Roch-Ramel *et al*., 1992). Indeed, several xenobiotic OCs that have been shown to be secreted *in vivo* such has amiloride, cimetidine, and mepiperphenidol are also countertransported for protons in isolated renal cortical luminal membrane vesicles (BBMV) (Rafizadeh *et al*., 1986; Takano *et al*., 1985; Wright and Wunz, 1989). Furthermore, functional integration of luminal OC/H^+ exchange in net tubular secretion of xenobiotic OCs has been demonstrated in perfused proximal tubules. In that preparation, the transepithelial flux of procainamide and cell-to-lumen flux of tetraethylamonium (TEA) are stimulated by acidification of the tubule lumen (McKinney, 1984; Dantzler and Brokl, 1988). These findings suggest a role for secretory exchange of xenobiotic OCs for protons across the luminal membrane in renal tubular secretion *in vivo*.

Transport of OCs across the luminal membrane is of particular interest, as it is the rate-limiting step in the secretion of these compounds. Proton antiport is thought to be a primary means by which OCs are translocated across the luminal membrane. Although the existence of the luminal OC exchanger is well documented, the physiological role of this transporter in renal excretion of OCs, endobiotic OCs in particular, is not fully understood. Available data suggest that OC/H^+ exchange may not be the sole means by which endobiotic OCs are transported across the luminal membrane. As demonstrated in rabbit BBMV, choline is poorly transported by the OC/H^+ exchange mechanism, but is transported by electrogenic facilitated diffusion (Wright *et al*., 1992). The role of the luminal OC exchanger in secretion of NMN is also quesitonable. Although countertransport of NMN for protons and OCs has been demonstrated in BBMV of several mammalian species (Holohan and Ross, 1981; Griffiths *et al*., 1992; Ott *et al*., 1991; Wright, 1985), in isolated perfused proximal tubules of rabbit and snake, secretion of NMN is unaltered by changes in luminal concentrations of protons or OCs (Besseghir *et al*., 1990; Dantzler and Brokl, 1987). Studies on rabbit BBMV also suggest that guanidine shares a common proton exchange pathway with TEA; however, the kinetic parameters of guanidine/H^+ exchange indi-

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ABBREVIATIONS: OC, organic cation; TEA, tetraethylammonium; NMN, N^1-methylnicotinamide; Darstine, mepiperphenidol; BBMV, brush-border membrane vesicle; K_m, Michaelis constant; V_{max}, maximal rate of uptake; V_{maxapp}, apparent maximal rate of uptake; K_{mapp}, apparent K_m; K_i, calculated inhibitor constant; pK_a, dissociation constant; M.W., molecular weight; T_m, maximum tubular transport rate.

cate that it may be transported by multiple carriers within the luminal membrane (Miyamoto *et al*., 1989). Other than guanidine, NMN, and choline, few endogenous OCs have been tested for transport by the luminal OC exchanger.

The objective of the present study was to elucidiate the role of the renal luminal OC exchanger in the transport of endobiotic OCs. To this end, the substrate specificity of the luminal OC exchanger for endobiotic and xenobiotic compounds was evaluated using an *in vitro* experimental model for renal luminal OC transport, avian BBMV (Villalobos and Braun, 1995). Endobiotic and xenobiotic OCs known to undergo net tubular secretion by the intact avian and mammalian kidneys (table 1; Rennick, 1981, Roch-Ramel *et al*., 1993, Wideman, 1988) were tested against a model substrate for the luminal OC exchanger, ^{14}C-TEA, which is poorly transported by the luminal multidrug-resistance transporter. The criteria for classifying an OC as a transported substrate were: 1) *cis* inhibition and 2) *trans* stimulation of carrier-mediated transport of the model substrate by the test OC (Holohan and Ross, 1981; Wilbrandt and Rosenberg, 1961). Accordingly, each OC was tested for its ability to *cis* inhibit ^{14}C-TEA/H^+ exchange and *trans*-stimulate ^{14}C-TEA efflux.

Materials and Methods

Animals. Six to 8-month-old White Leghorn hens, *Gallus domesticus* (1.5–2 kg), reared at the University of Arizona farm were used for all experiments. Before use, birds were given free access to water and commercial mash.

Brush-border membrane vesicle isolation. Kidneys were perfused *in situ* with ice-chilled homogenization buffer (50 mM mannitol, 20 mM Tris-HEPES, pH 7.4, excised, minced and weighed to the nearest 0.1 g. Kidney tissue from a single animal (8–20 g) was used for each BBMV preparation. Brush-border membranes were isolated from a crude renal homogenate by Ca^{++} precipitation and subsequent serial differential centrifugation as described previously (Villalobos and Braun, 1995). The final membrane fraction was suspended and preequilibrated in vesicle buffer containing 100 mM KCl, 200 mM mannitol and 10 mM HEPES-KOH (pH 6.0 or 7.5) for 60 min at 4°C. Any modifications of the vesicle buffer are listed in the figure legends. Protein content of the final membrane fraction was determined using the BioRad protein assay and bovine plasma γ-globulin as a standard. The mean protein yield was 0.35 ± 0.05% of the total protein in the initial homogenate. In the final membrane fraction, the specific activity of the brush-border membrane enzyme alkaline phosphatase was enriched ~18-fold over that in the initial homogenate; the specific activities of the basolateral and mitochondrial enzymes, Na^+-K^+,ATPase and succinate dehydrogenase, were not significantly greater than those in the initial homogenate.

Transport assay. Uptake of ^{14}C-TEA by BBMV was assayed by rapid filtration at room temperature (23–25°C) as outlined previously in detail (Villalobos and Braun, 1995). Vesicles were preincubated at room temperature for 20 min before the transport assay. In a 12 × 75 mm tube, uptake was initiated with the addition of 10 μl of vesicles to 90 μl of transport buffer containing 65 μM ^{14}C-TEA. Transport buffer also contained 100 mM KCl, 195–200 mM mannitol, 10 mM HEPES-KOH (pH 7.5); concentrations of test compounds in the transport buffer are listed in the tables and figure legends. Uptake was terminated with the addition of 1 ml of ice-chilled "stop" buffer (200 mM KCl, 2 mM $CaCl_2$, 0.1 mM $HgCl_2$ and 20 mM Tris-HEPES, pH 7.8). Under vacuum, 1 ml of this mixture was collected on a prewetted nitrocellulose filter (Millipore, HAWP 024, 0.45 μm pore size) that was then washed with 4 ml of ice-cold stop buffer. The filter was placed in a scintillation vial with 10 ml of EcoLite (ICN Biomedicals, Inc.), and the radioactivity was determined by a liquid scintillation system (Beckman LS 5801 spectrometer). Correction was made for nonspecific binding of isotope to the filter at each time point. The mean protein content per 10 μl vesicles was 50.1 ± 1.6 μg. Uptake was expressed as picomoles ^{14}C-TEA per mg vesicle protein. Unless otherwise stated, uptake was assayed at each time point in triplicate in at least three separate vesicle preparations (*i.e*., $n = 3$).

Efflux of TEA from BBMV was assayed by rapid filtration following a protocol similar to that for uptake with two major modifications. First, vesicles were preequilibrated with vesicle buffer (pH 7.5) that contained 150 μM ^{14}C-TEA for 60 min at 4°C, and efflux of ^{14}C-TEA was initiated with the dilution of 10 μl of BBMV with 90 μl of isotope-free transport buffer (pH 7.5) that contained 0 or 1 mM of test compound. Second, vesicular content of ^{14}C-TEA was expressed as a percent of total isotope contained within BBMV before the initiation of the efflux reaction. The initial vesicular ^{14}C-TEA content was assayed by diluting 10 μl of BBMV with a mixture of 1 ml ice-cold KCl stop buffer and 90 μl transport buffer. One milliliter of this suspension was collected on a filter, and following a wash with additional cold stop buffer, the radioactivity was determined by liquid scinitillation countings.

Chemicals. ^{14}C-Tetraethylammonium bromide (56 mCi/mmol) was purchased from Wizard Labs (West Sacramento, CA). Mepiper-

TABLE 1

Organic cations tested for substrate specificity in avian renal BBMV

Names, abbreviations or common name, dissociation constants (pK_a) and molecular weights (M.W.) are listed. Representative citations for net tubular secretion are given in parentheses.

	pK_a	M.W.	
Endobiotic compounds			
Acetylcholine (ACh)	13	146.2	Acara and Rennick (1972)
Choline	13.9	104.2	Acara and Rennick (1973)
Epinephrine	9.95	183.2	Rennick and Yoss (1962)
Guanidine	13.6	59.1	Sperber (1948)
N^1-Methylnicotinamide (NMN)	13	137.2	Sperber (1948)
Serotonin	11.1	176.2	Sanner and Wortman (1962)
Thiamine	9.2	301.8	Rennick *et al.* (1984)
Xenobiotic compounds			
Amiloride	8.7	229.7	Rennick *et al.* (1984)
Cimetidine	7.1	252.3	Rennick *et al.* (1984)
Isoproterenol	10.4	211.2	Lifshitz *et al.* (1973)
Mepiperphenidol (Darstine)	>13	370.4	Pilkington and Keyl (1963)
Procainamide	9.2	236.4	Christian *et al.* (1984)
Quinidine	8.8	324.4	Gerhardt *et al.* (1969)
Quinine	8.5	324.4	Haag *et al.* (1943)
Ranitidine	8.2	314.4	McNeil *et al.* (1981)
Tetraethylammonium	13	130.3	Rennick *et al.* (1984)

phenidol (Darstine) was contributed by Merck Sharp and Dome Laboratories (Rahway, NJ). All other chemicals were of the highest purity and obtained from standard sources.

Statistical analysis. In experiments in which *cis*-inhibition of ^{14}C-TEA uptake by OCs was tested, uptake was expressed as the absolute value of vesicular isotope content (pmol ^{14}C-TEA·mg vesicle protein^{-1}), and data were compared by analysis of variance (ANOVA). During the testing of *trans*-stimulation of ^{14}C-TEA efflux by OCs, it was determined that the absolute values for initial vesicular ^{14}C-TEA content as assayed in the presence of inwardly directed gradients of several test compounds (*e.g.*, amiloride, quinidine) were consistently greater than control values (*i.e.*, no test compound). Therefore, control and experimental values for vesicular ^{14}C-TEA content were converted to a percent of the initial ^{14}C-TEA content as assayed in the absence and presence of a given test compound, respectively, *i.e.* the fraction of ^{14}C-TEA retained. The percent of ^{14}C-TEA retained at 5 sec and 15 sec in the presence of each test compound was statistically compared to corresponding control values by three-way analysis of variance. Differences were deemed significant when the probability values were less than .05.

Results

***Cis*-inhibition of TEA/H$^+$ exchange by OCs.** Transmembranal transport of a model OC, TEA in these studies, by the renal OC exchanger requires that the cation bind to the substrate site on the carrier. Thus, binding of TEA to the exchanger and its subsequent transport would be inhibited by a second OC present at the same face of the membrane. Impedence of transport of a model substrate by a second substrate in this manner, referred to as *cis* inhibition, is an index of carrier specificity or affinity (Holohan and Ross, 1981; Wilbrant and Rosenberg, 1961). Based on this principle, the affinity of the luminal OC exchanger for several test OCs was assessed. Five-second uptake of 65 μM ^{14}C-TEA was assayed in the presence of an outwardly-directed proton gradient (pH_{in} 6.0: pH_{out} 7.5) and 100 μM of each endogenous and xenobiotic OCs, including unlabeled TEA, was assayed (fig. 1). Unlabeled TEA inhibited ^{14}C-TEA uptake by 20%. The endogenous cations, ACh, choline and guanidine each failed to inhibit TEA uptake ($P < .08$; fig. 1A). However, thiamine and serotonin moderately inhibited TEA transport, decreasing uptake by ~60% and ~45%. Epinephrine and NMN were less effective, reducing uptake by ~20%. In contrast, xenobiotic cations were potent inhibitors of TEA/H$^+$ exchange (fig. 1B). Amiloride and quinidine each inhibited ^{14}C-TEA uptake at least 90%; however, mepiperphenidol (Darstine) inhibited uptake ~60%, and isoproterenol decreased uptake only 30%. Equilibrium vesicular content of ^{14}C-TEA (2 hr; no inhibitor, 59.0 ± SE 1.1 pmol·mg protein^{-1}) was not altered by any test compound (*e.g.*, ranitidine, 62.5 ± SE 2.4 pmol·mg protein^{-1}·2 hr^{-1}). Excluding the endogenous OCs ACh, choline, and guanidine, the *cis* inhibitory potency of the OCs tested at 100 μM decreased in the order: quinidine > amiloride > quinine > cimetidine ≈ ranitidine > Darstine > thiamine > procainamide > serotonin > isoproterenol > TEA ≈ epinephrine ≈ NMN.

In preliminary studies, the effects of several test OCs on the kinetics of proton-driven TEA uptake were examined; each OC was tested in two separate vesicle preparations (n = 2). In the presence of serotonin (150 μM), the apparent K_m for TEA increased (*e.g.*, 260 *vs.* 545 μM); however, V_{max} remained constant. Thiamine (100 μM) also induced increases in apparent K_m for TEA (*e.g.*, 505 μM *vs.* 973 μM) without markedly changing V_{max}. These preliminary findings suggested that these endobitotics may competitively inhibit OC/H$^+$ exchange. Preliminary analyses were also conducted to evaluate inhibitor-induced changes in the kinetic parameters of TEA/H$^+$ exchange by amiloride, procainamide and quinidine. Amiloride (15 μM) decreased V_{max} for TEA (*e.g.*, 12 *vs.* 4 nmol·mg protein^{-1}·sec^{-1}); however, apparent K_m remained relatively constant (*e.g.*, 285 *vs.* 222 μM). Quinidine (5 μM) induced decreases in both apparent K_m and V_{max} (*e.g.*, 357 *vs.* 192 μM; 15 *vs.* 3 nmol·mg protein^{-1}·sec^{-1}). The effects of procainamide (75 μM) were not consistent between trials. In one trial, procainamide failed to markedly alter apparent K_m (213 *vs.* 265 μM) while decreasing V_{max} (12 *vs.* 4 nmol·mg protein^{-1}·sec^{-1}); in a second trial apparent K_m increased ~2-fold, while V_{max} was not altered. These preliminary findings suggested that inhibition by these three xenobiotic OCs may possibly involve binding to allosteric sites of the OC exchanger.

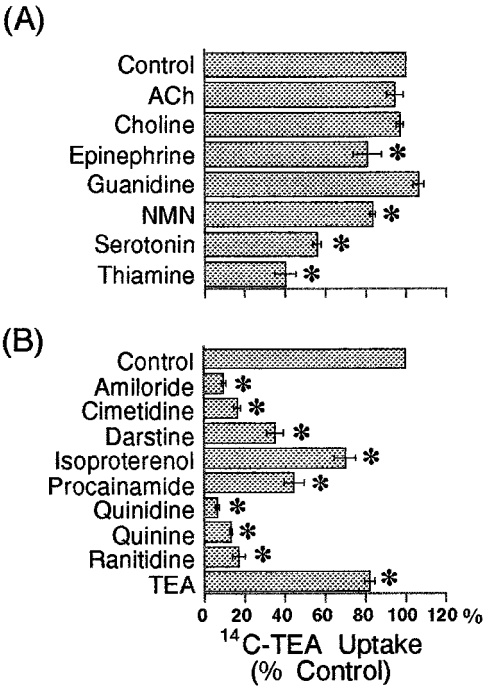

Fig. 1. *Cis* effects of 100 μM of (A) endogenous and (B) xenobiotic OCs on proton-driven TEA uptake by avian renal BBMV. Vesicles were pre-equilibrated in vesicle buffer (pH 6.0) for 60 min at 4°C. At 25°C, 5 sec uptake of ^{14}C-TEA was assayed by diluting 10 μl BBMV with 90 μl isosmotic transport buffer (pH 7.5) with 65 μM ^{14}C-TEA and 0 or 111.11 μM of a test OC. Vesicle and transport buffers each contained 100 mM KCl, 200 mM mannitol and 10 mM HEPES-KOH. Each OC was tested in triplicate in three separate BBMV preparations (*i.e.*, n = 3). Data are displayed as a percent of uptake in the absence of inhibitor (Control; mean ± SE); however, absolute values of ^{14}C-TEA uptake (pmol/mg protein-5 sec) were statistically analyzed by ANOVA. For Control *vs.* test OC: *$P < .05$. Control ^{14}C-TEA uptake values at 5 sec and 2 hr were 172.7 ± SE 5.7 and 59.0 ± SE 1.1 pmol/mg protein. Acetylcholine, ACh; mepiperphenidol, Darstine; N^1-methylnicotinamide, NMN.

***Trans*-stimulation of TEA efflux by organic cations.** Translocation of ions by an antiport or exchange mechanism involves physical coupling of the flux of one substrate to the counterdirected flux of a second. As demonstrated in this avian renal BBMV system, the OC exchanger mediates the exchange of TEA for either protons or OCs, TEA and NMN (Villalobos and Braun, 1995). Therefore, if a test OC were indeed a substrate for the OC exchanger, the mediated flux of

TEA should be stimulated in the presence of a counterdirected transmembrane gradient of the test OC. Augmentation of mediated transport of a model substrate by a second substrate in this manner, or *trans*-stimulation, is an index of the carrier's transport capacity. Therefore, effectiveness of test OCs to *trans*-stimulate TEA efflux from BBMV in the absence of a pH gradient ($pH_{in} = pH_{out} = 7.5$) was examined. Vesicles were preloaded with 150 μM ^{14}C-TEA, and then incubated with 0 or 1 mM of each test OC for 0, 5 and 15 sec (figs. 2 and 3). Under control conditions, vesicular ^{14}C-TEA content decreased over time, such that 71.5% ± SE 5.2% was retained at 5 sec, and 54.8% ± SE 4.2% at 15 sec. External 1 mM unlabeled TEA produced further loss of isotope; roughly 45% and 40% of the initial ^{14}C-TEA was retained at corresponding time points (fig. 3A). As a group, the endogenous OCs moderately stimulated time-dependent ^{14}C-TEA efflux (fig. 2A). However, external thiamine failed to stimulate TEA efflux (*i.e.* the percent of ^{14}C-TEA retained in BBMV was not significantly different than control; $P < .07$, fig. 2A). Although external ACh, choline and guanidine failed to inhibit TEA uptake, each stimulated TEA efflux, decreasing vesicular ^{14}C-TEA content through 15 sec by an additional 10% below control (*e.g.*, choline, 5-sec vesicular ^{14}C-TEA retention, 55.4% ± SE 5.4%; fig. 2B). External epinephrine, NMN, and serotonin also stimulated TEA efflux, as indicated by the ~15–10% decrease in vesicular isotope content with time (fig. 2B). Other than unlabeled TEA, isoproterenol was the only test xenobiotic OC to significantly *trans*-stimulate ^{14}C-TEA efflux, and like TEA, decreased 5 and 15 sec vesicular ^{14}C-TEA content ~20% and ~10% below control (fig. 3A). The remaining xenobiotics *trans*-inhibited, rather than *trans*-stimulated TEA efflux such that the percent of ^{14}C-TEA retained within BBMV was significantly greater than control. For example, in the presence of procainamide, 95.3% ± SE 5.3% of the initial vesicular TEA was retained at 5 sec (fig. 3B).

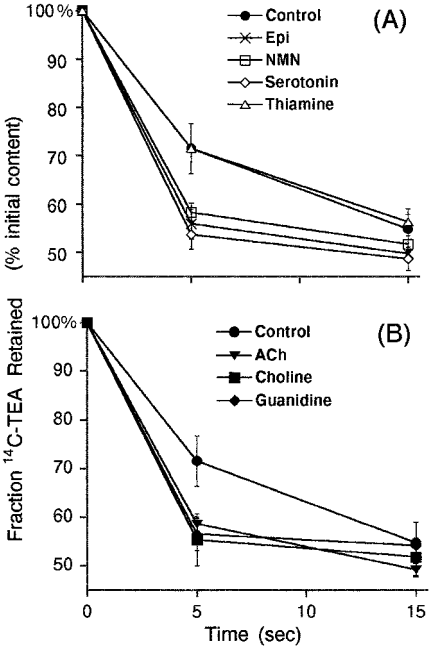

Fig. 2. *Trans* effects of 1 mM endogenous OCs on time-dependent efflux of TEA from avian renal BBMV. Following preequilibration with vesicle buffer (pH 7.5) containing 150 μM ^{14}C-TEA for 60 min at 4°C, 10 μl BBMV were diluted with 90 μl transport buffer (pH 7.5) containing 0 or 1.11 mM of a test OC. A, *Trans* effects of 1 mM epinepherine (Epi), N^1-methylnicotinamide (NMN), serotonin and thiamine. B, *Trans* effects of 1 mM acetylchoine (ACh), choline and guanidine. Each OC was tested in triplicate in three separate BBMV preparations ($n = 3$). Retention of ^{14}C-TEA by BBMV at 5 and 15 sec was expressed as a percent of the initial isotope content; data were analyzed by a three-way ANOVA. With the exception of thiamine, each test endogenous OC significantly decreased the fraction of ^{14}C-TEA retained below control values (*i.e.*, *trans*-stimulated efflux; $P < .05$).

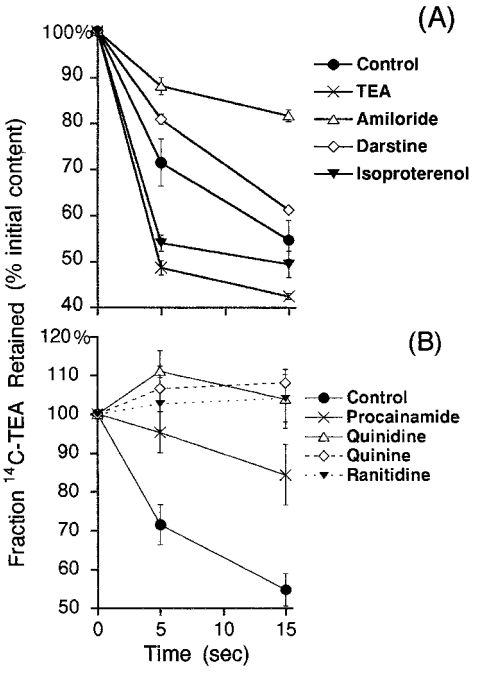

Fig. 3. *Trans* effects of 1 mM xenobiotic OCs on time-dependent efflux of TEA from avian renal BBMV. A, *Trans* effects of 1 mM TEA, amiloride, mepiperphenidol (Darstine) and isoproterenol. B, *Trans* effects of 1 mM procainamide, quinidine, quinine and ranitidine. Each OC was tested in triplicate in three separate BBMV preparations ($n = 3$). The assay for efflux of ^{14}C-TEA and statistical analysis of data were conducted as described for figure 2. Unlabeled TEA and isoproterenol significantly decreased the fraction of ^{14}C-TEA retained at 5 and 15 sec below control values (*i.e.,* time-dependent efflux was *trans*-stimulated; $P < .05$). However, for each of the remaining test xenobiotic OCs, the fraction of ^{14}C-TEA retained was significantly greater than control values (*i.e.,* time-dependent efflux was *trans*-inhibited; $P < .05$).

Stimulation of ^{14}C-TEA efflux in the presence of inwardly directed gradients of OCs may have been secondary to the generation of either an opposing negative diffusion potential by influx of cations or an inwardly directed H^+ gradient *via* OC/H^+ exchange. *Trans* effects of OCs on ^{14}C-TEA efflux under voltage-clamped conditions or in the presence of a proton ionophore (*e.g.*, FCCP) were not examined directly. However, in separate experiments, it was determined that an opposing negative membrane potential did not stimulate the efflux or initial uptake of ^{14}C-TEA in the absence of an opposing H^+ gradient (data not shown). Moreover, because of their size and polarity, these OCs are not likely to diffuse across the lipid bilayer at a rate sufficient to generate a membrane potential for the 15 sec duration of the transport reaction. At equilibrium (2 hr), neither absolute vesicular ^{14}C-TEA content (37.9 ± SE 2.3 pmol/mg protein) or percent of initial ^{14}C-TEA retained by BBM (31.2% ± SE 2.7%) was altered in the presence of external test OCs. This indicated alterations, if any, in either membrane permeability or membrane binding of ^{14}C-TEA were relatively uniform in the presence of the OCs tested.

Discussion

Extensive data from *in vitro* and *in vivo* experimental systems implicate a pH-dependent carrier system, presumably the luminal OC exchanger, in the renal secretion of many xenobiotic cations (*e.g.*, Baer *et al.*, 1956; Dantzler and Brokl, 1988). Although many vital endobiotic OCs, such as serotonin, ACh and thiamine, are also secreted by the renal tubules *in vivo* (Rennick *et al.*, 1984; Roch-Ramel *et al.*, 1992), few have been tested for transport efficacy by the renal luminal OC exchanger. Substrate specificity of the exchanger for these and other endobiotics was evaluated in avian luminal membrane vesicles (BBMV). Several endobiotic and xenobiotic OCs known to undergo net tubular secretion by the intact kidney (table 1) were tested for *cis* and *trans* effects on the transport of a model xenobiotic substrate, TEA. Although the data on *cis* inhibition of OC transport indicate that the relative affinity of the renal luminal OC exchanger was greater for xenobiotics (fig. 1), the data on *trans* stimulation indicate that xenobiotics were transported with lower relative transport capacity than were endobiotics (figs. 2 and 3).

Luminal OC exchanger's substrate affinity. A broad diversity in structural, physical, physiological, and pharmacological properties exists among the OCs secreted by the kidney. OCs that are known to undergeo net tubular secretion are all primary, secondary or tertiary amines or quarternary ammoniums that carry a positive charge on the amine nitrogen at physiological pH (Pritchard and Miller, 1993; Rennick, 1981; Roch-Ramel *et al.*, 1992). Previous investigators have used several homologous series of structural analogs to systematically quantify relationships of the affinty of the luminal OC exchanger to the chemical and physical properties of OCs based on inhibitory potency of test compounds. In isolated membranes, Wright *et al.* (1995) observed positive correlations of carrier affinity with the relative lipophilicity and the length of alkyl chain of R-groups attached to the parent structure and a negative correlation with the presence of hydrophilic moities for a series of structurally related quaternary ammonium compounds. Using an *in vivo* renal microperfusion system, David *et al.* (1995) tested several series of structurally related amines and quarternary ammoniums and observed positive correlations of carrier affinity with the substrate's molecular size, hydrophobicity and basicity, *i.e.*, pK_a values. Nevertheless, these correlations are general, considering the diversity of structural and chemical properties among OCs that inhibit or are transported by the OC exchanger. For the heterogenous series of OCs tested in the present study, a general relationship of affinity to molecular size was observed; larger compounds, such as amiloride and quinine, were more potent inhibitors of OC transport than were smaller compounds, such as NMN and guanidine (table 1, fig. 1). However, contrary to the previously observed correlation of affinity and pK_a values, compounds with lower pK_a values, such as amiloride and cimetidine, were more potent than compounds with pK_a values as great as 13, such as ACh and serotonin (table 1, fig. 1).

Rennick and colleagues demonstrated that the renal OC secretory pathway had a greater affinity for xenobiotics than endobiotics (Acara and Rennick, 1972; Besseghir and Rennick, 1981; Rennick *et al.*, 1977; Rennick *et al.*, 1984). Direct determination of a K_m for model substrates or assessment of *cis*-inhibition of OC exchange have demonstrated that xenobiotics, including those tested in the present study, are high-affinity substrates of the mammalian OC exchanger (table 2). In contrast, as determined by kinetic analyses in isolated renal BBMV and *in vivo* renal microperfusion, the affinity of the OC exchanger for endobiotic OCs is relatively low (table 2; David *et al.*, 1995). Minimal *cis*-inhibition of proton-driven TEA uptake by endobiotics indicates that the avian renal luminal exchanger also has preferential affinity for xenobiotic OCs (fig. 1). Exceptions to this generalization were observed. The inhibitory potency of thiamine was comparable to that of Darstine and procainamide, and the potency of epinephrine, NMN, or serotonin was equal or greater than that of isoproterenol. Previously, thiamine, as well as serotonin, were shown to *cis* inhibit proton-driven OC transport in mammalian renal BBMV (Sokol *et al.*, 1987; Takano *et al.*, 1993). Thus, thiamine inhibition of renal OC excretion observed *in vivo* may involve impedence of transport across the luminal membrane of the proximal tubule (Acara and Rennick, 1976; Rennick, 1958; Rennick *et al.*, 1984). Interestingly, several endobiotic and xenobiotic OCs tested in avian renal BBMV were shown to decrease renal OC clearance in the bird; moreover, similar orders of inhibitory potency were observed: cimetidine > raniditine > thiamine > procainamide > guanidine ≈ choline (fig. 1; Rennick *et al.*, 1984).

Transport of OCs by the luminal OC exchanger. In several *in vivo* and *in vitro* OC transport systems, the affinity of the luminal OC exchanger has been assessed based on relative *cis*-inhibitory potency of test compounds or inhibitor-induced changes in the K_m for a model substrate (David *et al.*,

TABLE 2
Kinetic parameters of organic cation transport in rabbit renal brush-border membrane vesicles

Organic Cation	K_m	V_{max}	
	μM	$nmol/mg/min$	
Amiloride	7.5	5	Wright and Wunz (1989)
Choline	10,000	38	Wright *et al.* (1992)
Cimetidine	4.6	0.4	Gisclon *et al.* (1987)
Guanidine	280	1.7	Miyamoto *et al.* (1989)
	3400	11.2	
MPP+	7.8	3.3	Lazaruk and Wright (1990)
NMN	630	5.1	Wright (1985)
	2620	7.0	Ott *et al.* (1991)
Procainamide	540	2.8	McKinney and Kunneman (1985)
Quinidine	2.5[a]		Ott *et al.* (1991)
Quinine	2.4[a]		Ott *et al.* (1991)
TEA	490	2.7	Miyamoto *et al.* (1989)

[a] Apparent K_i.

1995; Wright *et al*., 1995; fig. 1). However, the essence of an exchange or antiport mechanism is the physical coupling of the transmembrane flux of one substrate to the opposing flux of a second. Therefore, the relative transport capacity of the OC exchanger for a test substrate cannot be evaluated by *cis*-inhibitory interactions alone (Holohan and Ross, 1981; Wilbrandt and Rosenberg, 1961). Reorientation or turnover of the carrier is the rate-limiting event in mediated OC exchange. In theory, translocation of a model substrate should be stimulated in the presence of an opposing gradient of a second transported substrate. Thus, an OC transported by the luminal OC exchanger should not only *cis*-inhibit, but also *trans*-stimulate transport of the model substrate. The limited capacity of xenobiotics to *trans*-stimulate TEA transport indicated that exchanger's apparent transport capacity for these substrates is relatively low (fig. 3), despite the high affinity for such substrates (fig. 1B). Conversely, *trans*-stimlation of TEA transport by endobiotics indicated that they were transported with moderate transport capacity (fig. 2), despite modest affinity for these agents (fig. 1A). There were two exceptions. First, isoproterenol, a modest inhibitor of TEA uptake (fig. 1B), was the only xenobiotic to *trans*-stimulate TEA efflux, indicating it was transported by the OC exchanger (fig. 3A). Second, thiamine, a moderate inhibitor of TEA uptake (fig. 1), was the only endobiotic that did not *trans* stimulate TEA efflux, indicating it was a poorly transported substrate (fig. 2A). More specifically, for this series of OCs the ability to *trans*-stimulate OC transport was inversely related to the *cis*-inhibitory potency of a given OC. At the concentrations tested, OCs which *cis*-inhibited proton-driven TEA uptake by 40% or less (*i.e*., ACh, choline, epinephrine, isoproterenol, guanidine, NMN, serotonin, and unlabeled TEA) *trans*-stimulated TEA efflux. Conversely, OCs which *cis*-inhibited TEA uptake by 55% or more either failed to alter TEA efflux (*i.e*., thiamine) or *trans*-inhibited efflux (*i.e*., amiloride, cimetidine, mepiperphenidol, procainamide, quinidine, quinine, and ranitidine).

The transport capacity of the luminal OC exchanger may differ among species. Amiloride, procainamide and cimetidine, xenobiotics poorly transported by the avian OC exchanger in the absence of a H^+ gradient, are transported by OC/H^+ exchange in rabbit renal BBMV (table 2). Also contrary to the present observations, Darstine was shown to *trans*-stimulate OC uptake in dog and rabbit BBMV (Holohan and Ross, 1980; Rafizadeh *et al*., 1987). Nevertheless, the inverse relationship between substrate affinity and apparent transport capacity of the avian luminal OC exchanger observed *in vitro* was consistent with data on OC transport in BBMV from mammalian species, as well as the intact bird kidney. The reported kinetic parameters for OC transport in rabbit renal BBMV indicate an apparent inverse relationship between the exchanger's affinity for an OC and the maximal turnover rate of the OC-exchanger complex (table 2). For instance, whereas the K_m and V_{max} for amiloride are 7.5 μM and 3 nmol·mg^{-1}·min^{-1}, those for choline are 10 mM and 38 nmol·mg^{-1}·min^{-1}. The exception in the reported data is procainamide for which the OC/H^+ exchanger has a moderate affinity (K_m = 540 μM), but a transport capacity comparable to that for amiloride. The affinity of the avian OC exchanger for procainamide was also moderate (fig. 1B), and the apparent turnover rate in the presence of procainamide was low (fig. 3B). Similar trends are observed *in vivo*. Quinine, amiloride, and cimetidine, potent xenobiotic inhibitors of renal OC secretion, are secreted at lower rates by the kidney *in vivo*, as indicated by the reported maximum tubular transport rates (T_ms) in birds. Conversely, TEA, NMN, and choline, moderate inhibitors of renal OC secretion (Springate *et al*., 1987), are transported by the intact avian renal tubule at much higher rates (table 3).

Xenobiotics, such as amiloride, MPP^+, and quinine, that are transported at low rates by the mammalian luminal OC exchanger and compete for the OC binding site on the carrier, are known to *trans*-inhibit OC transport (Lazaruk and Wright, 1990; Rafizadeh *et al*., 1986; Sokol *et al*., 1987; Wright and Wunz, 1987). Likewise, several xenobiotics *trans*-inhibited TEA efflux from avian renal BBMV (fig. 3). The relatively low transport capacity for these and other xenobiotic substrates may be a direct, but adverse consequence of the high-affinity binding of the substrate to the exchanger. Kinetic analyses conducted on rabbit renal BBMV determined that amiloride and quinidine were competitive inhibitors of OC/H^+ exchange (Wright and Wunz, 1987; Ott *et al*., 1991). Preliminary kinetic analysis suggested that the inhibitory potency and low transport efficacy of amiloride, procainamide and quinidine may possibly involve allosteric interactions; however, based on these preliminary data, it cannot be said whether these xenobiotic OCs bound exclusively to allosteric sites on the avian OC exchanger. In practical terms, *trans*-inhibition of OC transport by high-affinity substrates most likely reflects the effective immobilization or physical impedence of the OC exchanger. Strong binding of an OC to the substrate site or an allosteric site on the exchanger could have several possible consequences: 1) modification of the affinity of the substrate site for the counter OC, 2) restriction of conformational changes in substrate-carrier complex necessary for an adequate rate of carrier reorientation from one side of the membrane to the other, or 3) reduction in the dissociation rate of the substrate-exchanger complex (Krupka, 1989). Hypothetically, efflux of substrate across the luminal membrane is the rate-limiting step in net transepithelial OC secretion by the proximal tubule. Thus, the low transport capacity of the luminal OC exchanger for high-affinity xenobiotics, such as amiloride and quinine, may explain in part the low renal clearance of these agents.

Based on the data on *trans*-stimulation, it was concluded that several endobiotic OCs were transported substrates of the renal luminal OC exchanger. Serotonin, epinephrine, isoproterenol, and NMN were the only test OCs to both *cis*-inhibit and *trans*-stimulate TEA transport (figs. 1–3). However, low-affinity substrates, ACh, choline, and guanidine, also *trans*-stimulated TEA efflux, indicating that these could nevertheless be transported by the luminal OC ex-

TABLE 3
Maximum tubular transport rate (T_m) values for some organic cations that undergo net tubular secretion by the chicken

Organic cation	T_m	
	μmol/kg/min	
Amiloride	0.012	Basseghir and Rennick (1981)
Choline	1.2	Acara and Rennick (1972)
Cimetidine	0.065	Springate *et al.* (1987)
NMN	2.5	Sperber (1948)
Quinine	Neglible	Volle *et al.* (1960)
TEA	0.5	Rennick *et al.* (1977)

changer (fig. 2B). The observation that endobiotic OCs were transported with greater apparent transport capacity by the OC exchanger than were xenobiotics suggests that the renal luminal OC exchanger could facilitate renal tubular secretion of these OCs observed experimentally. However, previous reports on renal transport of choline, NMN and guanidine indicate that the luminal exchanger may not be the predominant carrier that mediates transport of these OCs across the intact membrane. At supraphysiological plasma concentrations (>100 μM) choline undergoes net tubular secretion, sharing a common secretory pathway with TEA. However, at physiological levels (2–10 μM) it undergoes net tubular reabsorption (Acara and Rennick, 1973; Rennick *et al*., 1977). Wright and colleagues determined that choline is a low affinity substrate for the luminal OC exchanger (K_m for choline is ~10 mM *vs*. ~100 μM for TEA; Lazaruk and Wright 1990; Wright *et al*., 1992). Furthermore, they demonstrated that a high affinity electrogenic carrier (K_m = 100 μM), rather than a proton-driven exchanger, is likely the chief carrier mediating choline uptake across the luminal membrane of the proximal tubule (Wright *et al*., 1992). In the case of NMN, although the OC is transported by proton-driven exchange in BBMV from dog and rabbit (*e.g*., Holohan and Ross, 1981; Wright, 1985), an analogous mechanism apparently contributes little to transepithelial NMN secretion which remains relatively constant despite induction of aciduria *in vivo* or acidification of the tubular lumen *in vitro* (Besseghir *et al*., 1990; Dantzler and Brokl, 1987; Farah and Frazer, 1961). In contrast to the present findings, guanidine failed to *trans*-stimulate TEA transport in human renal BBMV (Ott *et al*., 1991). Furthermore, kinetic analyses and inhibition studies on rabbit renal BBMV indicated that although guanidine shares a common proton-exchanger with TEA, it may also be transported by another separate luminal carrier (Miyamoto *et al*., 1989). In speculating the significance of the luminal OC exchanger in renal transport of endogenous OCs, the descrepancy between carrier affinity and physiological plasma concentrations of substrates must also be considered. For example, the reported apparent K_m for serotonin transport by the luminal OC exchanger (2.4 mM, David *et al*., 1995) exceed measured plasma serotonin concentrations by several orders of magnitude (~5 nM, Anderson *et al*., 1987). Perhaps, as postulated for choline, the luminal OC exchanger is involved in renal transport of other endogenous OCs such as serotonin at supraphysiological, rather than physiological plasma concentrations.

In summary, the substrate specificity of the renal luminal OC/H^+ exchanger for a battery of test OCs was evaluated in membranes isolated from avian renal proximal tubule. As observed in isolated and intact renal luminal membranes of other vertebrates, the overall affinity of the OC exchanger in birds is greater for xenobiotic cations than for endobiotic cations. Amiloride, cimetidine, Darstine, procainamide, quinidine, quinine, raniditine, and thiamine, OCs for which the exchanger had relatively high affinity, were poorly transported in the absence of an opposing proton gradient. Despite their relatively low affinity, the endogenous OCs, ACh, choline, epinephrine, guanidine, NMN, and serotonin were transported by the OC exchanger with greater efficacy than xenobiotics. These data suggest that the renal luminal OC exchanger could play a physiological role in the net transport of endogenous compounds by the intact kidney. However, more studies in which the energetics of luminal membrane transport and transepithelial transport of endogenous substrates are characterized directly are necessary to further discern the role of luminal OC exchanger or other carriers in the renal transport of endobiotic OCs.

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