Functional analysis and molecular model of the human urate transporter/channel, hUAT

EDGAR LEAL-PINTO,1 B. ELEAZAR COHEN,2 MICHAEL S. LIPKOWITZ,1 AND RUTH G. ABRAMSON1

1Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York, New York, 10029; and 2Division of Extramural Activities, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

Received 1 November 2001; accepted in final form 12 February 2002

Leal-Pinto, Edgar, B. Eleazar Cohen, Michael S. Lipkowitz, and Ruth G. Abramson. Functional analysis and molecular model of the human urate transporter/channel, hUAT. Am J Physiol Renal Physiol 283: F150–F163, 2002—Recombinant protein, designated hUAT, the human homologue of the rat urate transporter/channel (UAT), functions as a highly selective urate channel in lipid bilayers. Functional analysis indicates that hUAT activity, like UAT, is selectively blocked by oxonate from its cytosolic side, whereas pyrazinoate and adenosine selectively block from the channel’s extracellular face. Importantly, hUAT is a galectin, a protein with two β-galactoside binding domains that bind lactose. Lactose significantly increased hUAT open probability but only when added to the channel’s extracellular side. This effect on open probability was mimicked by glucose, but not ribose, suggesting a role for extracellular glucose in regulating hUAT channel activity. These functional observations support a four-transmembrane-domain structural model of hUAT, as previously predicted from the primary structure of UAT. hUAT and UAT, however, are not functionally identical: hUAT has a significantly lower single-channel conductance and open probability is voltage independent. These differences suggest that evolutionary changes in specific amino acids in these highly homologous proteins are functionally relevant in defining these biophysical properties.

pyrazinoate; lactose; oxonate; glucose; adenosine; glycoporphin A

HUMANS, AS WELL AS BIRDS, reptiles, and some nonhuman primates lack functional uricase and, as a consequence, urate is the end product of the intracellular degradation of the purines adenine and guanine (3). Subsequent to its metabolic production by the enzyme xanthine oxidase (5, 40, 78), urate effluxes from cells by an unknown mechanism to enter the extracellular compartment. In the absence of degradation of urate to allantoin by hepatic uricase in these species, maintenance of urate homeostasis is entirely dependent on elimination of urate from the body by the kidneys (3) and, to a much lesser extent, the gastrointestinal tract (70, 71). Considering the very limited solubility of urate (80) within cells, plasma, and urine, it is apparent that the avoidance of urate crystallization in any of these compartments in humans is even more critically dependent on the efflux and excretory transporters than in species that have hepatic uricase to metabolize urate to the water-soluble compound allantoin (12, 25, 50). Although there are limited data on the mechanism(s) responsible for elimination of urate by the intestinal epithelium (70, 71), the handling (filtration, reabsorption, and secretion) and mechanisms of urate transport have been extensively evaluated in the kidney (3). Renal transport has been ascribed to both an electroneutral urate-anion exchanger (9, 20, 21, 28–30, 61) and an electrogenic urate uniporter (1, 2, 33, 61) in a number of species, including humans (61).

We recently cloned a cDNA from a rat renal expression library that encodes a 322-amino acid acid protein, prepared recombinant protein from the cDNA, and demonstrated that this protein functions as a highly selective, voltage-sensitive 10-pS urate transporter/channel in planar lipid bilayers (38). This protein, designated UAT, displays a number of characteristics (36) that suggest that this channel is the transporter responsible for urate efflux from systemic cells as well as the molecular representation of the rat renal electrogenic urate transporter. Moreover, recent studies in which UAT has been expressed as a chimeric protein documented that UAT is an integral plasma membrane protein with intracellular termini in a variety of renal and nonrenal epithelial cells derived from a number of species (59). However, it is noteworthy that UAT belongs to a family of proteins, the galectins, that have all been presumed to be soluble, cytoplasmic, or secreted proteins (4, 7, 10, 15–17, 22, 26, 48, 56). Equally importantly, galectins have been assigned multiple functions (4, 7, 10, 15–17, 22, 26, 48, 56), but none have ever been proposed to serve a transport function. The demonstration that UAT functions as a channel in synthetic lipid bilayers (36, 38) and resides as an integral plasma membrane protein in living cells (59) thus...
represents both a unique function and previously undescribed subcellular localization for a galectin.

Subsequent to our publication on the cloning of UAT (38), galectin 9 was reported in rats (76, 77), mice (76, 77) and humans (51, 52, 75). It is of note that the cDNAs for rat, mouse and human galectin 9 are, respectively, 99, 89, and 73% identical to UAT, and the translated proteins, like other members of the galectin family, are considered to be soluble, cytoplasmic, or secreted proteins (51, 75–77). Although a functional role has not been assigned to rat galectin 9 (76, 77), mouse galectin 9 has been proposed to serve a role in thymocyte-epithelial interactions (76, 77), whereas human galectin 9 is believed to participate in cellular interactions of the immune system (75) and in eosinophil chemotraction (51). In view of the high degree of homology between UAT (accession no. U67958) and human galectin 9/ecdectin (accession nos. Z49107, AB006782, and AB005894), we recently generated galectin 9 cDNA by RT-PCR from RNA of human white blood cells, prepared recombinant protein, and performed studies to evaluate the possibility that the apparent human homologue of UAT might serve a transport function (44). These studies demonstrated that recombinant human galectin 9, a 323-amino acid protein that is identical to accession no. AB006782 (minus the 32-amino acid insertion specific to the intestinal isoform of galectin 9), both functions as a highly selective urate channel in synthetic lipid bilayers and represents an integral plasma membrane protein with cytoplasmic NH2 and COOH termini in epithelium-derived cells (44). These observations led us to propose that the human homologue of UAT is also likely to represent the urate channel in plasma membranes of systemic cells and the electrogenic renal urate transporter in humans (44).

The present studies were conducted to evaluate the functional characteristics of the human urate transporter/channel, designated hUAT, to model its transmembrane organization and to assess the potential role in channel function of the two β-galactoside binding sites within hUAT, the signature amino acid sequence of a galectin. These studies demonstrate that hUAT channel activity displays a number of characteristics that suggest that the topologies of hUAT and UAT are quite similar. In contrast, single-channel conductance and voltage sensitivity of open probability of hUAT differ significantly from that of UAT, presumably as a consequence of some evolutionary divergence in critical amino acids in the respective sequences. Furthermore, these studies provide evidence to suggest that binding of the β-galactoside α-lactose to hUAT is not simply a confirmation that this protein contains the signature sequences for galectins but rather that such binding significantly influences hUAT channel activity. Finally, evidence is provided that glucose, the physiologically more relevant sugar, similarly has a significant modulating effect on hUAT channel activity.

MATERIALS AND METHODS

Preparation of Recombinant Protein

As previously reported, recombinant protein was made from cDNA prepared by RT-PCR of RNA that was harvested from human white blood cells (44). In brief, the full length of the coding sequence of hUAT in pBluescript was amplified by PCR using a sense primer with an XhoI site immediately 5′ to the start codon (5′-GCTTCGAGATGCGTGTCACGTTTCCCGAGCTCAG-3′) and an antisense primer with a HindIII site just 3′ to the stop codon (5′-GCAAGCTTCTATGTCCTGCCACATGGTGTCG-3′). The purified PCR product was subcloned into XhoI- and HindIII-digested pRSETA (Invitrogen, San Diego, CA) for subsequent production of a fusion protein with a six-histidine metal-chelating domain 5′ to the coding region of UAT. pRSETA-hUAT was isolated (Qiagen Plasmid Maxi kit, Qiagen, Chatsworth, CA) and used to transform BL21(DE3)pLysE cells (Novagen, Madison, WI). Colonies of BL21(DE3)pLysE cells containing pRSETA-hUAT were grown until the optical density reached 0.6–0.7. Thereafter, isopropyl-1-thio-β-D-galactopyranosidase was added to a final concentration of 0.4 mM, and the culture was grown for an additional 4 h and then centrifuged at 5,000 g for 20 min in a Sorvall RC-5B refrigerated centrifuge (DuPont). Cell pellets were stored at −70°C until recombinant protein was isolated. After cell lysis, the recombinant protein was harvested by metal-affinity chromatography on a nickel-chelating resin (Ni-NTA, Qiagen) in the presence of denaturants (6 M guanidine, 6 M urea), detergent (0.1% Triton X-100), a reducing agent (1 mM β-mercaptoethanol), and glycerol (10%) using a modification of a single-step purification/solubilization technique, in which denatured recombinant protein is solubilized in Tris-buffered saline and eluted in the same solution with EDTA (24). Eluate fractions containing hUAT were aliquoted and stored at −70°C until used in the lipid bilayer experiments.

Functional Evaluation of Recombinant hUAT

Formation of proteoliposomes. A 1:1 (wt/wt) mixture of bovine brain phosphatidylethanolamine (PE) and phosphatidylserine (PS; Avanti Polar Lipids, Birmingham, AL), each at a concentration of 10 mg/ml, were evaporated to dryness under a stream of nitrogen. The resultant pellet was suspended in 25 μl of 220 mM Cs2SO4 and 10 mM HEPES-NaOH at pH 7.4, after which 2 μl of recombinant hUAT protein were added. Proteoliposomes were formed by sonicating the suspension for 30 s at 80 kHz in a bath sonicator (Laboratory Supplies, Hicksville, NY) (36–38, 44). Fresh proteoliposomes were prepared for each experiment.

Lipid bilayer chamber, formation of lipid bilayer, and channel reconstitution. The lipid bilayer system was identical to that previously reported (36–38, 44). In all experiments, both chambers of the Plexiglas apparatus were filled with 1 ml of a solution containing 2.5 mM urate, 220 mM Cs2SO4, and 0.25 mM CaCl2 that was buffered to pH 7.4 with 10 mM HEPES-NaOH. Subsequently, a 50-μm hole in a Teflon film (type C-20, 12.5 μm thick, DuPont Electronics, Wilmington, DE) that had been tightly fitted between the two wells of the chamber was painted with lipids using a club-shaped glass rod. The lipids used to paint the bilayer were identical to those used to make the proteoliposomes (a 1:1 mixture of PE and PS, each at 10 mg/ml) but, after drying under nitrogen, the lipids were dissolved in n-decane (Sigma, St. Louis, MO) at a concentration approximating 50 mg lipid/ml. Junction potentials were corrected with the zero-adjust system of the patch-clamp amplifier (Axopatch 200B, Axon Instruments,
Burlingame, CA). The cis chamber is defined as the chamber connected to the voltage-holding electrode; all voltages are referenced to the trans (ground) chamber. Voltage was generated, clamped at different voltages (−100 to +100 mV), and controlled with the patch-clamp amplifier. When a stable resistance of at least 100 GΩ and a noise level of <0.1 pA were maintained, the experiments were initiated by addition of 5 μl of the hUAT-containing proteoliposomes to the trans chamber. The solution in the trans chamber was stirred until the proteoliposomes fused with the bilayer.

**Functional analysis of the channel.** In each experiment, the activity of the channel was initially assessed in the presence of symmetrical solutions of 2.5 mM urate in 220 mM Cs₂SO₄, 0.25 mM CaCl₂, and 10 mM HEPES-NaOH at pH 7.4 in the cis and trans chambers. Thereafter, the channel was reexamined in the symmetrical 2.5 mM urate solutions, but after the cis or trans chamber was pulsed with microliter volumes of one of the following reagents to achieve progressively increased concentrations in the bath: 2.5 mM α-lactose monohydrate (Sigma), 1.0 M D(+) glucose (Sigma), 1.0 M D(-) ribose (Sigma), 2.5 mM oxonate (Sigma), 2.5 mM pyrazinoate (PZA; Aldrich Chemical, Milwaukee, WI), or 1 mM adenosine (Sigma). All reagents were prepared in 220 mM Cs₂SO₄ and 10 mM HEPES-NaOH buffered to pH 7.4. In some experiments, channel activity was reexamined after the solution in the trans and/or cis chamber was replaced with reagent-free fresh urate solution.

**Data collection and analysis.** Current output of the patch clamp was filtered at 10 kHz through an eight-pole filter (Bessel filter, model 902, Frequency Devices, Haverhill, MA) that was digitized at 5 kHz (Digi Data 1200 series Interface, Axon Instruments). Data were analyzed with commercial software (pCLAMP, version 8.0, Axon Instruments) after additional digitized filtering at not less than 1 kHz.

**RESULTS**

**Characteristics of the Reconstituted Channel**

Figure 1 demonstrates single-channel activity of hUAT (evidenced by clear transitions between open and closed states) in the presence of symmetrical urate solutions after fusion of the hUAT-containing proteoliposomes with the lipid bilayer. Single-channel activity was evident in most experiments; however, multiple channels and apparent substate conductances were also observed. As is evident from the traces (Fig. 1, A and B), the open probability of hUAT is independent of

![Figure 1](http://ajprenal.physiology.org/)

**Fig. 1.** Human urate transporter/channel (hUAT) activity, open probability of the channel, and current-voltage relationship in symmetrical urate solutions. Channel activity was recorded in symmetrical solutions of 2.5 mM urate, 220 mM Cs₂SO₄, and 10 mM HEPES-NaOH, pH 7.4, after fusion of hUAT-containing proteoliposomes with the lipid bilayer. A: 1-min traces of channel activity obtained at various holding potentials. Vertical arrows, time at onset of 1-s traces; solid horizontal lines, closed state. B: 1-s traces recorded during the 1-min traces depicted in A. Solid horizontal lines, closed state. C: current-voltage relationship of the channel depicted in A. Solid line, best fit by linear regression analysis. G and R, slope conductance and correlation coefficient, respectively.
voltage and, in this experiment, the slope conductance was 2 pS (Fig. 1C). As previously reported, the mean single-channel slope conductance of hUAT, calculated by linear regression analysis, was $4.0 \pm 0.4$ pS ($n = 11$), rectification was not obvious in symmetrical urate solutions, and the channel was highly selective to urate (44). These combined findings indicate that two important properties of hUAT, its single-channel conductance and its voltage insensitivity, are distinctly different from those previously described for rat UAT (36, 38). Moreover, in contrast to rat UAT, hUAT channel activity generally displayed run-down.

**Effect of α-Lactose on Activity of hUAT**

The amino acid sequence of hUAT contains two highly conserved β-galactoside binding domains, H x N P R 7x V x N 6x W 2x E x R 5x F 2x G and H x N P R 6x V x N 6x W 2x E x R 7x F 2x G, where x represents any amino acid and the number indicates the number of variable amino acids (45). The initial β-galactoside binding domain is located within the first predicted extracellular domain (amino acids 61–96), and the other within the second predicted extracellular domain (amino acids 235–271) of hUAT (Fig. 2). Although these domains represent signature sequences for the galectins, and are known to bind selective sugars (6, 39, 45), the functional role, if any, of these domains has not been ascertained. To assess the possibility that these sites participate in the function of the urate channel, increasing concentrations of α-lactose, a well-known substrate for these binding sites (6), was added to the chambers bathing the cis or trans side of the channel. In the absence of α-lactose, the mean single-channel conductance approximated 2 pS (Fig. 3, A and B). However, as noted above and depicted in Fig. 3, several higher conductance levels were observed. Of note, simultaneous openings and/or closing to the higher conductance levels were seen intermittently, suggesting cooperativity between a number of subunits (31, 35). In the absence of α-lactose, the open probability of the channel was quite low (Fig. 3C), independent of the voltage applied (not depicted), and there was a rather rapid run down of channel activity over time. Addition of α-lactose to the cis side of the bilayer did not influence channel activity (Fig. 3, A and B). In distinct contrast, after addition of $70 \pm 14.6$ μM α-lactose to the trans chamber, the conductance of the channel increased significantly (Fig. 3, A and B), reaching a mean value of $8 \pm 1.9$ pS ($n = 7$). The increase in conductance to this level occurred in a progressive manner in association with increments in the concentration of lactose in the trans chamber (Fig. 3, A and B). Additionally, as in the control state, in the presence of α-lactose in the trans chamber simultaneous openings and closings to the higher conductance state were evident (Fig. 3, A and B). Finally, the presence of α-lactose resulted in a significant increase in the open probability of the channel (Fig. 3C) from 10.6 ± 5.1 to 58.3 ± 12.9% ($n = 7$) and reduced the likelihood of channel run down. This stabilization of channel activity at the higher conductance level (Fig. 3, A and B) could be consequent to a lactose-induced sustained cooperativity between hUAT subunits (multimerization) and/or modification in the conformation of the pore of the channel that results in a higher conductance state. On the basis of the assumption that α-lactose binds with the same or similar affinities to the two β-galactoside binding sites within hUAT, the observed unilateral effect of this substrate requires that the topology of hUAT is such that both binding sites must lie on the same side of the channel (Fig. 2). Moreover, in view of the consistency in the unilateral (trans) effect of α-lactose, it appears that hUAT must insert in the lipid bilayer in a specific orientation. A similar uniformity in the direction of lipid insertion was observed for UAT and, as previously noted, the consistent orientation of the channel in the bilayer likely reflects the nonsymmetrical distribution of electrical charges on the bilayer lipids (36).

**Effect of d(+)-Glucose, But Not d(−)-Ribose, on Activity of hUAT**

It has been presumed that the galactose moiety of α-lactose forms the major interaction with the β-galactoside binding sites in galectins (7, 39). The observation that there is at least a 100-fold higher affinity for α-lactose than galactose, however, has suggested that an interaction between the glucose moiety of α-lactose and the β-galactoside binding sites is also important (7, 39). To assess the possibility that glucose per se may
interact with hUAT, presumably via the β-galactoside binding sites, hUAT channel activity was examined in the presence of increasing concentrations of glucose (n = 6). In three of these studies, hUAT channel activity was first assessed in the presence of increasing concentrations of α-lactose, used as a control for a nonspecific sugar effect. As depicted in Fig. 4, A and B, addition of up to 50 mM D(-)-ribose to the trans side of the chamber failed to activate hUAT; open probability remained at < 1.0% for as long as 2 h after exposure of the channel to ribose. In distinct contrast, within minutes of addition of 5 mM D(+)-glucose to the trans chamber (Fig. 4, A and B) channel activity increased significantly to 11.7 ± 7.5% (n = 6). Of note, a further increase in glucose concentration in the trans chamber to 20 mM (Fig. 4, A and B) was associated with a further increase in the channel’s open probability to 25.8 ± 14.7% (n = 6). Although these studies demonstrate that hUAT has a much higher affinity for α-lactose (µM) than for glucose (mM) (Figs. 3 and 4) under these experimental conditions, it is apparent that glucose, like α-lactose, significantly modulates hUAT channel activity (Fig. 4), presumably via conformational changes secondary to an interaction with the β-galactoside binding domains in hUAT.

Local Block of Homology to Glycophorin A Within hUAT

On the basis of the data obtained after addition of α-lactose to the trans chamber that suggest that hUAT may multimerize (Fig. 3), the amino acid sequence of hUAT was assessed with the multiple protein sequence alignment program MACAW (65) to search for a local block of homology between hUAT (and rat UAT) and the extensively characterized dimerization motif within the single transmembrane domain of glycophorin A (GpA) (41–43, 46, 47, 62, 63, 66). As depicted in Fig. 5, the dimerization motif of GpA is formed by seven amino acids, Leu75, Ile76, Gly79, Val80, Gly83, Val84, and Thr87 (42, 43), with the G79xxxG83 sequence being described as the motif that is likely to be involved in high-affinity association of transmembrane α-helices (62, 66). Alignment of amino acids 18–33 of both rat UAT and hUAT [the block of residues that was
previously proposed to represent the first transmembrane domain of UAT (36) reveals significant homology to GpA (Fig. 5). In both UAT and hUAT, four residues are identical to the seven residues of the dimerization motif in GpA, including G24xxxG28 (Fig. 5). In UAT and hUAT, additional residues are homologous to those within the dimerization motif of GpA: three in UAT and two in hUAT (Fig. 5). Of interest, a second GxxxG sequence exists in UAT and hUAT (residues 19–23) that may be relevant to dimerization; however, alignment of G19xxxG23 with G79xxxG83 of GpA yields a lower overall homology between the 16-amino acid block of GpA (Fig. 5) and comparably sized blocks of UAT and hUAT.

**Effect of Oxonate on the Activity of hUAT**

Oxonate, a specific inhibitor of the enzyme uricase (14), both inhibits electrogenic urate transport in rat and rabbit renal cortical membrane vesicles (1, 2, 33) and blocks the activity of recombinant rat UAT that is reconstituted in the lipid bilayer system (36). As in other studies, multiple conductance states were detected in the absence of oxonate (Fig. 6, A and B).

Oxonate concentrations up to 188 μM in the trans chamber failed to influence hUAT activity (Fig. 6, A and B). In contrast, addition of increasing concentrations of oxonate to the cis chamber, to a concentration of 138 ± 30.5 μM (n = 7), progressively decreased the number of conductance states and ultimately virtually abolished channel activity (Fig. 6, A and B). As depicted (Fig. 6C), the effect of oxonate on the open probability of hUAT (in the presence or absence of lactose) was quite similar to its effect on the activity of UAT (36). This oxonate-induced block of hUAT was reversible in that channel activity was fully restored after the oxonate-containing solution in the cis chamber was replaced with a fresh oxonate-free urate solution (not depicted). Of note, the effect of oxonate on both rat UAT (36) and hUAT activity is restricted in that the oxonate-induced block is only observed when the cytoplasmic face of the channel is exposed to the reagent.

Because oxonate is a competitive inhibitor of uricase (14), and oxonate blocks hUAT channel activity (Fig. 6), the amino acid sequence of the human homologue...
was evaluated to determine whether it contains a block of homology to the substrate binding site in uricase. Importantly, the Q228 of *Aspergillus* uricase, which is critical to substrate binding (11) (presumably to oxonate as well as urate), is conserved within a 12-amino acid domain of porcine uricase, *Aspergillus* uricase, hUAT, rat UAT, and the intestinal isoform of galectin 9 (Fig. 7A). As depicted, alignment of a 12-amino acid block of porcine uricase (residues 231–242) and *Aspergillus* uricase (residues 224–235) with residues 158–169 of hUAT reveals that hUAT has 50% homology to both porcine and *Aspergillus* uricase (Fig. 7A). Alignment of residues 157–168 of rat UAT with residues 158–169 of hUAT reveals that this block of amino acids is highly conserved with 92% homology between the rat and human sequences (Fig. 7A). It is of note that a sequence for a gastrointestinal isoform of human galectin 9, which is inserted immediately after residue 148 of galectin 9, has been deposited in GenBank (accession no. AB006782). This domain in hUAT appears to be in part duplicated insofar as alignment of amino acids 4–15 of the 32-amino acid isoform sequence also has a high degree of homology to uricase, having 50 and 67% homology to porcine and *Aspergillus* uricase, respectively (Fig. 7).

**Effect of PZA on Activity of hUAT**

PZA, a potent inhibitor of urate transport in intact kidneys of multiple species (3) and an inhibitor of electrogenic urate transport in rat and rabbit membrane vesicles (1, 2, 33), also blocks channel activity of recombinant rat UAT (36). Comparable to observations made with recombinant rat UAT (36), despite the raising of the PZA concentration to 150 μM in the cis chamber, PZA failed to alter channel activity of hUAT (Fig. 8, A and B). Similar to the effect of PZA on rat UAT (36), PZA induced a dose-dependent block of hUAT activity (Fig. 8, A and B) and a reduction in open probability (Fig. 8C) when added to the trans chamber (in the presence or absence of lactose). The open probability of the channel was profoundly reduced at a concentration of 87.5 μM (n = 5). This block was completely reversed after the PZA-containing solution was replaced with a fresh PZA-free urate solution (not shown). Because PZA and oxonate only effectively block channel activity when in contact with the trans

---

**Fig. 6.** Channel activity in the presence of α-lactose and in the absence and presence of oxonate at a holding potential of 100 mV. A: 10-s traces of channel activity in symmetrical solutions of 2.5 mM urate, 220 mM Cs₂SO₄, and 10 mM HEPES-NaOH, pH 7.4, in the absence (top trace) and presence of the designated concentrations of oxonate in the trans (2nd trace) and the cis chambers (3rd and 4th traces) Solid horizontal lines, closed state. B: 1-s traces recorded during the 10-s traces depicted in A. The 1st, 2nd, and 4th traces represent the first second of the 10-s traces depicted in A; the 3rd trace was recorded at the time indicated by the arrow in A. Solid horizontal lines, closed state. C: open probability (% O.P.) of the channel during the traces depicted in A.
**DISCUSSION**

The present studies demonstrate that the human homologue of the urate transporter/channel, hUAT, (44) has characteristics that are both similar to and different from the highly homologous rat protein, rat UAT (36, 38). Three reagents that were previously documented to block rat UAT channel activity, oxonate, PZA, and adenosine (36), have been shown to similarly block hUAT channel activity (Figs. 5, 7, and 9). Moreover, with both the human and rat channels, each of these substrates only blocks channel activity when a specific side of the channel is exposed to the compound (Figs. 5, 7 and 9). There are, however, two important differences in the biophysical properties of these channels. First, the mean single-channel conductance of hUAT approximates one-half that of rat UAT (4.0 ± 0.4 vs. 9.5 ± 0.47 pS). Second, the open probability of hUAT is voltage independent (Fig. 1), whereas that of rat UAT is consistently voltage dependent (36, 38).

The concordance of findings observed with recombinant rat (36) and human homologues of UAT relative to the inhibitory effects of oxonate, PZA, and adenosine on channel activity (Figs. 5, 7 and 9), in conjunction with the identical sidedness of effects of the respective substrates, implies that the topologies of the human and rat transporters are similar. We previously proposed a molecular model for rat UAT that incorporated intracellular NH₂ and COOH termini and four transmembrane domains (36). This model, including the specific amino acid residues that represent the four transmembrane α-helices (36), was based on electrophysiological studies in lipid bilayers that revealed the sidedness of effects of these same three reagents, the location of local blocks of homology to the A₁/A₃ receptors and uricase within UAT, the hydrophobicity profile of UAT, and the detection of hydrophobic segments (long enough to span the membrane) with significant homology to transmembrane domain 2 in urate/xanthine permease (19), the α-helix documented to form transmembrane domain E in bacterial rhodopsin (58), and a portion of the α-helix reported to form transmembrane domain IX of subunit 1 of cytochrome c oxidase (74). By incorporating all of this information, the hydrophobic segments with homology to urate/xanthine permease, bacterial rhodopsin, and cytochrome c oxidase were modeled as transmembrane domains 1, 2, and 3, respectively, in rat UAT (36).

Confirmation that rat and human UAT are transmembrane proteins has been obtained in surface biotinylation studies of renal and nonrenal epithelia-derived cells transfected with the cDNA of rat and human
Moreover, recent evidence in support of the above-described model was obtained with immunofluorescent and confocal microscopy of nonpermeabilized and permeabilized epithelial cells subsequent to transfection with NH$_2$ or COOH FLAG-tagged UAT cDNAs; the NH$_2$ and COOH termini of both rat and human UAT were observed to reside on the intracellular side of the plasma membrane (44, 59). Additional strong support for this model is provided by the present studies. First, the high degree of homology that has been detected within amino acids 18–33 of both hUAT and UAT to the dimerization domain within the single transmembrane H$_{9251}$-helix of GpA (Fig. 5) (41–43, 46, 47, 62, 63, 66) supports our previous molecular model in which amino acids 15–35 of UAT were designated as transmembrane domain 1 (36). Second, we previously proposed that UAT contains two large extracellular domains, one located between transmembrane domains 1 and 2 and the second located between transmembrane domains 3 and 4 (Fig. 2). Importantly, hUAT contains two β-galactoside binding sites, one encompassed by residues 61–96 within the first putative extracellular domain and the second incorporated by residues 235–271 within the second putative extracellular domain. Of note, the specific amino acids involved in each of these sites are 100% conserved in human and rat UAT (the latter within residues 60–95 and 234–270). The present finding that α-lactose only influences hUAT channel activity when in contact with the extracellular face of the channel (Fig. 3) is thus consistent with our model. The combination of findings of intracellular locations of the NH$_2$ and COOH termini of hUAT and UAT (44, 59) and extracellular locations of the two β-galactoside binding sites could be consistent with two transmembrane α-helices. However, the unilateral intracellular block of channel ac-

![Fig. 8. Channel activity in the presence of α-lactose and in the absence and presence of pyrazinoate (PZA) at a holding potential of 75 mV. A: 10-s traces of channel activity in symmetrical solutions of 2.5 mM urate, 220 mM Cs$_2$SO$_4$, and 10 mM HEPES-NaOH, pH 7.4, in the absence (top trace) and presence of the designated concentrations of PZA in the cis (2nd trace) and trans chambers (3rd and 4th traces). Solid horizontal lines, closed state. B: 1-s traces recorded during the 10-s traces depicted in A. All 4 traces represent the first second of the 10-s traces depicted in A. Solid horizontal lines, closed state. C: open probability (%O.P.) of the channel during the traces depicted in A.](http://ajprenal.physiology.org/)

![Fig. 9. Local block of homology in hUAT to the adenosine A$_2$ receptors. The residue nos. of the individual proteins are indicated at the beginning and end of each line. Double lines between amino acids indicate identical residues; single lines indicate homologous residues as defined in Fig. 4.](http://ajprenal.physiology.org/)
tivity induced by the uricase inhibitor oxonate (Fig. 6) and the high degree of likelihood that this substrate interacts with amino acids 158–169 of the uricase-like domain in hUAT (Fig. 7) require that this site is exposed to the intracellular face of the channel. Because the uricase-like domain is located between the two β-galactoside binding sites, hUAT therefore must contain at least four rather than two transmembrane α-helices (Fig. 2), a model entirely compatible with our previously proposed molecular model of the rat urate transporter/channel (36).

We previously suggested that a local block of homology to uricase within UAT (36) is most likely responsible for the functional similarities between the electrogenic urate transporter and uricase (1, 2, 33, 34, 37) and the ability of our polyclonal antibody to porcine uricase to select the UAT clone from the rat cDNA library (38), react with recombinant UAT (38), block electrogenic urate transport in membrane vesicles prepared from rat kidney (34), and selectively block UAT channel activity from the cytoplasmic side of the channel (36). It was also suggested that the oxonate-induced block of UAT activity was most likely consequent to its interaction with the uricase-like domain in UAT (36). In previously aligning a local block of homology in rat UAT to the substrate binding site in uricase, Q156 in rat UAT was aligned with Q228 in Aspergillus uricase (36), the amino acid that has been identified by X-ray crystallography as being critically important in formation of the substrate-uricase complex (11). However, on the basis of additional sequence data, specifically hUAT (44), the human (51, 75), mouse, and rat sequences of galectin 9 (76), the pig sequence for a urate transporter/channel (72), and the intestinal isoforms of human (accession no. AB006782), mouse, and rat galectin 9 (76) and pig urate transporter/channel (72), an adjustment has been made in this alignment.

We now assign the substrate binding glutamine as Q161 in rat UAT as it is this glutamine, rather than Q156, that is conserved in the rat, human, pig, and mouse sequences (Fig. 7, A and B). In addition to conservation of this glutamine in the four mammalian species, it is evident that there is also an extremely high degree of homology within the block of amino acids that is located just proximal to the uricase-like domain in the intestinal isoform of these same species (Fig. 7B).

Despite the high degree of homology (Fig. 11) between and apparent similarities in the topology of the human and rat homologues of UAT, evidence has been...
obtained that two important biophysical properties of these proteins differ (voltage sensitivity of the open probability and single-channel conductance). The voltage sensitivity of channels has been ascribed to the presence of charged residues, specifically, basic residues localized in critical points in the structure of the channel (8). These residues are presumed to sense the electrical field across the membrane (8) and produce a significant change in the conformation of the protein that affects the open probability of the channel. We previously reported (36) that rat UAT contains two putative β-sheets (residues 96–104 and 111–119) linked by six amino acids (106–110) that carry a net positive charge (R106, E108, and K110) that carry a net neutral charge (S107, D109, and K111) – which are presumed to affect the open probability of the channel. We proposed that this segment of the protein could act as a mobile domain (69), interact with the membrane domains and thereby affect conductance. Induced mutations in hUAT to recapitulate the amino acids found in rat UAT in the first transmembrane α-helix of human and rat UAT (Fig. 5) may influence packing of the transmembrane domains and thereby affect conductance. Induced mutations in hUAT to recapitulate the amino acids found in rat UAT in the first transmembrane α-helix of human and rat UAT (Fig. 5) may alter the conformation of this domain and result in increased packing of the transmembrane α-helices. Insofar as transmembrane α-helices 2 and 3 participate in formation of the channel pore, closer packing of these α-helices may decrease the size of the channel pore and thereby reduce channel conductance in hUAT relative to that of rat UAT. Alternatively, the difference between the initial amino acid of the dimerization motif in the first transmembrane α-helix of human and rat UAT (Fig. 5) may influence packing of the transmembrane α-helices and thereby affect conductance.

Lactose, a β-galactoside, has previously only been utilized as a tool to isolate and purify galectins (4, 7, 10, 15–17, 22, 26, 48, 51, 56, 76). The ability to use this reagent for purification purposes is consequent to the fact that lactose binds to the highly conserved β-galactoside binding domains within galectins (7). Because various β-galactosides are found on glycolipids and glycoproteins on cell surfaces and extracellular matrix, some of which have also been shown to bind to the β-galactoside binding sites in galectins (39), it has been suggested that secreted galectins could function as biologically significant ligands that play a role in cell migration, cell proliferation, immune function, and adhesion (4, 7, 10, 15–17, 22, 26, 48, 56). To date, however, there is no direct evidence to support these proposals.

In contrast to the absence of functional data relative to lactose or the β-galactoside binding sites in galectins, the present study indicates that lactose, presumably by binding to the β-galactoside binding domains in hUAT, regulates the activity of hUAT by significantly increasing its conductance and open probability (Fig. 3). Although α-lactose per se would not be relevant to physiological function in vivo, it is important to note that both of lactose’s component sugars, galactose and glucose, interact with the β-galactoside binding do-
main in galectins (7), and therefore these sugars could regulate channel activity in vivo. The functional consequence of an increase in conductance and open probability of hUAT would be an increase in urate flux. On the basis of membrane potential and the likely prevailing electrochemical gradient for urate, this would represent an increase in urate efflux from systemic cells (inducing hyperuricemia) and an increase in urate excretion consequent to an increase in the rate of urate secretion in the renal proximal tubule and intestine (inducing hypouricemia). In this context, it is of interest that elevation of blood galactose levels in galactosemic patients (13) and rather modest elevations in blood glucose levels in diabetic patients (79) are associated with hyperuricemia. However, during periods of poor metabolic control hypouricemia and hyperuricemia are evident in diabetic patients (18, 49). Of note, the voltage sensor in voltage-dependent ion channels that elevation of blood galactose levels in galactosemic patients (13) and rather modest elevations in blood glucose levels in diabetic patients (79) are asso-

REFERENCES


AJP-Renal Physiol • VOL 283 • JULY 2002 • www.ajprenal.org


