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

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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 pyrazinolate 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.

pyrazinolate; 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 electronegative 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 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 electronegative 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

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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/ecalectin (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′-GCTCGAGATGGCCTTCAGCGGT-3′) and an antisense primer with a HindIII site just 3′ to the stop codon (5′-GCAAGCTTCATATGCTGACACAT-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-galactopyranoside 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 β-lactose monohydrate (Sigma), 1.0 M D(−)-glucose (Sigma), 1.0 M D(−)-ribose (Sigma), 2.5 mM oxonate (Sigma), 2.5 mM pyrazinomate (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...
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 NPR7xV x N 6x W 2x E x R 5x F 2x G and H x NPR6x 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 rundown 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 $10.6 \pm 5.1$ to $58.3 \pm 12.9$% ($n = 7$) and reduced the likelihood of channel rundown. 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 be located 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

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**Fig. 2.** Topological model of hUAT. Numbers 1–4 designate the NH$_2$-to-COOH terminal transmembrane domains. Numbers adjacent to the transmembrane domains indicate the amino acid residues at the beginning and end of each domain. The approximate location of the 2 β-galactoside binding sites within the 2 extracellular domains of hUAT, the site with homology to the A$_1$/A$_3$ adenosine receptors on the extracellular side of transmembrane domain 2, and the urate/oxinate binding site with homology to uricase in the cytoplasmic loop between transmembrane domains 2 and 3 are indicated by arrows. Brackets in the loop into the membrane from the extracellular face of the channel, pointed to by 2 arrows from the cytoplasmic side of the model, represent the location of the 2 β-sheets, which are connected by 6 amino acids that carry a net neutral charge in hUAT.
interact with hUAT, presumably via the β-galactoside binding sites, hUAT channel activity was examined in the presence of increasing concentrations of glucose. In three of these studies, hUAT channel activity was first assessed in the presence of increasing concentrations of D(−)-ribose, 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 such that the open probability of the channel 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, Ile 76, Gly 79, Val 80, Gly 83, 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 GxxxxG 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 than 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. As previously proposed with UAT (36), the distinct asymmetrical effect of oxonate implies that this compound interacts with a specific domain in hUAT that is consistently localized on the cis face of the channel. It is of note that in our bilayer system the cis side of the channel is exposed to changes in voltage, simulating an intracellular compartment; the cis chamber is connected to the voltage-holding electrode with all voltages referenced to the trans (ground) side. Insofar as the cis chamber represents an intracellular compartment, the domain in hUAT that interacts with oxonate, like the domain in UAT (36), must then reside on the cytoplasmic face of the channel. In this context, because lactose only influences hUAT when added to the opposite chamber of the bilayer (Fig. 3), the two β-galactoside binding sites must reside on the extracellular face of hUAT.

Local Block of Homology to Uricase Within hUAT

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

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**Fig. 5.** Local blocks of homology to glycophorin A in hUAT and rat UAT. The residue no. of the individual proteins is indicated at the beginning and end of each line. The arrows above the amino acid sequences indicate the amino acids that form the dimerization motif, i.e., Leu^75^, Ile^76^, Gly^79^, Val^80^, Gly^83^, Val^84^, and Thr^87^, in glycophorin A. Double lines between amino acids indicate identical residues; single lines indicate homologous residues. Homology is defined according to the Swiss-Prot data bank in which amino acids in each of the following groups are homologous: [S, T, A, G, P]; [N, D, E, Q]; [R, K, H]; [M, L, I, V]; and [F, Y, W].

**Fig. 4.** Channel activity in the presence of ribose and glucose 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_2SO_4, and 10 mM HEPES-NaOH, pH 7.4, in the presence of 50 mM ribose (top trace) and subsequent presence of the designated concentrations of glucose (2nd and 3rd traces) in the trans chamber. Solid horizontal lines, closed state. B: open probability (%O.P.) of the channel during the traces depicted in A.
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. 7B). 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...
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tially eliminated channel activity when the concentra-
with hUAT. As depicted in Fig. 10, adenosine essen-
potent blocker of UAT channel activity (36), the possi-
recombinant protein documented that adenosine is a
D132 of hUAT. Because functional studies with rat
N254, that bind adenosine and xanthine (32, 57). These
specific residues in the A1 receptor, P249, H251, and
amino acids is identical to or homologous with the
residues of the individual proteins are indicated at the begin-
ing and end of each line. Double lines indicate homologous
residues as defined in Fig. 4. B: alignment of the rat,
human, pig, and mouse homologues of UAT and their intestinal
isoforms with pig/rat uricase and Aspergillus uricase. The residue
numbers of the individual proteins are indicated at the begin-
ing and end of each line. gal, Galectin. The arrows in A and B
demonstrate conservation in all of these sequences of the amino acid Q 228,
which is critical to substrate binding in Aspergillus uricase.

and cis faces of the channel, respectively, the specific
domains that bind these substrates in hUAT, as in
UAT (36), must be located on opposite faces of the
channel. Insofar as oxonate binds to a site on the
cytoplasmic face of the channel, then the domain that
binds PZA in hUAT, as in the case of the domain in
UAT (36), must reside within an extracellular portion
of the channel.

Effect of Adenosine on Activity of hUAT

As demonstrated in Fig. 9, hUAT, like UAT, contains
a local block of amino acids that has 73 and 45%
homology to the adenosine A1 (60, 73) and A3 (64)
receptors, respectively. Importantly, this block of
amino acids is identical to or homologous with the
specific residues in the A1 receptor, P249, H251, and
N254, that bind adenosine and xanthine (32, 57). These
amino acids align with amino acids P127, H129, and
D132 of hUAT. Because functional studies with rat
recombinant protein documented that adenosine is a
potent blocker of UAT channel activity (36), the possi-
bility was evaluated that adenosine would also interact
with hUAT. As depicted in Fig. 10, adenosine essen-
tially eliminated channel activity when the concentra-
tion of adenosine in the trans chamber reached 14.2 ±
5.8 μM, producing a profound decrease in open proba-

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, oxo-
one, 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 chan-
nels. 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 recombi-
nant 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 pro-
posed a molecular model for rat UAT that incorporated
intracellular NH2 and COOH termini and four trans-
membrane domains (36). This model, including the
specific amino acid residues that represent the four
transmembrane α-helices (36), was based on electro-
physiological 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 recep-
tors and uricase within UAT, the hydrophobicity pro-
file of UAT, and the detection of hydrophobic segments
(long enough to span the membrane) with significant
homology to transmembrane domain 2 in urate/xan-
thine 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 transmem-
brane 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 cyto-
chrome c oxidase were modeled as transmembrane
domains 1, 2, and 3, respectively, in rat UAT (36).

Confirmation that rat and human UAT are trans-
membrane proteins has been obtained in surface bio-
tinylation studies of renal and nonrenal epithelia-de-
formed 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$_9$251-helix of GpA (Fig. 5) (41–43, 46, 47, 62, 63, 66) supports our previous molecular model in which amino acids 15–35 of UAT were designated as transmembrane domain 1 (36). Second, we previously proposed that UAT contains two large extracellular domains, one located between transmembrane domains 1 and 2 and the second located between transmembrane domains 3 and 4 (Fig. 2). Importantly, hUAT contains two β-galactoside binding sites, one encompassed by residues 61–96 within the first putative extracellular domain and the second incorporated by residues 235–271 within the second putative extracellular domain. Of note, the specific amino acids involved in each of these sites are 100% conserved in human and rat UAT (the latter within residues 60–95 and 234–270). The present finding that α-lactose only influences hUAT channel activity when in contact with the extracellular face of the channel (Fig. 3) is thus consistent with our model. The combination of findings of intracellular locations of the NH$_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.

![Figure 8](http://ajprenal.physiology.org/) by 10.220.32.247 on June 23, 2017

Fig. 9. Local block of homology in hUAT to the adenosine A$_1$/A$_3$ 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.
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-urate 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
The presence of charged residues, specifically sensitivity of channels has been ascribed to the probability and single-channel conductance. The voltage sensitivity of channels that are critical to the conformation of the pore. It is of interest that there appears to be a clustering of nonhomologous amino acids in the human and rat sequences within putative transmembrane α-helices 2 and 3 and, probably most significantly, in the block of cytoplasmic amino acids that connect these α-helices (Figs. 2 and 11). Importantly, within the hairpin turn between these transmembrane helices, the human channel contains three prolines (P150, P154, and P157), whereas rat UAT has only one (P153) (Fig. 11). Because prolines are known to induce turns in transmembrane segments and result in the formation of helical hairpins (53, 55), the increased number of prolines in the hairpin turn of hUAT may alter the conformation of this domain and result in increased packaging 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 domains and thereby affect conductance. Induced mutations in hUAT to recapitulate the amino acids found in rat UAT in the first transmembrane α-helix and/or the domain between transmembrane α-helices 2 and 3 will be required to assess the possibility that the difference in conductance in the rat and human homologues is consequent to naturally evolved divergences in their primary structure.

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 augmenting 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-

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Fig. 11. Comparison of amino acid sequences of the rat and human homologues of the urate transporter/channel. R and H, rat and human UAT sequences, respectively; shaded amino acids, those homologues of the urate transporter/channel. R and H, rat and human UAT sequences, respectively; shaded amino acids, those homologues of the urate transporter/channel.
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 (18) are evident in diabetic patients (18, 49). Of note, both are corrected when blood glucose is normalized (18). Consistent with the likelihood that increased proximal tubular fluid glucose concentration also has a direct effect on urate flux is the observation that the infusion of glucose induces an increase in urate clearance in humans that significantly exceeds that induced by mannitol at comparable osmolar clearances (54, 67, 68). Reports of this (18, 49, 54, 67, 68, 79), in conjunction with the observed effect of glucose on hUAT channel activity at concentrations comparable to physiological (5 mM) and pathological (20 mM) plasma levels in humans (Fig. 4), suggest that extracellular glucose may interact with urate transporter/channels that reside in systemic cells and the renal proximal tubule (27) and thereby exert a direct regulatory effect on the activity of this channel.

In summary, the present studies have provided evidence that recombinant protein that was prepared from the cDNA of hUAT has topological characteristics that are comparable to those of the rat homologue UAT. However, these proteins are not functionally identical: differences in their biophysical properties suggest that evolutionary changes in specificity of amino acids in these two highly homologous proteins are functionally relevant in defining these properties. Finally, the present data suggest that an interaction of selective sugars with the β-galactoside binding domains in hUAT may be responsible, at least in part, for regulating hUAT channel activity in vivo.

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