J. Membrane Biol. 26, 91–107 (1976)

Amide Transport Channels Across Toad Urinary Bladder

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Received 9 July 1975; revised 16 October 1975

Summary. Urea and other small amides cross the toad urinary bladder by a vasopressin-sensitive pathway which is independent of osmotic water flow. Amide transport has characteristics of facilitated transport: saturation, mutual inhibition between amides, and selective depression by agents such as phloretin. The present studies were designed to distinguish among several types of transport including (1) movement through a fixed selective membrane channel and (2) movement via a mobile carrier. The former would be characterized by co-transport (acceleration of labeled amide flow in the direction of net flow of unlabeled amide), the latter by counter-transport (acceleration of labeled amide flow in the opposite direction). Mucosal to serosal (M→S) and serosal to mucosal (S→M) permeabilities of labeled amides were determined in paired bladders. Unlabeled methylurea, a particularly potent inhibitor of amide movement, was added to either the M or S bath, while osmotic water flow was eliminated by addition of ethylene glycol to the opposite bath. Co-transport of labeled methylurea and, to a lesser degree, acetamide and urea with unlabeled methylurea was observed. Co-transport of the nonamides ethylene glycol and ethanol could not be demonstrated. Methylurea did not alter water permeability or transmembrane electrical resistance. The demonstration of co-transport is consistent with the presence of ADH-sensitive amide-selective channels rather than a mobile carrier.

In the normal mammalian kidney, urea plays a vital role in the elaboration of a concentrated urine, through a complex system in which regions of the nephron have widely different permeabilities to urea, sodium, and water. For this reason, an understanding of the mechanisms by which urea crosses cell membranes is of considerable importance. A method which permits the distinction among several modes of solute transport is based upon an analysis of interactions among passively transported solutes. Fig. 1 depicts four simple models which could be considered for a urea-transport pathway. They are (*A*) a simple diffusional barrier, (*B*) a model in which there exists a limited number of symmetrical

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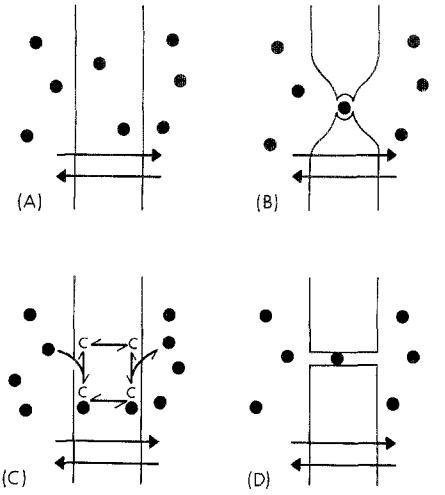

Fig. 1. Schematic of four simple membrane models: (*A*) diffusional barrier, (*B*) limited number of sites but no interactions among transported molecules, (*C*) carrier, (*D*) channel. Unidirectional permeabilities (→) of tracer solute (●) are all equal

transport sites, each of which may hold only a single solute molecule [9, 36], (*C*) a classical carrier, which binds to and passively transports solute molecules across the membrane [34, 35], and (*D*) a solute-selective channel [9, 10]. In the presence of tracer concentrations of solute, unidirectional isotope permeabilities are all equal, as depicted by the equal lengths

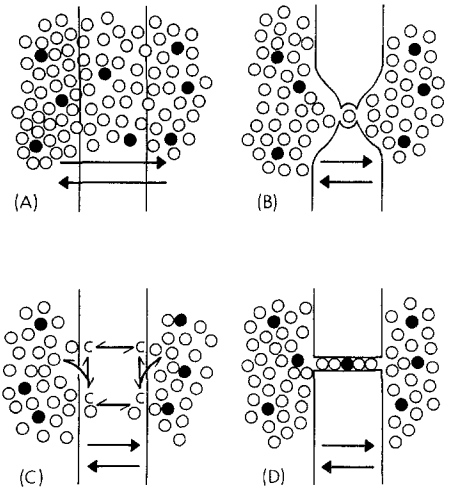

Fig. 2. Models from Fig. 1 when unlabeled solute (○) is added to labeled solute (●) on both sides of the membrane. Symmetrical depression of permeability is shown for models *B*, *C*, and *D*

of the paired arrows in the four models. Under the conditions shown in Fig. 1 the models cannot be distinguished from one another.

Fig. 2 shows the same models under a different set of conditions. Here, equal concentrations of unlabeled solute have been added to the labeled solute on both sides of the membrane. The diffusion membrane (*A*) will be unaffected by this change since the number of transport pathways is unlimited, while the other three models will demonstrate a decrease in isotope permeability in both directions compared to Fig. 1, as unlabeled solute competes with labeled solute for the limited number of transport sites. Expressed differently, saturation of unlabeled solute transport is observed under these conditions. As in Fig. 1, paired unidirectional isotope permeabilities are symmetrical in each of the four models.

Models *B*, *C*, and D can be distinguished as shown in Fig. 3. Here, unlabeled solute is added to only one side of the rate-limiting membrane. Again no alteration in isotope permeability is expected in *A*, the diffusional membrane. In *B* isotope permeability is diminished. However, since there exists only a single symmetrical transport site, membrane permeability in either direction depends only on the average solute concentration, and not on the distribution of solute between the two baths. No asymmetry of solute permeability is seen despite the asymmetry of solute distribution. In *C*, the mobile carrier, competition for the transport sites at the left side of the membrane is greater than competition on the right side, so that isotope permeability from left to right will be

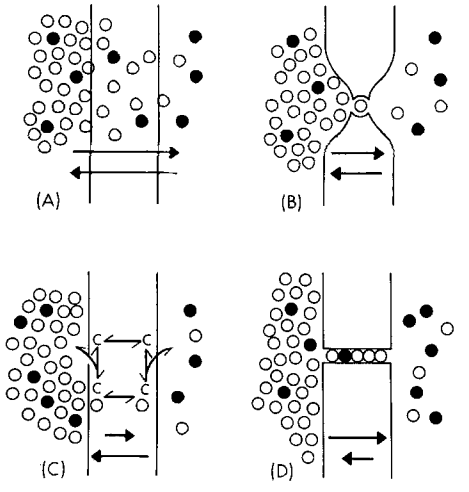

Fig. 3. Models from Fig. 1 with unlabeled solute added to only one side of the membrane. Permeabilities in *B* remain symmetrically depressed. Models *C* and *D* demonstrate counter-transport and co-transport, respectively

less than permeability from right to left. This is referred to as "counter-transport." In model *D*, however, the high unlabeled solute flux from left to right will carry along with it the labeled solute, so that isotope permeability measured from left to right will be greater than that from right to left. This coupling of solute flows is referred to as "co-transport."

In the present study the unidirectional permeabilities of labeled amides and nonamides across toad urinary bladder, an analog of the mammalian renal collecting duct, were measured in the presence of modest concentrations of unlabeled methylurea according to the outline above. Our results are consistent with model *D*, the amide-selective channel.

Materials and Methods

Female Dominican toads (National Reagents, Bridgeport, Conn.) were doubly pithed and glass bungs were tied into both hemibladders *in situ*. The bladders were excised, washed inside and out three times with glucose-free phosphate-buffered amphibian Ringer's solution (120 mM Na^+, 4 mM K^+, 0.5 mM Ca^{++}, 116 mM Cl^-, 5 mM phosphate, pH 7.40, 230 mOsm/kg), and finally filled with 8 ml and immersed in 35 ml of the test solutions. Stirring was provided by rotating magnets inside and out. Net water flow was determined by weighing the bladders [2]. Short-circuit current was determined in Lucite chambers with a central dividing partition. ^{14}C urea, acetamide, methylurea, and ethanol were obtained from New England Nuclear Corp., Boston, Mass.; ^{14}C ethylene glycol was supplied by ICN Pharmaceuticals, Inc., Cleveland, Ohio. Counting was done in a Tri-Carb liquid scintillation counter (Packard Instrument Co., La Grange, Ill.). In all experiments results obtained in one hemibladder were compared to those from its pair by the method of pair analysis [26]. Isotope permeability (K_{trans}) [20] was determined for a single 15-min period prior to vasopressin (Pitressin, Parke-Davis, Detroit, Mich.), and for two consecutive 15-min periods after vasopressin (86 mU/ml). The two latter periods were averaged into a single 30-min period for ease of presentation.

Determination of ^{14}C Urea Permeability with Unlabeled Amide on Both Sides of the Bladder

In order to choose the unlabeled amide which interacted most strongly with the transport of a labeled amide (^{14}C urea), a series of unlabeled amides was added to the Ringer's bathing both surfaces of the test bladders. M→S permeability of ^{14}C urea was measured and compared to that in control bladders containing and bathed in Ringer's alone. In one set of experiments in which osmotic water flow was determined, an osmotic gradient was created by decreasing the mucosal Ringer's to half-strength inside both bags.

Determination of Unidirectional Isotope Permeabilities with Unlabeled Methylurea on Only One Side of the Bladder

Of the unlabeled amides tested in the preceding set of experiments, methylurea proved to be the most potent inhibitor of ^{14}C urea transport. Methylurea was therefore used in all of the experiments designed to evaluate coupling of solute transport. Unlabeled methylurea was added to the Ringer's solution bathing either the mucosal or the serosal

surface of both paired hemibladders. In order to minimize net osmotic water flow, ethylene glycol was added to the opposite bath of both hemibladders. Both bladder halves therefore contained identical mucosal solutions and were bathed in identical serosal solutions in any given experiment. Isotope was added to one hemibladder's mucosal bath for M→S permeability measurements and to the other hemibladder's serosal bath for S→M measurements. Equal volumes were removed for permeability measurements from all four baths at 15-min intervals as noted above. Thus the paired hemibladders were treated identically throughout the experiment with the exception of the site of isotope addition.

It was found that when Ringer's alone bathed both sides of the bladder, a small loss of bladder volume (2.8 ± 0.3 µl/min, $n=10$ pairs) was consistently noted over the 45-min course of an experiment despite the absence of a significant hydroosmotic gradient. For this reason it was necessary to balance mucosal methylurea with a somewhat lower concentration of ethylene glycol than was needed to balance serosal methylurea. Serosal ethylene glycol concentrations were chosen to be 60% of mucosal methylurea concentrations, while mucosal ethylene glycol concentrations were held equal to those of serosal methylurea. Under these conditions net water flow averaged no more than 1 µl/min.

Results

Effects of Amide Solutions on K_{trans} ^{14}C Urea

The first set of experiments was carried out to determine the effect of a series of unlabeled amides, placed on both sides of the bladder,

Table 1. Effect of unlabeled amides on K_{trans} ^{14}C urea

Unlabeled amide	K_{trans} ^{14}C urea (cm/sec × 10^7)						Mean test / Mean control	
	Control bladder		Test bladder[a]		Δ (Control-Test)			
	I	II[b]	I	II	I	II	I (%)	II (%)
Urea, 150 mM (7)[c]	127 ± 36[d]	360 ± 64	81 ± 21	288 ± 46	46 ± 16[e]	73 ± 26[e]	64	80
Acetamide, 150 mM (10)	72 ± 18	210 ± 26	46 ± 13	154 ± 34	26 ± 8[f]	51 ± 21[e]	64	73
Propionamide, 150 mM (3)	23 ± 2	199 ± 26	13 ± 2	139 ± 21	10 ± 2[e]	61 ± 12[e]	57	70
Methylurea, 100 mM (7)	194 ± 71	439 ± 76	48 ± 19	180 ± 39	146 ± 52[e]	259 ± 46[f]	25	41

[a] Unlabeled amide added to both mucosal and serosal solutions of test bladder.
[b] Vasopressin added after period I. Period I, 15 min; period II, 30 min (mean of two 15-min periods).
[c] Numbers in parentheses indicate number of experiments.
[d] Mean ± 1 SEM.
[e] $p < 0.05$.
[f] $p < 0.01$.

Table 2. Effect of 100 mM methylurea on osmotic water flow[a]

$n=4$	Osmotic flow (μl/min/bladder)	
	I	II
Control bladder	1.9 ± 0.2	34.3 ± 4.3
Test bladder	2.0 ± 0.4	33.6 ± 3.1
Δ (Control-Test)	-0.1 ± 0.2 (NS)	0.7 ± 1.4 (NS)

[a] Data as in Table 1.
Test bladders received 100 mM mucosal and serosal methylurea.
All bladders contained half-strength Ringer's and were bathed in full-strength Ringer's.

on ^{14}C urea permeability. We have previously demonstrated [14] that 150 mM unlabeled acetamide under these conditions selectively depresses the permeability of the labeled amides urea and acetamide, but not the nonamides ethanol and ethylene glycol. The results of the present experiments are shown in Table 1. When added to both the mucosal and serosal baths, all of the amides tested led to a significant decrease in K_{trans} ^{14}C urea both before (period I) and after (period II) addition of vasopressin. Methylurea, at a concentration of 100 mM, is seen to be a markedly more potent inhibitor of K_{trans} ^{14}C urea than 150 mM of any of the other unlabeled amides tested. 100 mM methylurea did not alter osmotic water flow either before or after vasopressin (Table 2). Acetamide has previously been shown to be similarly innocuous [14].

Unidirectional ^{14}C Methylurea Permeabilities with Identical Mucosal and Serosal Baths – Validation of Experimental Technique

Because of its potent competitive effect, unlabeled methylurea was added to the mucosal or serosal baths in the experiments to follow, to determine whether co- or counter-transport of amides could be demonstrated. In order to determine the validity of measuring M→S and S→M isotope permeabilities in separate bladder halves, unidirectional ^{14}C methylurea permeabilities were measured in bladders in which the mucosal and serosal baths were closely matched in composition. This corresponds to the models as shown in Figs. 1 and 2. In the absence of active amide transport, measured unidirectional isotope permeabilities should be equal under these conditions. The baths consisted of Ringer's alone (M and S), Ringer's with 50 mM methylurea (M and S), or Ringer's

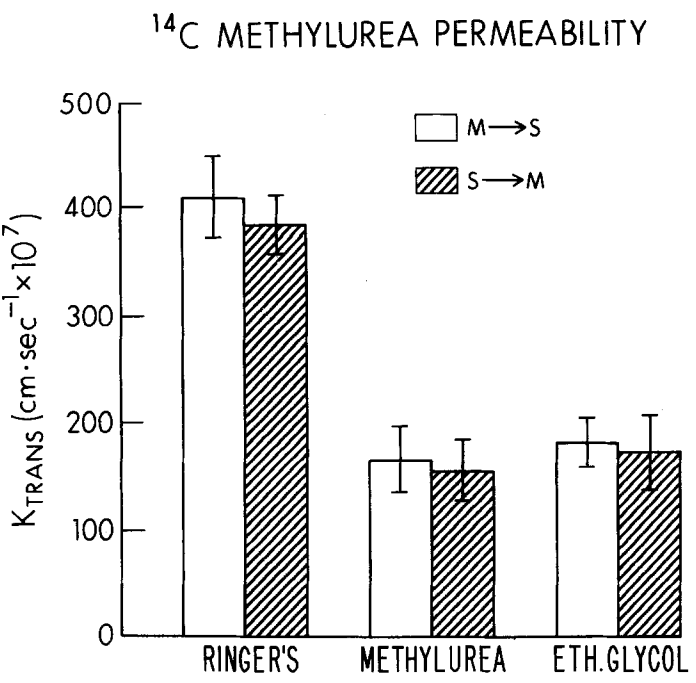

Fig. 4. Unidirectional K_{trans} ^{14}C methylurea after vasopressin in bladders in which mucosal (M) and serosal (S) baths were closely matched. Bathing media were Ringer's (ΔK_{trans} [M→S]−[S→M]=26±24×10^{-7} cm/sec, n=10), Ringer's with 50 mM methylurea (ΔK_{trans}=10±19, n=8), and Ringer's with ethylene glycol (ΔK_{trans}=8±11, n=4). No asymmetry of isotope permeability is seen. Data expressed as mean±1 SE

with 45 mM (M) and 30 mM (S) ethylene glycol. As noted above, a net volume loss approximating 2–3 μl/min was observed in the first two experiments, where identical mucosal and serosal baths were used. In the third, because of the slightly greater mucosal than serosal osmolality, water flow was only 0.2 μl/min. No asymmetry of unidirectional K_{trans} could be demonstrated in the absence of vasopressin or, as shown in Fig. 4, after the addition of vasopressin for any of the three experiments, providing validation of the experimental technique and confirming that active transport of methylurea does not exist[1].

1 Although Fig. 1 would seem to suggest that ethylene glycol might inhibit ^{14}C methylurea permeability to a degree comparable with 50 mM methylurea, four paired experiments designed to examine this possibility revealed no effect of 50 mM ethylene glycol (M and S) on K_{trans} ^{14}C methylurea either before or after vasopressin (control 191±44×10^{-7} cm/sec; ethylene glycol 211±66 after vasopressin). Thus the apparent difference in ^{14}C methylurea permeabilities with and without ethylene glycol seen in Fig. 4 appears to be the result of differences between toads, rather than an effect of ethylene glycol.

Effect of Asymmetric Distribution of Unlabeled Methylurea on ^{14}C Methylurea Permeabilities – Demonstration of Co-transport

In contrast to the symmetry of unidirectional ^{14}C methylurea permeabilities seen when identical mucosal and serosal solutions were used, addition of unlabeled methylurea to one side of the bladder resulted in a pattern of response consistent with co-transport of amides. Table 3 lists unidirectional ^{14}C methylurea permeabilities in bladders treated with 50 or 100 mM mucosal methylurea or with 50 or 100 mM serosal methylurea. Net osmotic water flow was minimized by the addition of ethylene glycol to the opposite bath. After vasopressin (period II and Fig. 5), a significant asymmetry of paired unidirectional fluxes was demonstrated in all four sets of experiments, consistent with coupling or co-transport of unlabeled and labeled methylurea. That is, ^{14}C methylurea permeability in the direction of net mass flow of unlabeled methylurea exceeded that in the opposite direction in the presence of either mucosal or serosal methylurea, at both 50 and 100 mM.

As seen in Table 3, mean isotope permeabilities in the direction of net unlabeled methylurea flow exceeded those in the opposite direction to approximately the same degree before vasopressin (period I) as after vasopressin (period II) in each set of experiments, suggesting the presence of comparable degrees of coupling of unlabeled and labeled methylurea

Table 3. Coupling of isotopic and unlabeled methylurea flows[a]

Solute added to Ringer's		Unidirectional K_{trans} ^{14}C Methylurea (cm/sec × 10^7)						K_{trans} ratio[f]	
Mucosal bath (mM)	Serosal bath (mM)	(M→S)		(S→M)		Δ(M→S)−(S→M)			
		I	II	I	II	I	II	I	II
MU[b] 50	EG 30 (10)	36±6	268±36	24±4	206±27	12±5[d]	63±16[e]	1.50	1.30
MU 100	EG 60 (6)	46±15	205±30	27±8	139±27	19±8[c]	66±18[d]	1.70	1.47
EG 50	MU 50 (10)	32±3	125±15	43±13	161±23	−11±9	−37±10[e]	1.34	1.29
EG 100	MU 100 (4)	18±4	90±25	33±9	144±41	−15±6[c]	−54±17[d]	1.83	1.60
MU 50	– (4)	43±9	209±40	34±6	142±36	8±4[c]	67±9[e]	1.26	1.47

[a] Data as in Table 1.
[b] MU: methylurea, EG: ethylene glycol.
[c] $p < 0.1$.
[d] $p < 0.05$.
[e] $p < 0.01$.
[f] K_{trans} in direction of not flow ÷ K_{trans} in opposite direction.

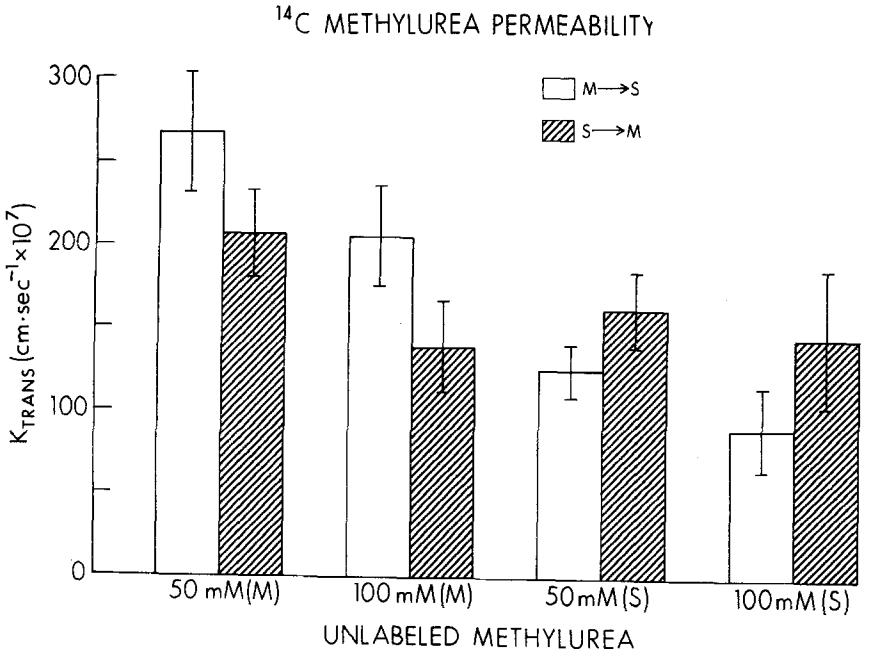

Fig. 5. Unidirectional K_{trans} ^{14}C methylurea in vasopressin-treated bladders with unlabeled methylurea in either mucosal or serosal bath. Significant coupling of labeled and unlabeled methylurea flows is observed under all conditions shown. Osmotic water flow balanced by ethylene glycol

in the absence and presence of vasopressin. Because of the low permeability of ^{14}C methylurea in the absence of vasopressin, however, and the consequent increased scatter of experimental data, statistical significance was achieved only in the experiments performed with 50 mM mucosal methylurea. Results of all experiments were statistically significant in the presence of vasopressin. To ensure that the asymmetry of labeled methylurea permeabilities was due to the unlabeled methylurea and not to the ethylene glycol, an additional set of experiments was performed, in which unidirectional ^{14}C methylurea permeabilities were measured in the presence of 50 mM mucosal methylurea but without ethylene glycol. Net water gain under these conditions was approximately 2 μl/min. As seen in Table 3, significant co-transport was observed under these conditions as well, confirming the coupling effect of methylurea.

Coupling of Unlabeled Methylurea and Labeled Amide Permeabilities

Can the coupling of labeled and unlabeled methylurea flows be extended to other amides? Fig. 6 shows the effect of unlabeled methylurea

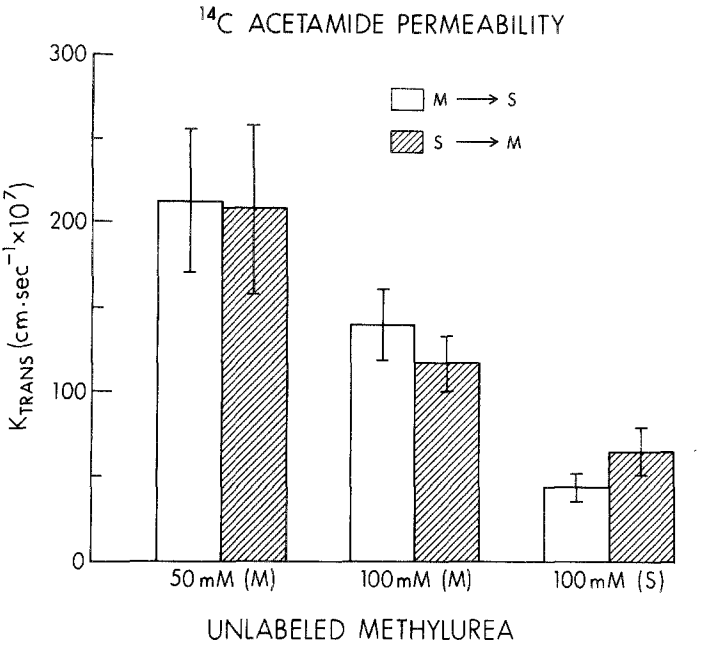

Fig. 6. Unidirectional K_{trans} ^{14}C acetamide in bladders treated as in Fig. 5. Significant coupling of labeled acetamide and unlabeled methylurea flows is observed with 100 mM mucosal or serosal methylurea. ΔK_{trans} [M→S]−[S→M]$=6\pm15\times10^{-7}$ cm/sec, $n=7$; 23 ± 7, $n=8$, $p<0.02$; -21 ± 7, $n=6$, $p<0.05$ for the three sets of experiments

on the unidirectional permeabilities of ^{14}C acetamide after addition of vasopressin. 50 mM mucosal methylurea caused no asymmetry of ^{14}C acetamide permeabilities, while co-transport could be demonstrated with 100 mM methylurea in either the mucosal or the serosal bath. No co-transport could be demonstrated in the absence of vasopressin. Thus the interaction of unlabeled methylurea and labeled acetamide within the membrane appears to be somewhat less strong than the interaction between unlabeled and labeled methylurea. Fig. 7 demonstrates a similar but weaker interaction between unlabeled methylurea and ^{14}C urea after vasopressin. Here only 100 mM mucosal methylurea led to significant coupling. Again, no coupling was observed in the absence of vasopressin.

Absence of Coupling Between Methylurea and Labeled Nonamides

Fig. 8 shows the effect of mucosal methylurea on the unidirectional permeabilities of ^{14}C ethylene glycol and ^{14}C ethanol after vasopressin. No co-transport of either isotope was observed with either 50 mM or 100 mM unlabeled methylurea either before or after vasopressin. This

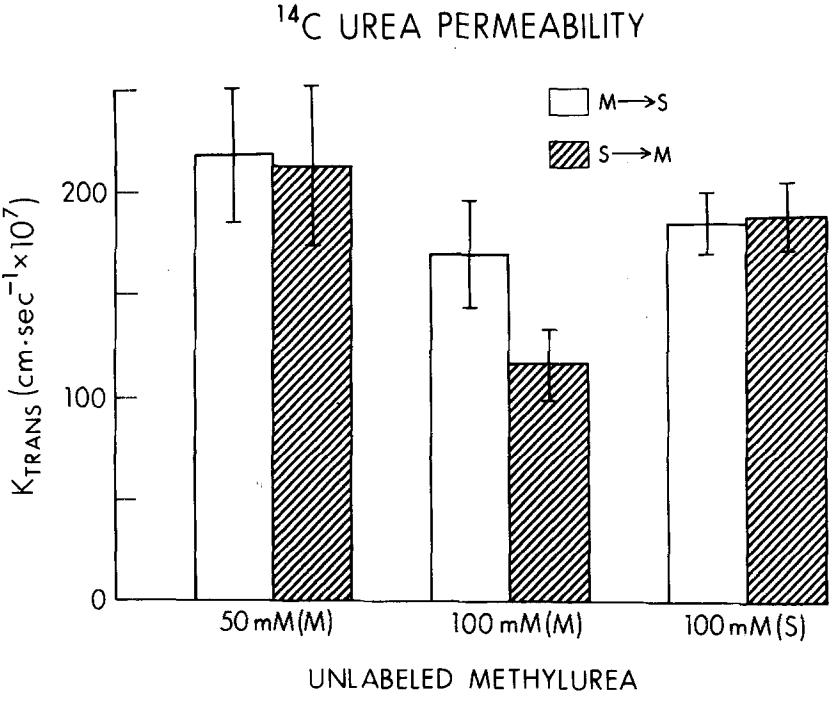

Fig. 7. Unidirectional K_{trans} ^{14}C urea in bladders treated as in Fig. 5. Significant coupling of labeled urea and unlabeled methylurea flows is observed with 100 mM mucosal methylurea only. ΔK_{trans} [M→S]−[S→M] = 5±21 × 10^{-7} cm/sec, n=5; 52±21, n=8, p<0.05; −3±23, n=7 for the three sets of experiments

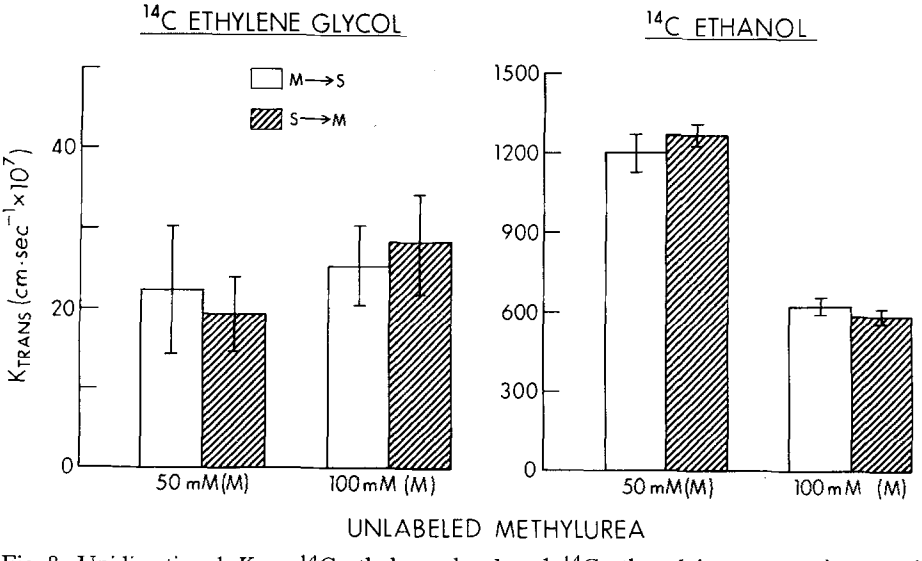

Fig. 8. Unidirectional K_{trans} ^{14}C ethylene glycol and ^{14}C ethanol in vasopressin-treated bladders receiving unlabeled mucosal methylurea and serosal ethylene glycol. No coupling is seen. (n=6, 7, 4, 5 for the four sets of experiments)

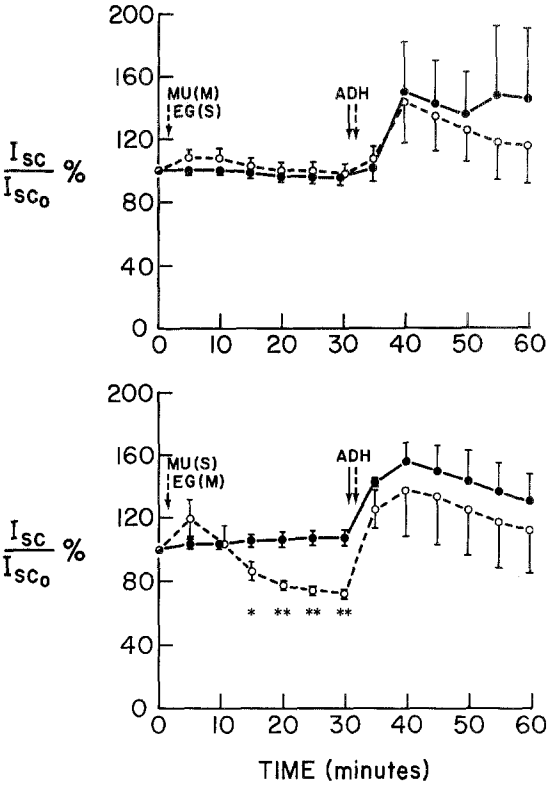

Fig. 9. *Top:* I_{sc} in three test bladder segments (o——o) receiving 100 mM mucosal methylurea and 60–100 mM serosal ethylene glycol at 0 min compared with untreated controls (●——●). Vasopressin 80–100 mU/ml added to both segments at 30 min. *Bottom:* Same data for three bladder segments receiving 100 mM serosal methylurea and 100 mM mucosal ethylene glycol (* $p < 0.05$; ** $p < 0.02$)

is consistent with our earlier demonstration that ethylene glycol and ethanol do not utilize the amide transport pathway [12, 13].

Effect of Experimental Conditions on Bladder Electrical Parameters

To demonstrate bladder integrity further during the experimental conditions examined, short-circuit current (I_{sc}) and transmembrane resistance (calculated as open-circuit potential difference/I_{sc}) were measured. After a stable baseline, methylurea and ethylene glycol in Ringer's were added to the test portion of the bladder, as noted in Fig. 9. Ringer's alone was added to the control portion. Thirty minutes later vasopressin was added to both serosal baths. Bladders were kept short-circuited except for a short period at intervals for measurement of transmembrane

potential and resistance. With 100 mM mucosal methylurea and 60–100 mM serosal ethylene glycol in the experimental portion no difference was seen between experimental and control data either before or after vasopressin. With 100 mM serosal methylurea and 100 mM mucosal ethylene glycol, a small rise, then a fall in I_{sc} is seen. The response to vasopressin is, however, entirely unimpaired. Transmembrane resistance measured at 15-min intervals was also entirely unaltered compared to control. These results suggest that the concentrations of methylurea and ethylene glycol used for determination of coupling cause little alteration in bladder electrical parameters.

Discussion

It was originally proposed that the stimulatory effect of vasopressin on water and urea permeabilities in amphibian skin and urinary bladder could be attributed to a widening of pores in the epithelial cell membrane, permitting a simultaneous increase in both water and urea permeabilities through a common channel [1, 12]. Recent studies have brought this idea into question, and suggest that solutes such as urea cross the toad bladder by a pathway separate from that for water flow. First, the permeabilities of the bladder to urea and to water can be independently altered by a number of agents. Phloretin, tannic acid, and the oxidizing agents periodate, permanganate, and chromate selectively depress amide permeability, leaving water permeability unaltered [13, 23, 25][2]. Second, several general anesthetic agents—including the gases methoxyflurane and halothane and the short-acting barbiturate methohexital—have the opposite effect: depression of vasopressin-stimulated water permeability without altering amide permeability [15, 16]. Third, transport of amides, but not nonamides, has been shown to exhibit saturation in the presence of high concentrations of acetamide, while bladder integrity, evaluated by measurement of potential difference, short-circuit current, osmotic water flow, and nonamide permeability is unaltered [14]. A similar phenomenon has been described in the presence of thiourea [6].

Further definition of the amide-transport pathway has been approached by studies in amphibian skin and urinary bladder which describe coupling between flows of solute and water (solvent drag) or solutes and each other. In order to achieve measurable degrees of interaction, these studies have generally utilized large osmotic gradients or

2 *Also:* Franki, N., Einhorn, R., Hays, R.M. Selective inhibition of urea transport by oxidizing agents. *J. Membr. Biol. (in press).*

very high solute concentrations. Although co-transport has been demonstrated under these conditions, it may not reflect the conditions occurring in the untreated tissue. Increasing the osmolality of the medium bathing the outer or apical surface of amphibian skin above that of the inner surface with any of several solutes, including electrolytes, sucrose, urea, and dimethylsulfoxide, has been shown to lead to a large increase in the inflow permeability of test solutes present at low concentration, with little change in outflow permeability [3, 7, 18, 29]. This is seen despite the large efflux of water which results from the apical bath hypertonicity and has been attributed to coupling of flows of test solute with those of the hypertonic solute. The coupling effect of basal (inner) bath hypertonicity is much less pronounced [3]. The interpretation of these alterations in tissue permeability is complicated by the observation that apical bath hypertonicity leads to a marked fall in electrical resistance across the skin [30], with deformation of the apical intercellular junctions [4]. Similar histologic and electrical alterations have been observed in toad urinary bladder when the apical (mucosal) bath is made hypertonic to the basal (serosal) bath, accompanied by an increase in the permeability of the intercellular junctions, and presumably an increase in solute flow through intercellular rather than intracellular pathways [5, 28, 33]. Quite possibly, apical hypertonicity opens large intercellular channels in toad skin which are not present *in vivo*, and in which the observed nonspecific solute flow coupling occurs. Furthermore, urea transport across amphibian skin may occur largely via intercellular pathways, rather than intracellularly, even in the absence of apical hypertonicity. In contrast to its striking inhibitory effect on urea permeability in the erythrocyte [19] and toad urinary bladder [13], phloretin does not inhibit urea permeability in frog skin [21], suggesting that urea transport across skin and bladder occur via fundamentally different pathways.

Of particular interest with regard to the present study, the electrical and structural alterations seen with apical hypertonicity do not appear to be caused by hypertonicity *per se*, but rather by the gradient between apical and basal baths [5, 33]. Thus basal hypotonicity results in the same alterations as apical hypertonicity, while identical basal and apical hypertonic solutions cause no change in histology or resistance. Balancing the osmotic activity of mucosal methylurea by serosal addition of a second solute as is done in the present study would be expected to have two advantages. First, net water movement is minimized, so that no solvent drag should occur. Second, the structural and electrical alterations caused by relative mucosal hypertonicity will be prevented, as

demonstrated by the lack of change in transmembrane electrical resistance and vasopressin-stimulated short-circuit current.

The present study presents data showing a high degree of interaction between the transport of the amide methylurea and that of labeled methylurea and other labeled amides, but not nonamides. This interaction is qualitatively comparable in the presence and absence of vasopressin, and, with the exception of ^{14}C urea, whose transport is more strongly influenced by mucosal than by serosal methylurea, is similar whether methylurea is added to the mucosal or serosal bath. Under the experimental conditions utilized, alterations in parameters other than those specifically investigated are minimized. These observations suggest that the results obtained are truly representative of the state of the amide transport system *in vivo* and not significantly contributed to by the experimental maneuvers.

Our conclusions are consistent with the findings of Lief and Essig [18], who demonstrated coupling of labeled and unlabeled urea flows across toad bladder in the presence of asymmetrical addition of high urea concentrations. In their study, however, attempts at balancing water flows with hypertonic sucrose led to abolition of the previously observed coupling. Our conclusions are also consistent with the observations of Eggena [6] that glutaraldehyde fixation of the toad bladder's mucosal surface in the absence of vasopressin fixes urea permeability at a low value, while treatment of the vasopressin-stimulated bladder with glutaraldehyde fixes urea permeability at a high level which is not reduced after removal of vasopressin, supporting the presence of channels rather than a mobile carrier.

The structure of the amide transport channel cannot be well established from the present study. Galey and Van Bruggen [8] and Van Bruggen *et al.* [31] have demonstrated coupling of solute fluxes across porous synthetic membranes, in general correlating the degree of coupling with the size of the solute and inversely with the size of the membrane pore, but suggest that factors such as pore length, tortuosity, surface charge, bound water, solute shape, and hydrogen bonding may also play an important role. The degree of interaction of labeled and unlabeled methylurea in the present study is greater than that of labeled urea with unlabeled methylurea. This may be in part due to the larger size of methylurea as noted above. Lief and Essig [18] have suggested that amide concentration within the transport channel may be higher than in the bathing solutions in order to achieve the degree of interaction necessary for demonstration of co-transport.

It is important to recognize that kinetic analyses such as the one presented here cannot uniquely define the transport site at which coupling occurs [17, 22, 32]. Although a "channel" is a rather simple and convenient model which demonstrates this type of interaction, more complex models such as a three-dimensional anisotropic network of sites [9] which demonstrate the same pattern of coupling can be formulated.

The role of urea co-transport in the renal medulla, which, like the toad bladder, has an amide-selective transport system [24], may be quite significant. During antidiuresis, urea gradients as high as 400 mM have been postulated between the tubular fluid at the beginning of the inner medullary collecting duct and its surrounding interstitium [11, 27]. In the face of this large gradient, co-transport could lead to a net urea efflux from the tubule considerably higher than would be predicted from the transtubular urea concentrations and isotopic urea permeability.

Presented in part at the 32nd annual meeting of the American Federation for Clinical Research, Atlantic City, New Jersey, May 1975. This work was supported by a Grant-in-Aid from the New York Heart Association and NIH Grant HL-13979. Dr. Levine is the Molly Berns Senior Investigator of the New York Heart Association. The authors are grateful to Dr. Richard M. Hays for his advice and encouragement.

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