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Membrane Pathways for Water and Solutes in the Toad Bladder: I. Independent Activation of Water and Urea Transport

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Summary. Vasopressin activates a number of transport systems in the toad bladder, including the systems for water, urea, sodium, and other small solutes. Evidence from experiments with selective inhibitors indicates that these transport systems are to a large extent functionally independent. In the present study, we show that the transport systems can be separately activated. Low concentrations of vasopressin (1 mU/ml) activate urea transport with virtually no effect on water transport. This selective effect is due in part to the relatively greater inhibitory action of endogenous prostaglandins on water transport. Low concentrations of 8-bromoadenosine cyclic AMP, on the other hand, activate water, but not urea transport. In additional experiments, we found that varying the ratio of exogenous cyclic AMP to theophylline activated water or urea transport selectively. These studies support the concept of independently controlled systems for water and solute transport, and provide a basis for the study of individual luminal membrane pathways for water and solutes in the accompanying paper.

In tissues such as the toad bladder, vasopressin elicits a broad response, in which the movement of water, urea, sodium, and other small solutes is increased (Leaf & Hays, 1962; Hays, 1976*a*). Studies with a number of inhibitory agents acting at the luminal surface of the bladder have shown that the luminal pathway for urea entry can be blocked, with no effect on osmotic water flow (Hays, 1976*b*). Thus, a clear separation of the entry steps for water and urea has been possible. There is also evidence that earlier steps in the adenylate cyclase-cyclic AMP sequence can be selectively inhibited (Peterson & Edelman, 1964; Levine *et al.*, 1976*a*; Carvounis, Levine & Hays, 1979). Functional separation of vasopressin-stimulated transport systems may therefore include steps close to the binding of vasopressin itself.

In the studies to be reported, we have found that urea or water transport across the toad bladder can be separately activated by low

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concentrations of vasopressin or 8-bromoadenosine cyclic AMP, respectively. The relative insensitivity of the water transport system to vasopressin rests in part on the more pronounced inhibitory effect of prostaglandins on water transport when compared to urea. We have also found that by varying the ratio of exogenous cyclic AMP to theophylline, we could activate the water or urea response selectively. In the accompanying paper, we have used this capability of independent activation to examine the physical properties of the water and solute transport pathways in the luminal membrane.

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 and washed inside and out three times with amphibian phosphate-buffered Ringer's solution (120 mM Na^+, 4 mM K^+, 0.5 mM Ca^{++}, 116 mM Cl^-, 5 mM phosphate, 230 mosmol/kg, pH 7.4). They were finally filled with 8 ml Ringer's diluted 1:10 and suspended in a bath containing 35 ml of full-strength Ringer's solution. Stirring was provided by magnetic stirrers, inside and out, and aeration by bubbling in the serosal bath. Osmotic water flow was determined gravimetrically (Bentley, 1958). ^{14}C urea or phenytoin permeability (K_{trans}) was determined by placing the isotope in the luminal medium and sampling the serosal and luminal solution at 15 min intervals. Samples were pipetted into Aquasol (New England Nuclear Corp., Boston, Mass.) and counted in a liquid scintillation counter (Tri-Carb, Packard Instrument Co., LaGrange, Ill.). Short-circuit current (SCC) was determined in Lucite chambers with a central dividing partition (Sharp & Leaf, 1964). Naproxen was a gift from the Syntex Corp., Palo Alto, Calif.

Most of the experimental protocols involved a single 15-min period prior to stimulation with argenine vasopressin 3′,5′-cyclic AMP (cAMP) or 8-bromoadenosine 3′,5′-cyclic monophosphate (8-Br-cAMP) (Sigma Chemical Co., St. Louis, Mo.), while the post-treatment period represents the average of two consecutive 15-min periods after stimulation. Vasopressin, unless stated otherwise, was added to the serosal bath of the control hemibladder at a saturating concentration of 86 mU/ml. The experimental hemibladder was stimulated simultaneously with a lower concentration of vasopressin, cAMP, 8-Br-cAMP or theophylline. The exact conditions for each set of experiments will be indicated in *Results*.

When the results are expressed as percent of maximum stimulation, this refers to the increment from basal levels seen after stimulation in the experimental hemibladder, compared to that seen in the respective control.

In all experiments, results obtained in the test bladders were compared to those of the paired control bladders by the method of paired analysis (Snedecor & Cochran, 1967).

Results

Effects of Low Concentrations of Vasopressin and Cyclic AMP on Urea Permeability and Water Flow

We first examined the effects of low concentrations of vasopressin on water and urea movement in comparison to paired hemibladders

Table 1. Comparative effects of low and saturating concentrations of vasopressin on water flow and K_{trans} urea

Vasopressin concentration (mU/ml)	Response to low vasopressin concentration			Response to 86 mU/ml vasopressin			$\frac{\Delta \text{ low vaso}}{\Delta \text{ 86 mU/ml}}$
	Basal	Vasopressin	Δ	Basal	Vasopressin	Δ	(%)
			Water flow (μl/min)				
0.2 (3)[a]	3.4	2.5	−0.9±0.6[b]	3.0	56.1	53.1±7.1	−2±2
0.5 (14)	2.4	5.9	3.5±1.6	2.4	57.0	54.6±4.2	6±3
1.0 (7)	2.5	8.6	6.1±2.8	2.7	57.6	54.9±2.6	11±5
3.0 (3)	7.6	52.1	44.5±1.9	9.1	64.7	55.6±5.6	80±5
			K_{trans} urea (cm/sec × 10^7)				
0.2	8	37	29 ±22	8	372	364 ±42	8±6
0.5	36	208	172 ±46	31	377	346 ±57	50±7
1.0	11	240	229 ±28	12	340	328 ±19	70±8
3.0	13	373	359 ±54	12	408	396 ±55	91±5

[a] Number of experiments.
[b] ±SEM.

receiving a saturating (86 mU/ml) concentration of hormone (Table 1). The results are shown diagramatically in Fig. 1; urea transport (vertical axis) and water flow (horizontal axis) are expressed as percentages of maximal movements in the paired hemibladders. It can be seen that urea transport is activated before there is an appreciable increase in water flow. At 0.5 mU/ml of vasopressin, urea permeability is half maximal, and at 1 mU/ml 70% of maximum, while water flow has reached only 6–11% of its maximum rate. As vasopressin concentrations increased, there was little additional increase in urea permeability, while water flow increased sharply. The same pattern was seen when arginine vasotocin (the native amphibian hormone) oxytocin, and the vasopressin analogue dDAVP were substituted for vasopressin.

A different pattern was seen when the effect of increasing concentrations of cyclic AMP were compared to saturating concentrations of vasopressin (Fig. 2, Table 2). Here the experimental points fell close to the identity line, indicating that the two transport systems were approximately equally responsive to exogenous cAMP. To confirm the difference between the vasopressin and cAMP patterns, we directly compared, in a separate set of experiments, concentrations of cAMP and vasopressin that produced equal activation of water flow. Under these conditions,

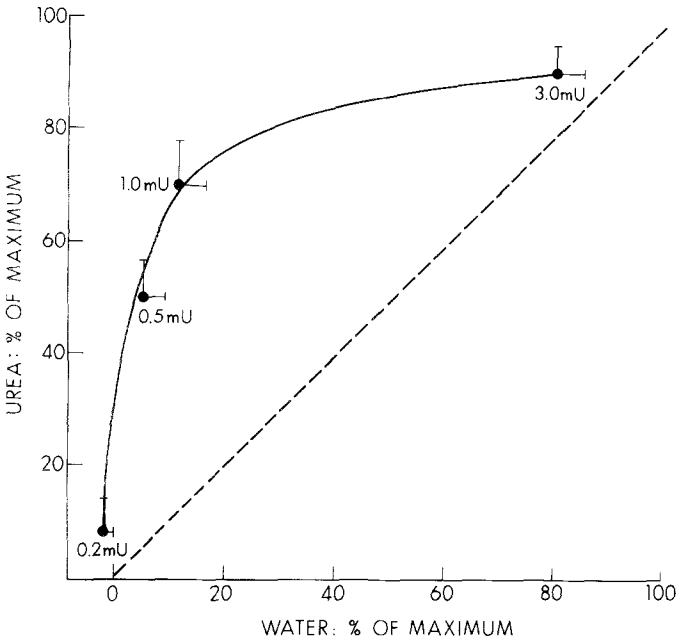

Fig. 1. Comparison of responses of urea and water transport to low concentrations of vasopressin, expressed as percentages of maximum response determined in paired hemibladders. Vasopressin concentrations indicated as mU/ml. Bars indicate ± 1 SEM. Dashed line indicates identical responses of urea and water

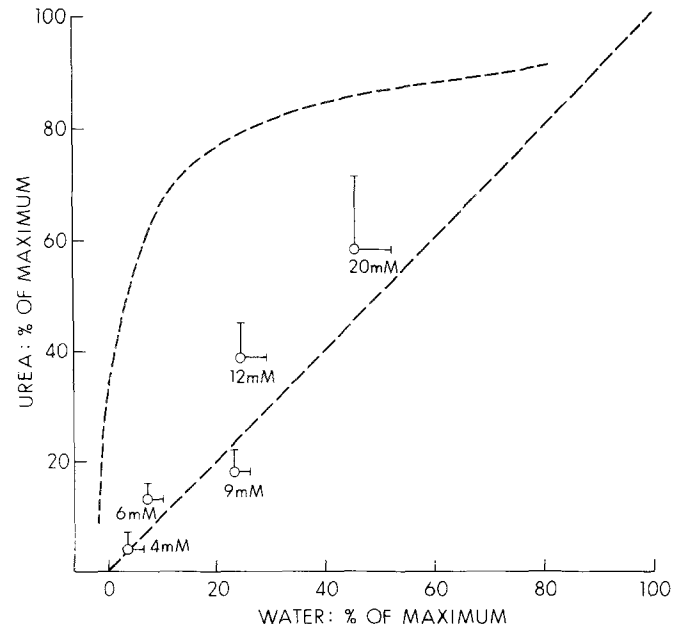

Fig. 2. Comparison of responses of urea and water transport to exogenous cyclic AMP. Cyclic AMP concentrations (mM/liter) are shown. The upper dashed line indicates the response to vasopressin shown in Fig. 1

Table 2. Comparative effects of graded cAMP concentrations and saturating concentration of vasopressin

cAMP concentration (mM)	Response to cAMP			Response to 86 mU/ml vasopressin			Δ cAMP / Δ Vasopressin
	Basal	cAMP	Δ	Basal	Vasopressin	Δ	(%)
				Water flow (µl/min)			
4 (3)	3.2	4.5	1.3±1.4	3.6	48.6	45.0±6.0	3± 3
6 (9)	3.2	6.2	3.0±1.3	3.0	44.5	41.5±1.6	7± 3
9 (12)	2.9	14.2	11.3±2.2	3.2	52.3	49.1±2.5	23± 4
12 (15)	3.3	14.8	11.5±2.1	2.9	50.9	48.0±3.1	24± 5
20 (9)	3.2	27.5	24.3±4.1	3.7	57.8	54.1±1.8	45± 7
				K_{trans} urea (cm/sec × 10^7)			
4	199	208	9 ±15	236	599	363 ±15	3± 4
6	46	80	34 ± 9	46	310	264 ±37	13± 3
9	56	113	57 ±15	59	376	317 ±36	18± 4
12	120	238	118 ±18	118	419	301 ±26	39± 6
20	113	289	176 ±44	99	402	303 ±24	58±13

Table 3. Direct comparison of effects of cAMP and vasopressin on transport in paired hemibladders

Period	Group 1: identical water flows (n=6)			Group 2: identical K_{trans} urea (n=6)		
	cAMP (9 mM)	Vasopressin (1 mU/ml)	P value	cAMP (12 mM)	Vasopressin (0.5 mU/ml)	P value
	Water flow (µl/min)			Water flow (µl/min)		
Basal	3.1±0.5	3.2±0.3	NS	3.2±0.4	2.9±0.4	NS
Stimulated	17.6±3.2	16.4±3.5	NS	27.7±3.5	15.0±3.7	<0.02
Δ	14.5±3.6	13.2±3.3	NS	24.5±3.4	12.1±3.8	<0.02
	K_{trans} urea (cm/sec × 10^7)			K_{trans} urea (cm/sec × 10^7)		
Basal	47 ±16	39 ±13	NS	28 ±9	29 ±11	NS
Stimulated	127 ±38	211 ±47	<0.05	153 ±38	159 ±25	NS
Δ	80 ±36	172 ±44	<0.02	125 ±14	130±31	NS

urea transport was considerably higher in the vasopressin-treated than in the cAMP-treated hemibladders (Table 3, Group *I*). When the experimental protocol was reversed, comparing the response to concentrations of cAMP and vasopressin selected to give equal activation of urea transport, water flow was significantly higher in the cAMP-treated hemibladders (Table 3, Group *II*).

Role of Prostaglandins in the Water and Urea Response

In the next series of experiments, we determined the extent to which a selective inhibitory effect of the prostaglandins might be responsible for the blunted response of water flow to low concentrations of vasopressin. Vasopressin has been shown to increase prostaglandin synthesis by the toad bladder (Zusman, Keiser & Handler, 1977); prostaglandins, in turn, inhibit vasopressin-stimulated water flow (Orloff, Handler & Bergstrom, 1965).

The response of the bladder to low concentrations of vasopressin was first examined in the presence and absence of 10^{-6}M naproxen, an agent that blocks prostaglandin biosynthesis (Zusman *et al.*, 1977). In these studies, one hemibladder (control) received a low dose of vasopressin and then a saturating dose (86 mU/ml). The paired hemibladder received the identical low dose and saturating dose, but had been pretreated for an hour with 10^{-6} M naproxen. The percentage stimulation of water and urea transport (low dose/saturating dose of vasopressin) was calculated for each hemibladder and compared. The results are shown in Fig. 3. Naproxen enhanced the water flow response far more than the urea response, with a resulting movement of the experimental points closer to the identity line. This suggested that urea transport is less

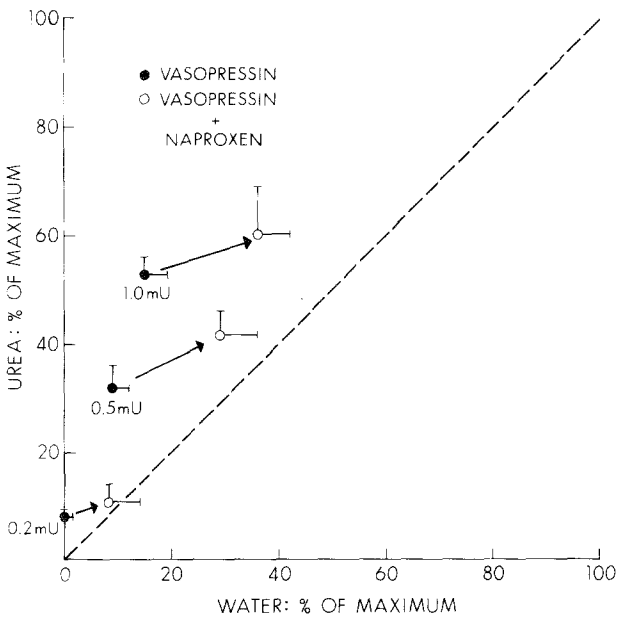

Fig. 3. Urea and water transport in paired bladders in response to vasopressin (filled circles), and vasopressin plus naproxen (open circles)

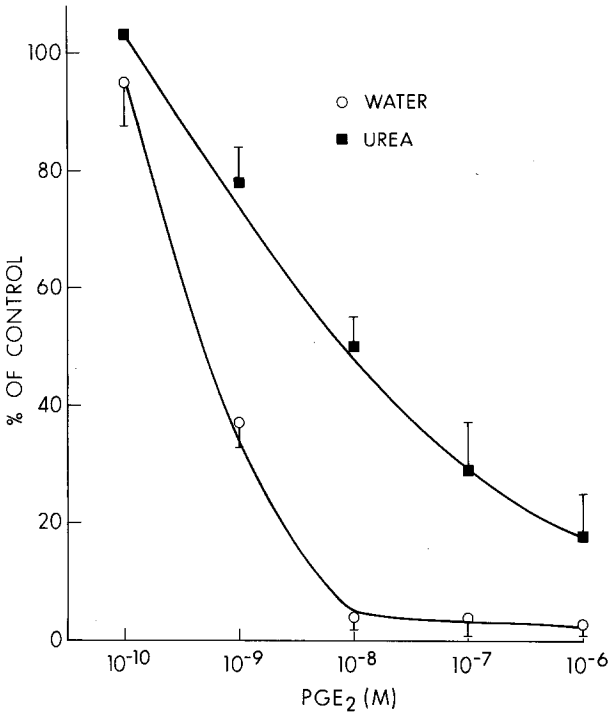

Fig. 4. Inhibition of water flow (open circles) and urea transport (filled squares) by PGE_2. Data are shown as percentages of paired control bladders not receiving PGE_2

sensitive than water flow to the inhibitory action of the prostaglandins. Accordingly, the effect of exogenous prostaglandins on urea and water transport was tested in paired hemibladders, one of which received prostaglandins, the other acting as a control. Both experimental and control hemibladders were pretreated for 1 hr with 10^{-6}M naproxen to eliminate endogenous prostaglandin synthesis. Prostaglandin was then added to the serosal bath of the experimental bladder, followed 30 min later by 1 mU/ml vasopressin to both hemibladders. Figure 4 shows that over a wide range of PGE_2 concentrations, water flow was significantly more inhibited than urea transport. Similar results were obtained with PGE_1, and $PGF_{2\alpha}$. Thus, the relative unresponsiveness of water flow to low concentration of vasopressin appears to be due in part to the greater sensitivity of the water flow system to endogenous prostaglandins.

Effects of 8-Bromo cAMP and cAMP plus Theophylline

8-bromoadenosine cyclic AMP, a potent analogue of cyclic AMP, affected water flow and urea transport in a manner opposite to that

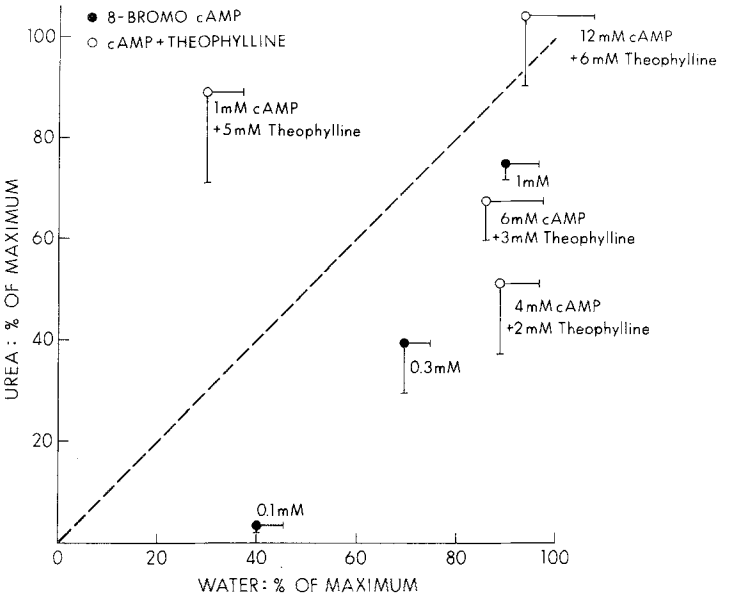

Fig. 5. Comparison of response of urea and water transport to 8-bromo cyclic AMP (filled circles) and to combinations of cyclic AMP and theophylline (open circles). *See* text for details

Table 4. Comparative effects of graded 8-Bromo cAMP concentrations and saturating concentration of vasopressin

8-Bromo cAMP concentration (mM)	Response to 8-Br cAMP			Response to 86 mU/ml vasopressin			$\frac{\Delta \text{ 8-Br cAMP}}{\Delta \text{ vasopressin}}$
	Basal	8-Br cAMP	Δ	Basal	Vaso-pressin	Δ	(%)
				Water flow (μl/min)			
0.1 (4)	4.5	30.4	25.9±4.8	6.4	70.9	64.5±7.0	40±6
0.3 (5)	3.3	38.8	35.5±4.9	3.8	54.2	50.4±4.0	70±4
1.0 (3)	2.6	51.8	49.2±6.5	2.9	57.4	54.5±3.4	90±7
				K_{trans} urea (cm/sec × 10^7)			
0.1	94	114	20 ±8	103	588	485 ±41	4±1
0.3	41	161	120 ±39	41	386	345 ±86	39±10
1.0	85	296	211 ±53	75	355	280 ±68	75±2

of vasopressin: a preferential stimulation of water flow. This was most pronounced at 0.1 mM 8-bromo cyclic AMP, which increased water flow to 40% of maximum, with virtually no effect on urea transport (Fig. 5, Table 4). At 0.3 and 1.0 mM 8-Bromo cAMP the experimental points continued to fall to the right of the identity line.

Table 5. Direct comparison of two combinations of cAMP and theophylline on transport in 6 paired hemibladders

Period	cAMP 5 mM, theophylline 1 mM	cAMP 1 mM, theophylline 5 mM	*P* value
	Water flow (μl/min)		
Basal	2.9±0.3	3.3±0.4	NS
Stimulated	40.8±2.5	28.2±3.3	<0.01
Δ	37.9±2.4	24.9±3.4	<0.01
	K_{trans} urea (cm/sec × 10^7)		
Basal	38 ±15	41 ±14	NS
Stimulated	105 ±47	138 ±42	<0.05
Δ	67 ±33	97 ±30	<0.05

In additional experiments, also shown in Fig. 5, we found that water flow or urea transport could be preferentially activated by varying the ratio of exogenous cAMP and theophylline. When the ratio of cAMP to theophylline was 2:1, water flow was preferentially stimulated at 4 and 6 mM cAMP, but not 12 mM cAMP. When 5 mM theophylline and 1 mM cAMP were used, urea transport was 90% of maximum, while water flow was less than 30%. To confirm the finding that the cAMP-theophylline ratio determined which pathway was stimulated, paired experiments were carried out, which showed that 5 mM cAMP plus 1 mM theophylline activated water flow to a greater extent, and urea transport to a lesser extent than 1 mM cAMP plus 5 mM theophylline (Table 5).

Effect of Vasopressin on the Permeability of a Lipophilic Solute

To determine whether the effect of vasopressin on urea transport is representative of its effect on all solutes, we studied its effect on phenytoin (Dilantin®), a highly lipophilic solute, again in comparison to water flow (Fig. 6, Table 6). Vasopressin, cAMP, and cAMP plus theophylline all increased phenytoin permeability to the same extent as water flow, with the experimental points falling on the identity line.

Effect of Vasopressin on Sodium and Urea Transport

In a limited number of experiments, the effect of 2 mU/ml of vasopressin on sodium and urea transport was compared to the effect of 86 mU

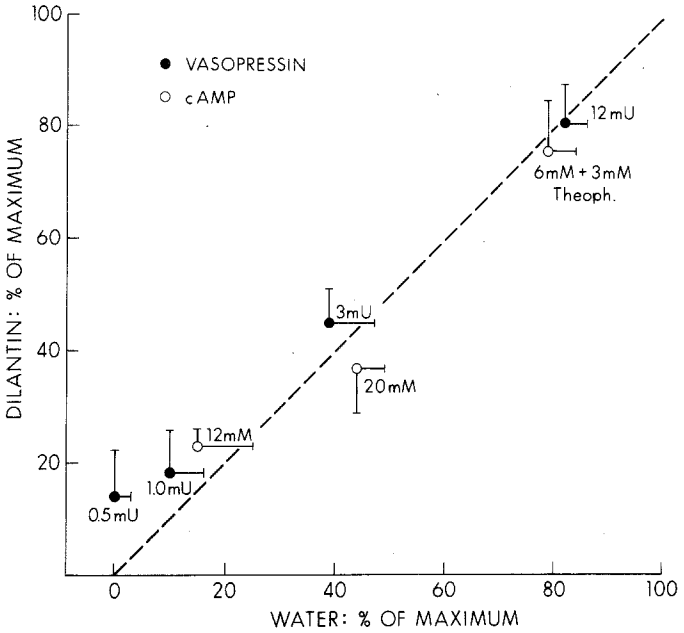

Fig. 6. Response of phenytoin (Dilantin) and water transport to vasopressin filled circles) and cyclic AMP (open circles)

Table 6. Comparative effects of low and saturating concentrations of vasopressin on water flow and K_{trans} phenytoin

Vasopressin concentration (mU/ml)	Response to low vasopressin concentration			Response to 86 mU/ml vasopressin			$\frac{\Delta \text{ low conc.}}{\Delta \text{ 86 mU/ml}}$
	Basal	Vasopressin	Δ	Basal	Vasopressin	Δ	(%)
				Water flow (μl/min)			
0.5 (5)	2.7	2.3	−0.4±0.7	3.3	39.2	35.9±5.1	0±3
1.0 (5)	2.6	6.9	4.3±3.3	3.3	46.1	42.8±5.9	10±6
3.0 (6)	2.6	20.0	17.4±3.8	3.1	47.9	44.8±1.8	39±8
12.0 (3)	2.9	32.5	29.6±2.7	3.0	39.0	36.0±2.1	82±4
				K_{trans} phenytoin (cm/sec × 10^7)			
0.5	60	73	13 ±6	57	148	91 ±16	14±8
1.0	36	51	15 ±7	38	122	84 ±27	18±7
3.0	38	66	28 ±6	39	101	62 ±10	45±6
12.0	34	66	32 ±6	31	70	39 ± 4	82±7

per ml. These experiments were carried out in a Lucite chamber with a central dividing partition, permitting direct comparison of the low and saturating dose of vasopressin. Isotonic Ringer's solution bathed

Table 7. Comparative effects of graded cAMP concentrations and saturating concentration of vasopressin on water flow and K_{trans} phenytoin

cAMP concentration (mM)	Response to cAMP			Response to 86 mU/ml vasopressin			$\frac{\Delta \text{ cAMP}}{\Delta \text{ vasopressin}}$
	Basal	cAMP	Δ	Basal	Vasopressin	Δ	(%)
				Water flow (μl/min)			
12 (3)	2.4	10.0	7.6± 5.0	2.8	53.7	50.9± 3.4	15±10
20 (6)	2.9	27.2	24.3± 3.4	2.1	57.3	55.2± 2.2	44± 5
6+3 mM theoph. (3)	3.2	46.2	43.0± 2.2	3.2	57.4	54.2± 1.4	79± 5
				K_{trans} phenytoin			
12	70	91	21 ± 8	59	145	86 ±22	24± 3
20	58	91	33 ± 7	65	155	90 ±12	37± 8
6+3 mM theoph.	75	130	55 ±10	64	137	73 ± 6	76± 9

both sides of the bladder. In 5 paired experiments, urea transport following 2 mU vasopressin was 66±12% of maximum, while the peak rise of short-circuit current was 57±18% of maximum. Thus, the sensitivity of the sodium transport response was comparable to that of urea. Water flow was not determined; however, Ferguson and Twite (1974) have reported that sodium transport responds to vasopressin prior to water flow in the toad bladder.

Discussion

The complexity of the response to vasopressin varies from tissue to tissue. In the mammalian cortical collecting duct, only water flow appears to be accelerated by vasopressin (Grantham & Burg, 1966; Rocha & Kokko, 1974), while permeability to both water and urea may be increased in the medulary segment (Morgan, Sakai & Berliner, 1968). In the toad bladder, water, sodium, amides, and the majority of small hydrophilic and lipophilic molecules increase their transcellular movement following vasopressin (Leaf & Hays, 1962; Pietras & Wright, 1974; Hays, 1976*b*). Current evidence indicates that several vasopressin-sensitive transport systems are involved in water and solute transport. The luminal membrane pathway for water may well exclude all solutes, even those as small as urea (Levine, Franki & Hays, 1973; Finkelstein, 1976). This conclusion is based on the observation that phloretin, permanganate,

and other luminally-acting agents inhibit urea transport and have no effect on water flow (Levine *et al.*, 1973; Franki, Einhorn & Hays, 1975). Morphologically, the water pathway is identified with the intramembranous particle aggregates that appear in the luminal membrane following vasopressin (Chevalier, Bourguet & Hugon, 1974; Kachadorian, Wade & DiScala, 1975). Studies with selective inhibitors or stimulators of water and solute transport have supported the view that the particle aggregates transport water only (Kachadorian *et al.*, 1977; Levine, Kachadorian & Schlondorff, 1979). The nature and number of luminal membrane pathways for solutes is not clearly established, nor has any structural component of the membrane involved in solute transport been identified.

The concept of "separate pathways" for water and for classes of solutes involves more than the events at the luminal membrane. There is evidence that early steps in the adenylate cyclase-cyclic AMP sequence may be directed towards water or solute transport. The anesthetic agents methohexital and methoxyflurane inhibit vasopressin-stimulated, but not cyclic AMP-stimulated water flow, with no effect on urea or sodium transport (Levine *et al.*, 1976*a*). This indicates that the action of the anesthetics is at a step prior to the generation of cyclic AMP. Recent studies (Levine *et al.*, 1976*b*) have shown that these agents are potent inhibitors of toad bladder adenylate cyclase. Similar results have been obtained with the metabolic inhibitors rotenone and dinitrophenol, which selectively inhibit water flow at an early step in the cyclic nucleotide sequence; methylene blue, on the other hand, inhibits urea transport, but not water flow, and appears to act prior to the generation of cyclic AMP (Hays, Franki & Ross, 1979). These early selective effects are not confined to inhibitory agents; hydrazine, which stimulates toad bladder adenylate cyclase, selectively enhances vasopressin-stimulated water flow, with no change in urea or sodium transport (Levine *et al.*, 1977). Thus, on the basis of a series of studies with pharmacologic agents, there is evidence for functional independence of the adenylate cyclase-cyclic AMP systems involved in water and solute transport.

In the present studies, we have shown that selective responses of transport systems in the toad bladder can be produced under physiologic conditions, by using low concentrations of vasopressin. Urea transport appears to be far more sensitive to vasopressin than is water flow. At 1 mU/ml vasopressin, for example, urea transport reached 70% of its maximum rate, while water flow had barely begun. A number of vasopressin analogues also activated urea transport before water transport. The possibility of separate receptors deserves further study.

The preferential activation of urea transport produced by vasopressin was not seen when cyclic AMP was employed, suggesting that the relative sensitivity of the urea transport system resides in a step or steps activated by vasopressin, but not cyclic AMP. It appears that at least part of the difference in sensitivity can be attributed to the greater inhibitory effect of prostaglandins on vasopressin-stimulated water flow, when compared to prostaglandin inhibition of urea transport. This was shown in two ways: first, in bladders treated with naproxen, an inhibitor of prostaglandin synthesis, the responsiveness of water flow increased to a relatively greater extent than of urea transport. Second, in experiments directly testing the inhibitory effects of prostaglandins E_1, E_2, and $PGF_{2\alpha}$, inhibition of water flow was significantly greater than that of urea transport. Since vasopressin stimulates endogenous prostaglandin synthesis in the toad bladder (Zusman *et al.*, 1977), we would propose the selective inhibition of water flow by endogenously produced prostaglandins as one factor contributing to the sequential response of urea and water to vasopressin. At low concentrations of vasopressin, urea transport would be activated prior to water transport, permitting equilibration of urea across bladder or (by analogy) across the collecting duct. This may be advantageous for the maintenance of a high medullary concentration of urea in the mammalian countercurrent system, and for the subsequent reabsorption of water.

The observation that prostaglandins inhibit water flow to a greater extent than urea transport suggests that the adenylate cyclase step is important in determining the relative selectivity of the two systems to vasopressin. This conclusion is based on the assumption that prostaglandins inhibit adenylate cyclase in the toad bladder. There is some evidence that this is the case: Omachi *et al.* (1974), for example, have shown that cyclic AMP levels following vasopressin are reduced by prostaglandins. However, these experiments were carried out at concentrations of vasopressin plus theophylline which would have stimulated water flow maximally, and it is not certain that prostaglandins were effective as inhibitors of water flow under these conditions. Attempts to demonstrate a direct inhibitory action of prostaglandins on toad bladder adenylate cyclase have had limited success (Lipson, Hynie & Sharp, 1971; Bar *et al.*, 1970). Therefore, in the absence of conclusive evidence for the site of action of prostaglandins in the toad bladder, the role of adenylate cyclase in determining sensitivity remains speculative.

In addition to urea, we studied the response of phenytoin (Dilantin) a highly lipophilic solute, to graded concentrations of vasopressin and

cyclic AMP. In contrast to urea, phenytoin exhibited the same sensitivity as water, suggesting that lipophilic solutes move across the luminal membrane by a pathway which is distinct from the amide pathway. It is possible that lipophilic solutes penetrate the lipid phase of the membrane directly, and that the small increase in the permeability of phenytoin following vasopressin reflects the small change in membrane fluidity reported by Pietras and Wright (1974) and Masters, Yguerabide and Fanestil (1978).

The relative response to vasopressin of sodium and water transport across the toad bladder has been studied by Ferguson and Twite (1974), who found that sodium transport was activated prior to that of water. In our own studies, we examined the relative response of sodium and urea transport and found that they showed almost identical sensitivity to low concentrations of vasopressin. This is in accord with the observation of Ferguson and Twite, since the water response lags significantly behind that of urea. Despite the similarity of the sodium and urea response in our studies, we would not conclude that urea and sodium share the same luminal membrane pathway, since a number of agents (including phloretin, permanganate, and chromate) block the response of urea to vasopressin and cyclic AMP but not that of sodium (Levine *et al.*, 1973; Franki *et al.*, 1975). It is entirely possible that separate membrane pathways exist for water, amides, lipophilic solutes, and sodium. How hydrophilic solutes other than the amides cross the membranes has not been extensively studied, but will be discussed in the accompanying paper.

The intricacy of the system controlling water and solute permeability is apparent when we consider the effects of 8-Bromo cAMP and combinations of cAMP and theophylline on water and urea transport. 8-Bromo cAMP, a synthetic analogue of cAMP (Ikehara & Uesugi, 1969; Muneyama *et al.*, 1971), stimulated water flow to 40% of its maximum value, with no effect on urea transport. Stadel and Goodman (1978) have recently reported a similar effect of 8-*p*-chlorophenylthio-cAMP, another potent analogue of cAMP. The mechanism of action of 8-Br cAMP in the toad bladder has not been completely characterized; preliminary studies (D. Schlondorff, N. Franki, S.D. Levine & R.M. Hays, *unpublished*) have shown that it stimulates cAMP-dependent protein kinase and has little inhibitory effect on phosphodiesterase. The ability of 8-Br cAMP to stimulate water flow alone suggests that alternate pathways exist at and beyond the kinase step which regulate water and solute permeability.

Although exogenous cAMP alone did not preferentially stimulate

water or urea transport, we found that cAMP in combination with theophylline activated one or the other transport systems, depending on the ratio of the two agents. A 2:1 ratio of cAMP to theophylline preferentially stimulated water flow while 1:5 cAMP/theophylline preferentially stimulated urea transport. It is not possible to give any detailed interpretation of these findings, except to say that they strengthen the case for independent control systems. In the following paper, we have utilized these techniques for independent stimulation to investigate the properties of the membrane pathways involved in water and solute transport.

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