Stimulation of Osmotic Water Flow in Toad Bladder by Prostaglandin E_1

EVIDENCE FOR DIFFERENT COMPARTMENTS OF CYCLIC AMP

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ABSTRACT The effect of prostaglandin E_1 (PGE_1) on osmotic water flow across toad bladder and cyclic AMP content of the mucosal epithelial cells has been determined under basal conditions and in the presence of either theophylline or antidiuretic hormone (ADH). Under basal conditions and with PGE_1 concentrations from 10^{-8} to 10^{-5} M no evidence of stimulation of water flow was observed, and with 10^{-7} M PGE_1 a significant inhibition was found. Cyclic AMP content under control conditions was 8 pmol/mg protein. It was 9 at 10^{-8} M PGE_1, 13 at 10^{-7} M, 16 at 10^{-6} M, and 23 at 10^{-5} M. In the presence of theophylline, 10^{-8} and 10^{-7} M PGE_1 inhibited the theophylline-induced water flow as expected. In contrast, 10^{-6} and 10^{-5} M PGE_1 enhanced the rate of water flow. Theophylline increased cyclic AMP content from 8 to 18 pmol/mg protein. PGE_1 in the presence of theophylline caused marked increases in cyclic AMP content. The content was 23 at 10^{-7} M, 41 at 10^{-6} M, and 130 at 10^{-5} M. Thus PGE_1 stimulates theophylline-induced water flow at cyclic AMP concentrations somewhere between 23 and 41 pmol/mg. Further evidence along these lines was obtained from experiments in which the effects of PGE_1 on ADH-induced water flow were studied. Inhibitory effects of PGE_1 were not observed at concentrations of PGE_1 which raised the level of intracellular cyclic AMP to 30 pmol/mg protein or higher.

These results were obtained despite the fact that all four concentrations of PGE_1 tested were found capable of inhibiting ADH-induced water flow under appropriate conditions or, in other words, were inhibiting the adenylate cyclase controlling water flow. Thus the increase in cyclic AMP content in response to PGE_1 is not derived from this enzyme. Thus the stimulation of water flow by PGE_1 in the presence of theophylline is thought to be caused by cyclic AMP spilling over from one compartment to the water flow compartment. No evidence was obtained to directly suggest spillover into the sodium transport compartment. Furthermore evidence is discussed to suggest that most of the cyclic AMP generated in the tissue does not originate from the enzyme controlling sodium transport. As cyclic AMP-stimulated water flow and sodium transport are thought to occur in one cell type, the granular cells, distinct pools of cyclic AMP are thought to be present in one and the same cell type. Thus one pool controls water flow and one controls sodium transport. With high concentrations of PGE_1 in the presence of theophylline or high concentrations of ADH, the adenylate cyclase responsible for water flow is inhibited. However, PGE_1 can stimulate a tissue adenylate cyclase to sufficiently high levels that cyclic AMP spills over into the "water flow compartment" and thus stimulates water flow.

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INTRODUCTION

Two major functions of the toad urinary bladder, sodium transport and water reabsorption, are stimulated by antidiuretic hormone (ADH)[1] (1). Both are mediated by adenosine 3',5'-monophosphate (cyclic AMP) resulting from the stimulation of adenylate cyclase by

[1] *Abbreviations used in this paper:* ADH, antidiuretic hormone; PGE_1, prostaglandin E_1; TCA, trichloroacetic acid.

ADH (2–5). Evidence for the existence of two distinct adenylate cyclases, one controlling sodium transport and one osmotic water flow has been presented previously (6–8). For instance, the water flow effect of ADH can be dissociated from that of sodium transport. In the presence of a high concentration of calcium in the bathing medium, the ADH-induced water flow is inhibited, leaving the effect on sodium transport intact (6, 7). As high calcium in the medium does not affect the response to exogenous cyclic AMP the effect of calcium must be to inhibit an adenylate cyclase responsible for water flow. Additional evidence has been gained from studies on prostaglandins. Prostaglandin E_1 (PGE_1) has been shown to stimulate sodium transport by stimulation of adenylate cyclase and to inhibit ADH-induced water flow by inhibition of adenylate cyclase (8). These findings are compatible with the existence of two adenylate cyclases controlling sodium transport and water flow separately. Since sodium transport and water flow appear to be functions of the same cell type (granular cells of the mucosal epithelium), both adenylate cyclases would have to be present in this cell type. Thus compartmentalization of cyclic AMP within the cell must also be present in order that dissociation of the effects of ADH on sodium transport and water flow can exist in the presence of Ca^{++} or for prostaglandins to stimulate sodium transport while inhibiting water flow.

We have examined the question of compartmentalization further by studying the effects of prostaglandins on water flow in the presence and absence of theophylline and in the presence and absence of ADH, while correlating the effects with cyclic AMP concentrations in the mucosal epithelial cells. We have also studied the interaction of prostaglandins and ADH on sodium transport.

METHODS

Experiments were performed on bladders of the toad *Bufo marinus* (obtained from National Reagents, Bridgeport, Conn.). The Ringer solution used had the following composition: NaCl, 113.5 mM; KCl, 3.5 mM; $NaHCO_3$, 2.4 mM; $CaCl_2$, 0.9 mM; pH = 8.0, osmolality = 220–225 mosmol/kg water.

Water flow. Osmotic water flow was measured in paired half bladders mounted on glass tubes according to the technique of Bentley (9). Ringer solution diluted 1:5 with distilled water was placed on the mucosal surface of the half bladder. The serosal surface was bathed by full strength isotonic Ringer solution. After an initial stabilization period, water flow was measured in paired half bladders by weighing the bladders at the beginning and end of each test period. Specific details of the test situations are given in Results and in the legends. The results are expressed in milligrams H_2O lost by the half bladder per 30 min.

Sodium transport. Sodium transport across the bladders was measured by the short circuit current technique of Ussing and Zerahn (10) using double chambers so that one half bladder provided both an experimental and a control tissue (11). All bladders were allowed to stabilize before addition of PGE_1, ADH, or vehicle. When combinations of PGE_1 and ADH were tested the agents were added simultaneously. In order to normalize the results between different bladders, sodium transport was expressed as the peak current in response to the addition of test agents minus the starting current divided by the starting current and then multiplied by 100 (i.e., percent increase in current).

Cyclic AMP. Toad bladders were cut into portions of approximately equal size. After preincubation in Ringer solution the pieces were incubated during 30 min in control Ringer or Ringer containing different concentrations and combinations of PGE_1, theophylline, and ADH (Pitressin, Parke, Davis & Co., Detroit, Mich.). Immediately after incubation, the mucosal epithelial cells were rapidly scraped off the bladders and immersed in liquid nitrogen. Cyclic AMP was extracted from the frozen cells by 1 ml of 8% trichloroacetic acid (TCA) containing a small amount of [^{3}H]cyclic AMP for recovery calculation. Thorough Vortexing was followed by centrifugation at 1,200 *g* for 15 min. The supernate was purified by chromatographic columns (0.8 × 8 cm) containing 4 ml Bio-Rad AG 50 W-X4 (200–400 mesh) resin (Bio-Rad Laboratories, Richmond, Calif.) in water (1:1 vol/vol) previously equilibrated with 0.1 N HCl. With this technique cyclic AMP eluted in the 4th to 6th ml of water added to the column. The eluent was dried at 50°C in a Rotary Evapo-Mix (Buchler Instruments Div., Searle Analytic, Inc., Fort Lee, N. J.) and the resulting powder diluted with 50 mM Tris buffer (pH 7.4). 100-μl aliquots of this solution were counted for calculation of the recovery (70–80%). Similar volumes were assayed in duplicate for cyclic AMP by the protein binding method of Brown, Albano, Ekins, Sgherzi, and Tampion (12). The incubation tubes contained 100 μl of sample, 50 μl of [^{3}H]-cyclic AMP containing a total of 1 pmol (sp. act, 24 Ci/mmol), and 100 μl of a 1:20 dilution of binding protein prepared according to Brown et al. (12). The incubation took place at 4°C during 90 min and was terminated by adding 200 μl of a suspension containing 20 mg of activated charcoal (Norit A., J. T. Baker Chemical Co., Phillisburg, N. J.) and 2% bovine albumin. After centrifuging at 1,800 *g* for 15 min, 200 μl of the supernate was counted in a liquid scintillation spectrometer. The concentration of cyclic AMP is expressed in picomoles of cyclic AMP per milligram protein. Protein was measured by the method of Lowry, Rosebrough, Farr, and Randall (13) after dissolving the pellets in 0.5 N NaOH.

Statistics. All were performed by Student's *t* test for paired tissues.

RESULTS

Effects of PGE_1 on osmotic water flow

Basal flow. Basal flow was measured in paired half bladders for 30 min. One of the paired half bladders received PGE_1 (four concentrations were tested) while the other was used as a control. From the results shown in Table I it can be seen that PGE_1, at a concentration of 10^{-7} M significantly inhibited basal water flow during the test period relative to the control half bladder. At 10^{-8}, 10^{-6}, and 10^{-5} M PGE_1, basal water flow was not lowered significantly.

TABLE I

Effect of PGE_1 on Basal Water Flow

PGE_1	Basal water flow: Control	Basal water flow: Test	Δ	*P*	*n*
M		*mg/30 min*			
10^{-8}	29±4	24±5	−5±6	0.5	7
10^{-7}	33±5	21±5	−12±5	<0.05	7
10^{-6}	33±3	25±5	−8±5	<0.2	7
10^{-5}	35±5	28±8	−7±10	0.5	7

Basal water flow was measured in paired half bladders in the presence and absence of PGE_1. Statistical analysis was performed by Student's *t* test on paired half bladders.

Water flow in the presence of theophylline. Basal water flow was measured in paired half bladders for 30 min. Then one of the halves received 10 mM theophylline and the other, theophylline plus PGE_1. Four concentrations of PGE_1 from 10^{-8} to 10^{-5} M were again tested. The results are presented in Fig. 1. Theophylline caused a stimulation of water flow in all four series. The effect of PGE_1 at 10^{-8} and 10^{-7} M, shown in the two left-hand panels of Fig. 1, was to inhibit the theophylline-induced water flow. In contrast, at concentrations of 10^{-6} and 10^{-5} M PGE_1 (right-hand panels) a significant stimulation of theophylline-induced water flow occurred. Thus both inhibitory and stimulatory effects of PGE_1 on water flow have been detected.

Effects of PGE_1 on cyclic AMP content in the presence and absence of theophylline

The amount of cyclic AMP in mucosal epithelial cells was measured in response to different concentrations of PGE_1 in the presence and absence of theophylline. In the absence of theophylline, 10^{-7} M PGE_1 was the lowest concentration to significantly increase cyclic AMP levels (from 8 to 13 pmol/mg protein). 10^{-6} and 10^{-5} M PGE_1 caused cyclic AMP to increase to 16 and 23 pmol/mg protein, respectively. In the presence of theophylline the stimulatory effects of PGE_1 were observed over the same concentration range (10^{-7}–10^{-5} M). Theophylline alone increased the cyclic AMP to 18 pmol/mg protein. PGE_1 at concentrations of 10^{-7}, 10^{-6}, and 10^{-5} M, in the presence of theophylline caused increases in cyclic AMP to 23, 41, and 130 pmol/mg protein, respectively. The results are shown in Fig. 2.

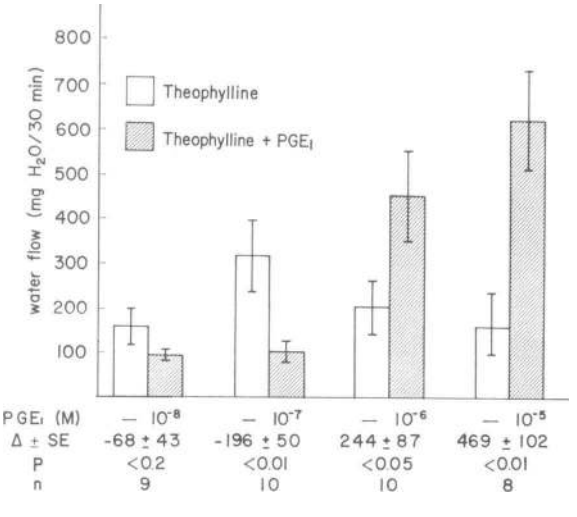

FIGURE 1 The effect of PGE_1 on theophylline-induced water flow. Results are expressed in milligrams H_2O/30 min per half bladder. Vertical lines represent ±SEM.

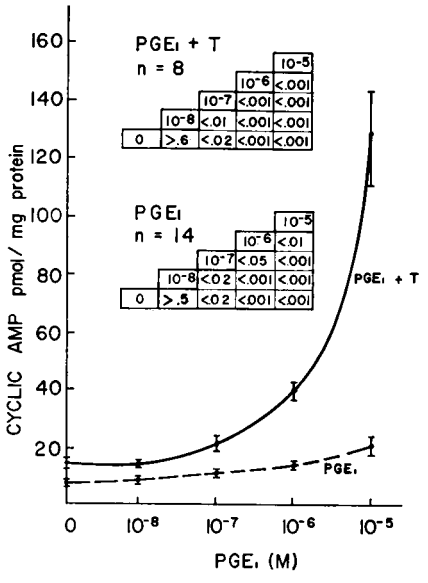

FIGURE 2 The effect of PGE_1 on cyclic AMP content of mucosal epithelial cells of toad bladder in the presence and absence of 11 mM theophylline. Results are expressed as picomoles cyclic AMP per milligram TCA-precipitable protein. Vertical lines represent ±SEM.

Effects of PGE_1 on ADH-stimulated osmotic water flow

When the effects of PGE_1 were tested in the presence of 25 mU/ml ADH, a significant inhibition of the ADH-induced water flow was observed with 10^{-8}, 10^{-7}, and 10^{-6} M PGE_1 (see Fig. 3). No significant inhibition was observed with 10^{-5} M.

In order to test whether 10^{-5} M PGE_1 can inhibit ADH-induced water flow, this concentration was tested against three different concentrations of ADH. In these experiments (Fig. 4) 10^{-5} M PGE_1 significantly inhibited the effect of 2.5 mU/ml ADH but had no effect when tested against 25 and 250 mU/ml ADH. That these high concentrations of ADH can be inhibited by PGE_1 is shown in Fig. 5, in which 10^{-7} M PGE_1 was inhibitory to 2.5, 25, and 250 mU/ml ADH.

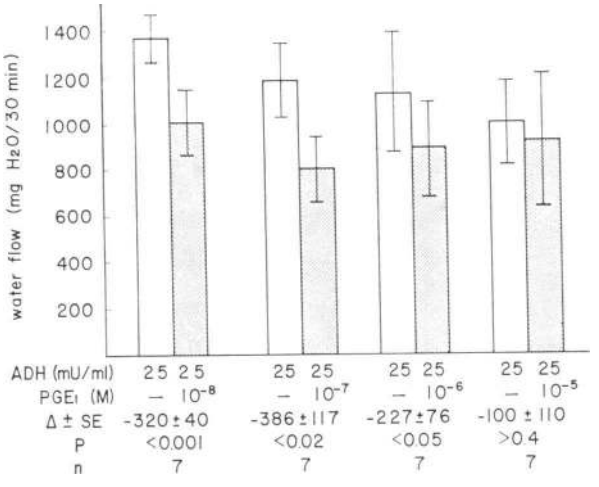

FIGURE 3 The effect of different concentrations of PGE_1 on the water flow response to 25 mU/ml ADH. Vertical lines represent ±SEM.

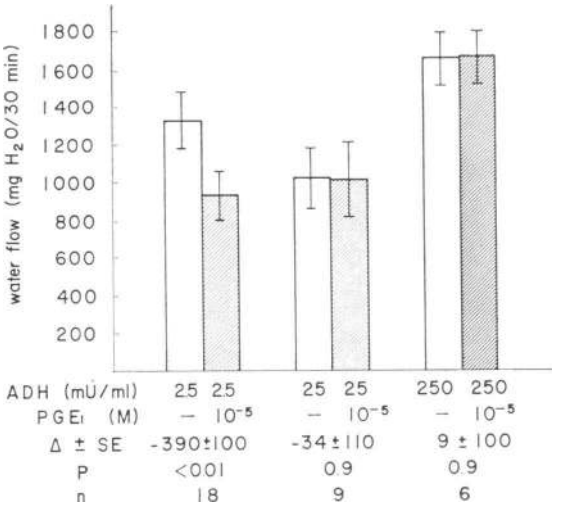

FIGURE 4 The effect of 10^{-5} M PGE_1 on the water flow response to 2.5, 25, and 250 mU/ml ADH. Vertical lines represent ±SEM.

Effects of ADH on cyclic AMP content in the presence and absence of 10^{-5} M PGE_1

2.5, 25, and 250 mU/ml ADH all caused a significant increase in cyclic AMP content from a control value of 11 to 15, 15, and 20 pmol/mg protein, respectively (see Fig. 6). 10^{-5} M PGE_1 increased the content to 21 pmol/mg and the combination of PGE_1 plus ADH showed further concentration-dependent increases of cyclic AMP. With 2.5, 25, and 250 mU/ml ADH and 10^{-5} M PGE_1 the cyclic AMP contents were 24, 30, and 38.0 pmol/mg protein, respectively.

Effects of PGE_1 on ADH-stimulated sodium transport

In preliminary experiments ADH at 5 mU/ml was tested for its ability to stimulate sodium transport relative to the effect of 25 mU/ml. The stimulation due to 5 mU/ml was 64% that of 25 mU/ml ($n = 5$), demonstrating that this concentration was submaximal. Two series of experiments were then performed. In the first series 4 mU/ml ADH was tested in the presence and absence of PGE_1 at three concentrations 2.5×10^{-7}, 2.5×10^{-6}, and 2.5×10^{-5} M. None of these concentrations of PGE_1 had any effect upon the response to ADH. In the second series 1×10^{-6} and 1×10^{-5} M PGE_1 was tested against ADH and again no difference was detected in the response. The results are presented in Table II.

DISCUSSION

The results in this paper present evidence that PGE_1 can stimulate osmotic water flow in toad bladder. This occurred with 10^{-6} and 10^{-5} M PGE_1 in the presence of theophylline. Stimulation of the water flow response to theophylline by 1.7×10^{-6} M PGE_1 was mentioned in an earlier report by Orloff, Handler, and Bergstrom (14) when they used the bag technique of Bentley (9). It is interesting that using a chamber method for the measurement of water flow 1.7×10^{-10}, 10^{-9}, and 10^{-7} M PGE_1 significantly inhibited theophylline-induced water flow while no effect was detected with 1.7×10^{-6} M. As with our experiments the increase in water flow seen with high concentrations of PGE_1 contrasts with the inhibitory effect of 10^{-7} M on the theophylline-in-

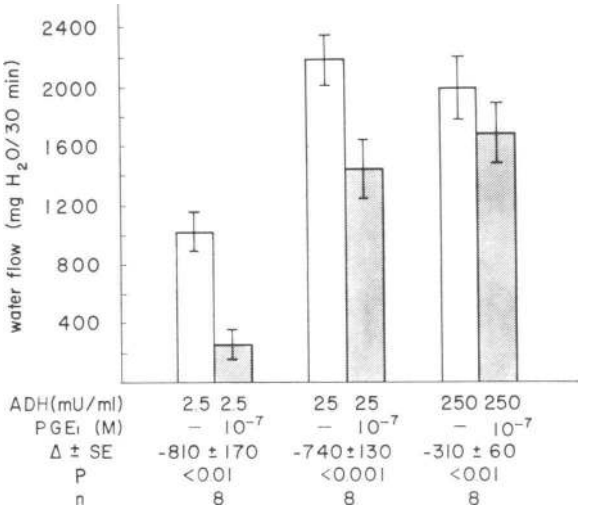

FIGURE 5 The effect of 10^{-7} M PGE_1 on the water flow response to 2.5, 25, and 250 mU/ml ADH. Vertical lines represent ±SEM.

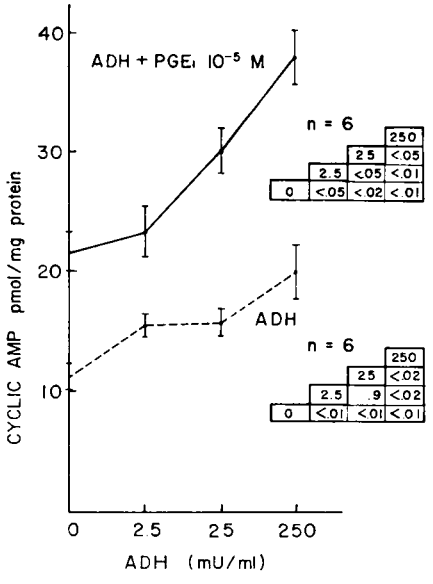

FIGURE 6 The effect of different concentrations of ADH on cyclic AMP content of mucosal epithelial cells of toad bladder in the presence and absence of 10^{-6} M PGE_1. Results are expressed as picomoles cyclic AMP per milligram TCA-precipitable protein. Vertical lines represent ±SEM.

duced water flow. That the increase cannot be due to stimulation of the adenylate cyclase responsible for water flow is shown by the results obtained with PGE_1 alone, in which no stimulation of basal water flow was observed. The lack of significant inhibition (except for 10^{-7} M) may well reflect the inherent difficulty of measuring the small changes in water flow under basal conditions and is similar to our previous results (8), in which no significant decreases were seen.

One explanation for the stimulation of water flow by high concentration of PGE_1 in the presence of theophylline could be that PGE_1 is an antagonist of ADH-stimulated adenylate cyclase at low concentrations but is an agonist at high concentrations. This we have specifically ruled out by demonstration of an inhibitory effect of 10^{-6} M PGE_1 against 25 mU/ml ADH (Fig. 3) and of 10^{-5} M PGE_1 against 2.5 mU/ml ADH (Fig. 4). Thus we must seek an alternative explanation for the stimulation of osmotic water flow seen with 10^{-6} and 10^{-5} M PGE_1 in the presence of theophylline.

TABLE II

Effect of PGE_1 on ADH-Stimulated Sodium Transport

ADH	PGE_1	Sodium transport			P	n
		Control	Test	Δ		
mU/ml	*M*					
4	2.5×10^{-7}	49±12	43±8	−6±7	>0.3	8
4	2.5×10^{-6}	41±8	39±7	−2±4	>0.6	11
4	2.5×10^{-5}	44±8	45±9	1±3	>0.7	7
4–5	1×10^{-6}	50±9	52±13	2±13	>0.9	6
4–5	1×10^{-5}	67±14	74±14	7±14	>0.6	7

Sodium transport was measured in paired quarter bladders, and the results are expressed as: 100 × (peak short circuit current − initial short circuit current)/initial short circuit current. Statistical analysis was performed by Student's *t* test on paired quarter bladders.

The concentrations of PGE_1 which increase cyclic AMP levels in the cells are from 10^{-7} to 10^{-5} M. This concentration range corresponds with the dose-response characteristics of the stimulation of sodium transport by PGE_1 in toad bladder (8) and with the range over which stimulation of adenylate cyclase in toad bladder cell homogenates is observed (15). Thus it is possible that the rise in cyclic AMP in the cells in response to PGE_1 could be ascribed to stimulation of the adenylate cyclase responsible for sodium transport, and the possibility exists that cyclic AMP from a sodium transport compartment can spill over into the water flow compartment. Consideration of the amounts of cyclic AMP in the tissue under conditions of PGE_1 stimulation in the presence and absence of theophylline give support to this idea. 10^{-5} M PGE_1 alone gave a value of 23 pmol cyclic AMP/mg protein. No stimulatory effect on water flow was seen. In the presence of theophylline, however, concentrations of PGE_1 which enhance water flow, 10^{-6} and 10^{-5} M, increased cyclic AMP levels to values in excess of 23 pmol/mg protein, the levels being 41 and 130 pmol, respectively. Therefore, at levels of cyclic AMP somewhere between 23 and 41 pmol/mg, the concentration of cyclic AMP is sufficient to stimulate water flow. It is difficult, however, to equate the PGE_1-stimulated rise in cyclic AMP levels with the stimulation of sodium transport. In a previous study we found that maximal stimulation by PGE_1 was only 40% of the stimulation that could be achieved by ADH. Similarly, in our present experiments no enhancement by PGE_1 of the stimulation of sodium transport by submaximal concentrations of ADH was observed. Indeed, Albert and Handler (16) have presented evidence that a small inhibition of sodium transport can be detected in the presence of PGE_1. Thus the *large* stimulation of cyclic AMP content in the mucosal epithelial cells of toad bladder would not appear to be directly related to the stimulation of sodium transport. Thus, although spillover of cyclic AMP would appear to be the cause of the PGE_1-induced water flow, it seems unlikely that the source of the cyclic AMP is the pool responsible for sodium transport.

Further evidence for a spillover of cyclic AMP from some compartment to the water flow compartment comes from a combination of the effects of PGE_1 and ADH on osmotic water flow and intracellular cyclic AMP content. 25 mU/ml stimulates osmotic water flow maximally and 2.5 mU/ml was a submaximal concen-

tration (tested for this batch of vasopressin, results not shown). When different concentrations of PGE_1 were tested against 25 mU/ml ADH, inhibition of water flow was obtained with 10^{-8}, 10^{-7}, and 10^{-6} M PGE_1 but not with 10^{-5} M PGE_1. The combination of 10^{-5} M PGE_1 and 25 mU/ml ADH gave a value of 30 pmol cyclic AMP/mg protein for the intracellular content (i.e., in the spillover range). When tested with 2.5 mU/ml ADH inhibition by 10^{-5} M PGE_1 was observed and the cyclic AMP content in this case was 24 pmol/mg protein. This corresponds with the 23 pmol/mg protein obtained with the combination of 10^{-7} M PGE_1 and theophylline with which inhibition of water flow was seen (i.e., this concentration of cyclic AMP is insufficient for spillover). It should be noted further that the combinations of 10^{-5} M PGE_1 with 25 mU/ml ADH and 250 mU/ml ADH do not show increased osmotic water flow relative to ADH alone because the two concentrations of ADH are both producing maximal effects on water flow. Thus with cyclic AMP levels for 10^{-5} M PGE_1 plus 25 mU/ml ADH and 10^{-5} M PGE_1 plus 250 mU/ml ADH at 30 and 37 pmol/mg protein, respectively, maximal stimulation of water flow was observed, presumably due to spillover of cyclic AMP.

Several points of interest arise from this work. There is indirect evidence from previous studies that two compartments of cyclic AMP exist within one cell type. For instance, sodium transport responsive to ADH (and therefore cyclic AMP) occurs in the granular cells, the cells which make up some 85% of the mucosal epithelial cell layer (17, 18). This is because membranes of the granular cells make up more than 90% of the surface area of the apical side of the bladder (19). Thus, micropuncture studies identifying the action of ADH to stimulate sodium transport as a change in resistance of the apical membranes of the mucosal epithelial cells must necessarily be recording effects in the granular cells (20). Similarly there is evidence that ADH stimulates osmotic water flow only in the granular cells (21–23). If, therefore, the ADH stimulation of osmotic water flow and sodium transport takes place in one cell type, then two compartments of cyclic AMP must also be within these cells. Certainly it seems unlikely that cyclic AMP generated in a minority cell type could pass to the granular cells in sufficient quantity to give the effects reported in this paper unless an efficient intercellular transfer via gap junctions is involved. Calculation of the cyclic AMP concentration in cell water from our results gives a value of around 5×10^{-6} M for a cyclic AMP content of 40 pmol/mg protein (assuming a cell protein content of 10% and a cell water content of 80% by weight). This concentration, if present in the Ringer's solution bathing the cells, fails to gain sufficient entry to stimulate either sodium transport or water flow; in fact concentrations in the millimolar range are required. Thus evidence points to the existence of two distinct adenylate cyclases controlling sodium transport and water flow and to the presence of two distinct pools of cyclic AMP within the same cell.

From the results presented in this paper it seems unlikely that the cyclic AMP generated by PGE_1 (or at least the bulk of it) is derived from the adenylate cyclase responsible for sodium transport. Similarly, it seems unlikely that it is generated from the enzyme responsible for water flow. Thus the origin of the cyclic AMP generated by PGE_1 is obscure. Nevertheless, high concentrations of PGE_1, which can be demonstrated to inhibit ADH-stimulated water flow, stimulate water flow in the presence of theophylline. Evidence that this is due to spillover from some compartment (perhaps unrelated to transport functions) has been presented. One might also consider the possibility, in view of the inhibition of ADH-stimulated sodium transport observed by Albert and Handler (16), that PGE_1 normally inhibits the adenylate cyclase controlling both sodium transport and water flow and that the stimulation of sodium transport that has been detected is due to spillover from a PGE_1-responsive, nontransport cyclase.

In conclusion, the results show that water flow in the toad bladder can be stimulated as a result of the generation of cyclic AMP in large amounts by PGE_1 treatment. As the concentrations of PGE_1 which stimulate water flow in the presence of theophylline actually inhibit responses due to ADH, it is likely that this is spilling over from some compartment in the tissue which is normally unrelated to the control of water flow. As we do not believe that this spillover occurs as a result of stimulation of the adenylate cyclase responsible for the stimulation of sodium transport, the location of this PGE_1-stimulatable adenylate cyclase remains to be determined.

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