Vasopressin-Stimulated Prostaglandin E Biosynthesis in the Toad Urinary Bladder

EFFECT ON WATER FLOW

RANDALL M. ZUSMAN, HARRY R. KEISER, and JOSEPH S. HANDLER,
Hypertension-Endocrine Branch and Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT Prostaglandin E biosynthesis and its effect on water permeability were investigated in the toad urinary bladder. Arginine vasopressin (1 mU/ml) increased prostaglandin E (PGE) biosynthesis from 0.5±0.1 to 5.0±0.4 pmol/min per hemibladder (mean ±SEM, $n = 8$, $P < 0.001$). Maximal vasopressin-stimulated PGE biosynthesis, 6.4±0.2 pmol/min per hemibladder, occurred at vasopressin concentrations in excess of 3 mU/ml. Half-maximal stimulation of PGE biosynthesis occurred at a vasopressin concentration of approximately 0.7 mU/ml, whereas half-maximal stimulation of water flow occurred at a vasopressin concentration of approximately 5 mU/ml. Vasopressin-stimulated PGE biosynthesis did not depend on water flow along an osmotic gradient or upon sodium transport. Thin-layer chromatographic analysis of the lipids released from hemibladders labeled with tritium-arachidonic acid revealed that vasopressin stimulates the release of arachidonic acid from intracellular lipid stores without affecting the percentage of free arachidonic acid converted to PGE. Neither cyclic AMP nor theophylline stimulated PGE biosynthesis although they mimic arginine vasopressin (AVP) in stimulating water permeability. Biosynthesis of PGE was inhibited by mepacrine, a phospholipase inhibitor, and by agents that inhibit arachidonic acid oxygenase. The inhibition of PGE biosynthesis resulted in augmented vasopressin- and theophylline-stimulated water flow, but had no effect on cyclic AMP-stimulated water flow. We interpret these results to mean that endogenous PGE inhibits basal and vasopressin-stimulated adenylate cyclase activity. In contrast to the effects of AVP on permeability and transport, AVP stimulates PGE biosynthesis by a mechanism that does not depend on an increase in cellular cyclic AMP levels. The water permeability response of the toad urinary bladder to vasopressin is inhibited by PGE synthesized by the bladder in response to vasopressin.

INTRODUCTION

Arginine vasopressin increases water flow along an osmotic gradient across the toad urinary bladder, the renal collecting tubule, and certain other epithelial membranes. This effect is secondary to stimulation of adenylate cyclase by arginine vasopressin (AVP)[1] and the resultant accumulation of cyclic AMP (1). The increase in cellular cyclic AMP results in increased permeability to water and can be reproduced by exogenous cyclic AMP or theophylline, an inhibitor of cyclic nucleotide phosphodiesterase (2).

Prostaglandins E_1 and E_2 inhibit the water permeability response of the toad urinary bladder to vasopressin and to theophylline but do not inhibit the response to cyclic AMP (3, 4). The mechanism of inhibition by prostaglandin E (PGE) of the water permeability response to AVP is probably the inhibition of adenylate cyclase (5, 6). On the basis of observations in toad bladder (3) and studies on the rabbit renal cortical collecting tubule, Grantham and Orloff suggested that endogenous PGE modulates the water permeability response to vasopressin (7). This theory

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Dr. Zusman's present address is: Massachusetts General Hospital, Medical Services, Boston, Mass. 02114.

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[1] *Abbreviations used in this paper*: AVP, arginine vasopressin; PG, prostaglandin (used variously according to the identification of a given prostaglandin, i.e., PGE, PGE_1, PGE_2, and $PGF_{2\alpha}$).

was supported in subsequent studies in which it was found that indomethacin, an inhibitor of PGE biosynthesis, increased the water flow response to AVP (8, 9).

Recent studies in our laboratory have shown that AVP stimulates prostaglandin E_2 biosynthesis in rabbit renomedullary interstitial cells in tissue culture (10). In these cells, vasopressin stimulates an intracellular acylhydrolase (phospholipase), which results in the increased release of endogenous arachidonic acid, the precursor of PGE_2, and a subsequent increase in the synthesis of PGE_2 (10). Mepacrine, a phospholipase inhibitor, diminishes AVP-stimulated PGE_2 biosynthesis in renomedullary interstitial cells by decreasing phospholipase activity and thereby reducing the release of arachidonic acid. In contrast, indomethacin, ibuprofen, meclofenamic acid, and naproxen decrease PGE_2 biosynthesis by inhibiting the enzyme that catalyzes the addition of molecular oxygen to arachidonic acid (11).

The purpose of this investigation was to study PGE biosynthesis in the toad urinary bladder and the role of endogenous PGE in vasopressin-stimulated water flow.

METHODS

Toads, *Bufo marinus*, were obtained from National Reagents, Bridgeport, Conn., and kept on moist San-i-cell. Urinary bladders were removed from doubly-pithed toads, and the hemibladders were mounted as sacs on bungs. The serosal surface was bathed with 15 ml of amphibian Ringer's solution, 90 mM sodium chloride, 25 mM sodium bicarbonate, 1 mM calcium chloride, 3 mM potassium chloride, 0.5 mM potassium dihydrogen phosphate, 0.5 mM magnesium sulfate, 5 mM glucose, and 200 mg/liter kanamycin sulfate, and gassed with 97% O_2-3% CO_2. The mucosal surface was bathed with 3 ml of Ringer's solution, diluted 1:5 with distilled water. Water flow was measured gravimetrically according to the method of Bentley (12). Basal water flow was measured for 30 min. Stimulated water flow was measured for 30 min, beginning 10 min after the addition of AVP, cyclic AMP, or theophylline. PGE in the serosal solution was measured by radioimmunoassay as previously described (10). We have been unable to identify unequivocally as either PGE_1 or PGE_2 the actual prostaglandin produced by the toad urinary bladder. Although we feel that it is most likely PGE_2, we refer to it in the paper merely as prostaglandin E for the sake of accuracy. In those experiments in which we gave arachidonic acid, the specific precursor of PGE_2, we refer to the product as PGE_2. In view of the fact that cellular accumulation of prostaglandins has not been demonstrated (11), the appearance of PGE in the serosal solution is a measure of PGE biosynthesis. The rate of appearance of PGE in the serosal solution is 20 times that in the mucosal solution. All agents were added to the solution bathing the serosal surface of the hemibladders. Freshly-prepared serosal solutions containing the appropriate agents were added immediately before the basal and test periods. In all experiments, paired hemibladders from the same toad were used. Experiments were performed at room temperature. Statistical analyses were done with Student's *t* test for paired observations (13).

The effect of different concentrations of AVP on water flow and PGE biosynthesis. The 24 hemibladders from 12 toads were assigned to six equal groups so as not to have the two hemibladders from one toad in the same group. Each group of hemibladders was incubated with one concentration of AVP, 0.1, 0.3, 1.0, 3.0, 10.0, or 25.0 mU/ml, and water flow and PGE synthesis were measured.

Identification of the step in arachidonic acid metabolism affected by AVP. Hemibladders were incubated overnight in 15 ml Ringer's solution containing 20 mM glucose and [^{3}H]arachidonic acid, 62 Ci/mmol (New England Nuclear, Boston, Mass.) to label the arachidonic acid storage pool. The following day the serosal and mucosal Ringer's solution were changed three times over a period of 30 min, and the experimental hemibladders were incubated with mepacrine, a phospholipase inhibitor (0.1 mM), or naproxen, an oxygenase inhibitor (0.1 mM), for 180 min before the basal period. After the basal period, the serosal solution was changed to a solution containing vasopressin (6 mU/ml) with or without the inhibitors, and water flow was measured. The serosal solutions from the basal and vasopressin periods were each extracted with chloroform, and the extracted lipids were isolated by thin-layer chromatography as previously described (10).

The effect of arachidonic acid, prostaglandin E_2, phospholipase inhibition, and oxygenase inhibition on vasopressin-stimulated water flow and prostaglandin E biosynthesis. 1.6 mM arachidonic acid (NuChek Prep) was dissolved in a buffered solution consisting of 4 parts amphibian Ringer's solution as described above and 1 part fetal calf serum. The control hemibladder was incubated in buffer containing fetal calf serum without arachidonic acid. Arachidonic acid was added to the experimental hemibladder 30 min before the basal period.

The agents used in these studies were: arginine vasopressin (ICN Pharmaceuticals, Inc., Cleveland, Ohio), theophylline (ICN Nutritional Biochemicals Div., Cleveland, Ohio), cyclic AMP (Sigma Chemical Co., St. Louis, Mo.), mepacrine (K & K Laboratories Inc., Plainview, N. Y.), indomethacin (Merck, Sharp & Dohme, West Point, Pa.), ibuprofen (The Upjohn Co., Kalamazoo, Mich.), meclofenamic acid (Parke-Davis & Co., Detroit, Mich.), and naproxen (Syntex Laboratories, Inc., Palo Alto, Calif.).

RESULTS

The effect of AVP, cyclic AMP, and theophylline on water flow and PGE biosynthesis (Table I). Arginine vasopressin (1 mU/ml) increased water flow and PGE biosynthesis. To examine the time course of PGE accumulation after AVP stimulation, samples were obtained every 10 min for 40 min. PGE accumulation was the same in each period. Although cyclic AMP (10 mM) increased water flow, it did not affect prostaglandin E biosynthesis. Theophylline (10 mM) increased water flow but did not affect PGE biosynthesis. In view of the failure of cyclic AMP and theophylline to stimulate PGE biosynthesis, it is likely that the increase in PGE elicited by AVP does not involve cyclic AMP. The stimulation of sodium transport and the stimulation of osmotic water flow by arginine vasopressin are associated with secondary effects on the epithelial cells. The increased rate of sodium transport is accompanied by increased cellular metabolism (14); the

TABLE I

The Effect of AVP, Theophylline, and Cyclic AMP on Water Flow and PGE Biosynthesis in the Toad Urinary Bladder

Agent added	Water flow*		PGE biosynthesis*	
	Control	Experimental	Control	Experimental
	mg/min per hemibladder		*pmol/min per hemibladder*	
AVP (1 mU/ml)	0.2±0.1	17.7±1.7‡	0.5±0.1	5.0±0.4‡
Cyclic AMP (10 mM)	0.3±0.3	19.2±1.1‡	0.8±0.1	0.9±0.2
Theophylline (10 mM)	0.2±0.3	18.5±6.3‡	0.4±0.1	0.3±0.1

* Each value represents the mean±SEM ($n = 8$).
‡ $P < 0.01$.

increased osmotic water flow results in swelling of the epithelial cells (15). To test whether the AVP-elicited increase in PGE biosynthesis is dependent on the sodium transport response or the water flow response, the two responses were eliminated. 0.1 mM amiloride in a mucosal solution eliminates net sodium transport by the bladder (16), but had no effect on basal or AVP (1 mU/ml)-stimulated PGE biosynthesis, 0.4±0.1, and 4.0±0.2 pmol/min per hemibladder, respectively. Similarly, basal and AVP-stimulated PGE biosynthesis, 0.4±0.1, and 4.2±0.4 pmol/min per hemibladder, respectively, were not altered in bladders in which osmotic water flow was eliminated by the use of full-strength Ringer's solution on the mucosal and serosal surfaces.

The effect of different concentrations of AVP on water flow and PGE biosynthesis. Vasopressin-stimulated water flow increased progressively in the range of 0.1–10 mU/ml, reaching maximal flow at 25 mU/ml (Fig. 1). No further increase in water flow was observed at 50 mU/ml. Half-maximal stimulation of water flow occurred at a vasopressin concentration of ≅4.8 mU/ml.

Vasopressin-stimulated PGE biosynthesis increased progressively in the range of 0.1–1.0 mU/ml. Half-maximal stimulation of PGE biosynthesis occurred at a vasopressin concentration of ≅0.7 mU/ml (Fig. 2).

Identification of the step in arachidonic acid metabolism affected by AVP (Fig. 3). After 18 h of incubation with tracer [^{3}H]arachidonic acid, 28% (mean, $n = 24$) of the tritium tracer remained in the serosal solution. After removal of tracer-containing solution, vasopressin administration increased the rate of release of arachidonic acid from the intracellular lipid storage pool and increased the rate of appearance of [^{3}H]prostaglandin E_2. Mepacrine diminished basal and vasopressin-stimulated arachidonic acid release by 65% but had no effect on the ratio of released PGE_2 to released arachidonic acid. Naproxen, a prostaglandin synthetase (oxygenase) inhibitor, had no effect on vasopressin-stimulated arachidonic acid release, but inhibited PGE_2 biosynthesis by 99%. These data indicate that AVP stimulates acylhydrolase activity, which results in an increased release of arachidonic acid from lipids in the cell.

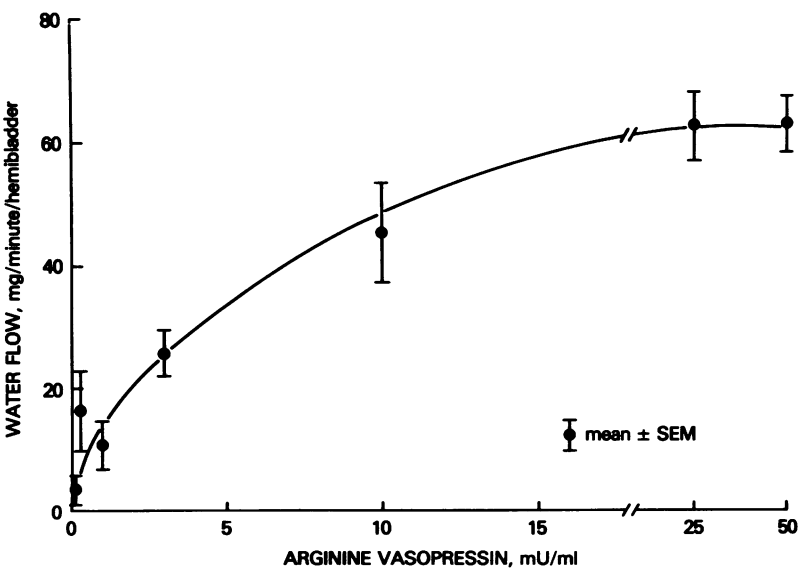

FIGURE 1 Water flow in the toad urinary bladder in response to different concentrations of arginine vasopressin. Half-maximal water flow occurred at ≅5 mU/ml, and maximal flow at concentrations > 25 mU/ml.

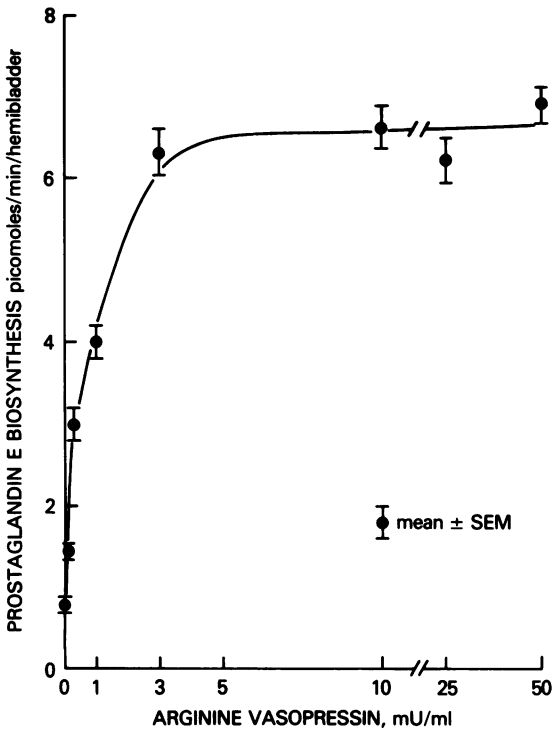

FIGURE 2 PGE biosynthesis in the toad urinary bladder in response to different concentrations of vasopressin. Half-maximal stimulation of PGE biosynthesis occurred at ≅0.7 mU/ml, and maximal PGE biosynthesis occurred at concentrations >3 mU/ml.

The effect of mepacrine, arachidonic acid, PGE_2, and indomethacin on vasopressin-stimulated water flow and PGE biosynthesis (Table II). Because exogenous prostaglandin E_1 and E_2 inhibit vasopressin- and theophylline-stimulated water flow, we investigated the effect of alterations in the rate of PGE biosynthesis on vasopressin-, cyclic AMP-, and theophylline-stimulated water flow. Basal water flow was unaffected by mepacrine, arachidonic acid, prostaglandin E_2, or indomethacin (data not shown).

Mepacrine decreased basal and vasopressin-stimulated PGE biosynthesis and increased vasopressin-stimulated water flow. When mepacrine was added to control and experimental hemibladders and the inhibition by mepacrine of arachidonic acid release was bypassed by the addition of 1.6 mM arachidonic acid to the experimental hemibladder, PGE_2 biosynthesis by arachidonic acid-treated hemibladders was markedly increased and AVP-stimulated water flow was markedly inhibited. The effect of arachidonic acid on vasopressin-stimulated water flow was reversible. When the experimental hemibladder was incubated with indomethacin and arachidonic acid was added to the control and experimental hemibladders, indomethacin markedly inhibited PGE_2 biosynthesis. Water flow in response to vasopressin increased markedly in the hemibladder exposed to indomethacin. The addition of 1 μM PGE_2 inhibited the water permeability response to AVP, confirming the observation of Urakabe et al. (4). Similarly, in the presence of indomethacin, PGE_2 inhibits the water permeability response to AVP (data not shown). Ozer and Sharp (17) suggested that arachidonic acid has inhibitory properties on water flow independent of those of PGE_2. We conclude, however, that the inhibition of vasopressin-stimulated water flow by arachidonic acid is secondary to the marked stimulation of PGE_2 biosynthesis.

The effect of oxygenase inhibitors on vasopressin-stimulated water flow and PGE biosynthesis (Table III). Basal water flow was unaffected by ibuprofen, indomethacin, meclofenamate, and naproxen (data not shown). Each of the oxygenase inhibitors eliminated PGE biosynthesis and increased vasopressin-stimulated water flow.

The effect of mepacrine and oxygenase inhibitors on cyclic AMP-stimulated water flow and PGE biosynthesis (Table IV). Mepacrine, ibuprofen, meclofenamate, and naproxen had no effect on cyclic AMP-stimulated water flow. In contrast, 1 μM indomethacin increased cyclic AMP-stimulated water flow from 15.7±4.8 to 38.0±3.0 mg/min per hemibladder ($n = 6$, $P < 0.01$). The augmentation of cyclic AMP-stimulated water flow by indomethacin has been reported previously (8, 9). All the oxygenase inhibitors eliminated PGE synthesis, yet only indomethacin affected cyclic AMP-stimulated water flow. Therefore, it is apparent that the enhancement of cyclic AMP-stimulated water flow by indomethacin is unrelated to the inhibition of prostaglandin synthesis. It is possible that inhibition of cyclic nucleotide phosphodiesterase by indomethacin (9) is responsible for the enhancement of cyclic AMP-stimulated water flow.

The effect of mepacrine and naproxen on theophylline-stimulated water flow and PGE biosynthesis (Table V). Mepacrine and naproxen each decreased PGE biosynthesis and increased theophylline-stimulated water flow. We interpret these observations to mean that PGE produced in the absence of AVP inhibits basal adenylate cyclase activity and the resulting accumulation of cyclic AMP in the epithelial cells of the theophylline-treated hemibladders.

DISCUSSION

In 1965, Orloff et al. demonstrated that PGE_1 diminished the water permeability response of the toad urinary bladder to AVP (3). Prostaglandins E_1 and E_2 inhibit theophylline- as well as vasopressin-stimulated water flow but have no effect on cyclic AMP-stimulated water flow (3, 4, 8, 18). This pattern

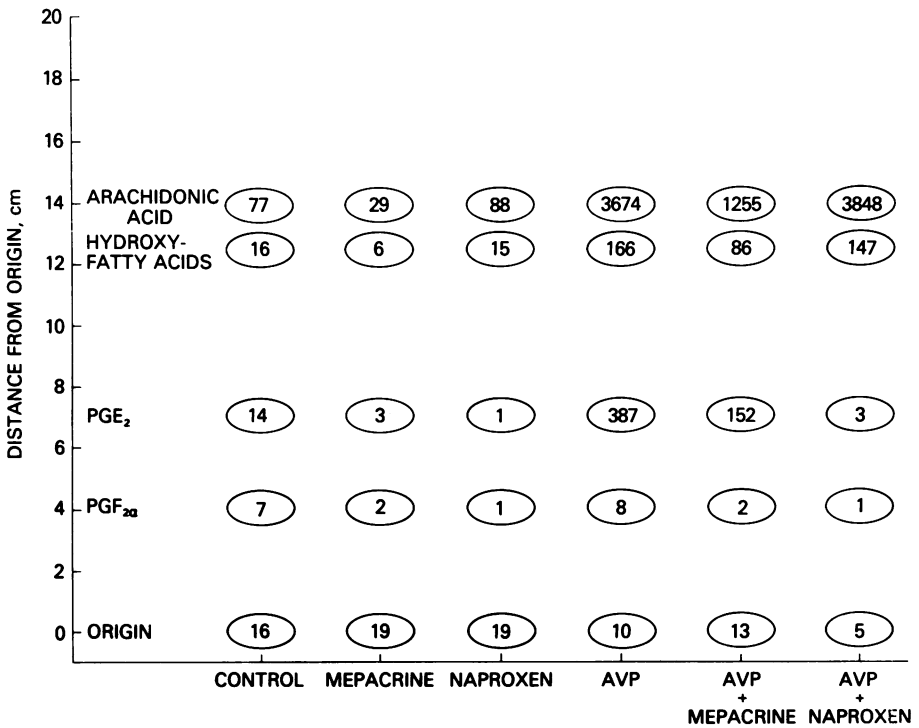

FIGURE 3 Thin-layer chromatogram of lipids released from the toad urinary bladder after vasopressin stimulation. When used, 0.1 mM naproxen or 0.1 mM mepacrine were added 180 min before the basal (control) period. Vasopressin was used at a concentration of 6 mU/ml. Each value represents the counts per minute of lipid released per minute per hemibladder, and is the mean of six determinations. Arachidonic acid, PGE_2, and $PGF_{2\alpha}$ standards were used on each thin-layer chromatographic plate. The R_f values for arachidonic acid, PGE_2, and $PGF_{2\alpha}$ in this system were 0.70, 0.35, and 0.20, respectively. The hydroxy-fatty acids and the material remaining at the origin were not identified.

of inhibition of water flow led Grantham and Orloff (7) to conclude that PGE inhibits vasopressin-stimulated adenylate cyclase activity. Grantham and Orloff (7) demonstrated the inhibitory effect of prostaglandin E_1 on vasopressin-stimulated water flow in the isolated rabbit renal collecting tubule, an analogous vasopressin-responsive epithelial membrane, and suggested that endogenous PGE biosynthesis modulates vasopressin-stimulated water flow. The concept of PGE biosynthesis inhibiting adenylate cyclase activity

TABLE II

The Effect of Mepacrine, Arachidonic Acid, PGE_2, and Indomethacin on Vasopressin-Stimulated Water Flow and PGE Biosynthesis

Agents added		Water flow			PGE biosynthesis		
Control	Experimental		Control	Experimental		Control	Experimental
		n	*mg/min per hemibladder*		*n*	*pmol/min per hemibladder*	
—	Mepacrine	20	13.4±2.1	22.5±2.1*	8	4.9±0.2	1.5±0.1*
Mepacrine	Mepacrine and arachidonic acid	12	15.4±3.4	1.3±0.4*	8	1.6±0.2	61.8±5.2*
—	Arachidonic acid	12	22.1±2.1	1.2±0.4*	8	4.3±0.3	72.3±2.9*
Arachidonic acid	Arachidonic acid and indomethacin	12	2.7±0.3	37.7±2.2*	8	66.9±7.6	0.8±0.1*
—	PGE_2	8	28.6±3.7	2.0±0.3*		—	—

1 mU/ml AVP was used in all experiments. 0.1 mM mepacrine was added 210 min before the test period. 1.6 mM arachidonic acid was added 60 min before the test period. 0.1 mM indomethacin was added 210 min before the test period. 1 μM PGE_2 was added 60 min before the test period.

* $P < 0.001$.

TABLE III

The Effect of Oxygenase Inhibitors on Vasopressin-Stimulated Water Flow and PGE Biosynthesis

Agent added	Water flow			PGE biosynthesis		
		Control	Experimental		Control	Experimental
	n	*mg/min per hemibladder*		*n*	*pmol/min per hemibladder*	
Ibuprofen	4	33.9±6.9	63.1±5.7*	4	4.9±0.2	0.04±0.02*
Indomethacin	4	19.2±4.4	39.3±4.8*	4	5.8±0.3	0.02±0.01*
Meclofenamic acid	8	13.4±2.6	30.6±3.0*	4	6.0±0.6	0.01±0.01*
Naproxen	8	17.7±1.7	32.6±2.9*	4	4.9±0.2	0.09±0.04*

1 μM ibuprofen, indomethacin, meclofenamate, or naproxen was added to the experimental hemibladder 60 min before the test period. 1 mU/ml AVP was used in all experiments.
* $P < 0.005$.

was supported by the finding of increased vasopressin-stimulated water flow in toad urinary bladders incubated with indomethacin, a prostaglandin synthetase (oxygenase) inhibitor (8, 9). The present investigation provides direct evidence that the toad bladder synthesizes PGE and that endogenous PGE inhibits the adenylate cyclase-dependent water permeability response. Furthermore, in this investigation we demonstrate the stimulation of PGE biosynthesis by AVP.

The concentration-response relationship between arginine vasopressin-stimulated water flow and prostaglandin E biosynthesis is of particular interest. Half-maximal water flow occurred at a vasopressin concentration of 5 mU/ml with maximal flow at concentrations > 25 mU/ml. In contrast, half-maximal PGE biosynthesis occurred at <1 mU/ml and reached a maximum at vasopressin concentrations >3 mU/ml; vasopressin-stimulated PGE biosynthesis is maximal at a concentration of AVP that is half-maximal for vasopressin-stimulated water flow. Endogenous PGE is of greatest significance in inhibiting the response to low concentrations of vasopressin. For example, when the concentration-response curve was repeated in the presence and absence of naproxen, an inhibitor of PGE biosynthesis, water flow at a vasopressin concentration >15 mU/ml (55.8±2.7 mg/min per hemibladder, $n = 16$) was unaffected by naproxen. At 0.2 mU/ml vasopressin-stimulated water flow was 3.6±1.3 mg/min per hemibladder in control hemibladders, and 15.2±1.9 mg/min per hemibladder ($n = 4$, $P < 0.005$) in naproxen-treated bladders. At 0.6 mU/ml vasopressin-stimulated water flow was 17.0±4.6 mg/min per hemibladder in control hemibladders, and 37.4±2.3 mg/min per hemibladder ($n = 4$) in naproxen-treated bladders.

We have recently demonstrated arginine vasopressin-stimulated prostaglandin E_2 biosynthesis in rabbit renomedullary interstitial cells. Vasopressin-stimu-

TABLE IV

The Effect of Mepacrine and Oxygenase Inhibitors on Cyclic AMP-Stimulated Water Flow and PGE Biosynthesis

Agent added	Water flow			PGE biosynthesis		
		Control	Experimental		Control	Experimental
	n	*mg/min per hemibladder*			*pmol/min per hemibladder*	
Mepacrine	6	22.4±4.2	23.9±5.5		0.5±0.04	0.2±0.02*
Ibuprofen	4	15.0±4.8	16.8±3.8		0.5±0.03	0.07±0.03*
Indomethacin	6	15.7±4.8	38.0±3.0*		0.5±0.03	0.04±0.01*
Meclofenamic acid	4	13.0±3.7	11.4±3.4		0.5±0.04	0.04±0.01*
Naproxen	6	12.9±2.5	11.6±1.8		0.4±0.03	0.04±0.01*

0.1 mM mepacrine was added to the experimental hemibladder 210 min before the test period. 1 μM ibuprofen, indomethacin, meclofenamate, or naproxen was added to the experimental hemibladder 60 min before the test period. Cyclic AMP was used in all experiments at a concentration of 10 mM.
* $P < 0.01$.

TABLE V

The Effect of Mepacrine and Naproxen on Theophylline-Stimulated Water Flow and PGE Biosynthesis

Agent added	Water flow			PGE biosynthesis		
		Control	Experimental		Control	Experimental
	n	*mg/min per hemibladder*		*n*	*pmol/min per hemibladder*	
Mepacrine	6	23.0±3.8	44.6±3.7*	6	0.6±0.1	0.2±0.03*
Naproxen	8	15.4±2.9	32.7±2.9*	4	0.4±0.04	0.04±0.01*

0.1 mM mepacrine was added to the experimental hemibladder 210 min before the test period; 1 μM naproxen was added to the experimental hemibladder 60 min before the test period. 10 mM theophylline was used in both experiments.
* $P < 0.01$.

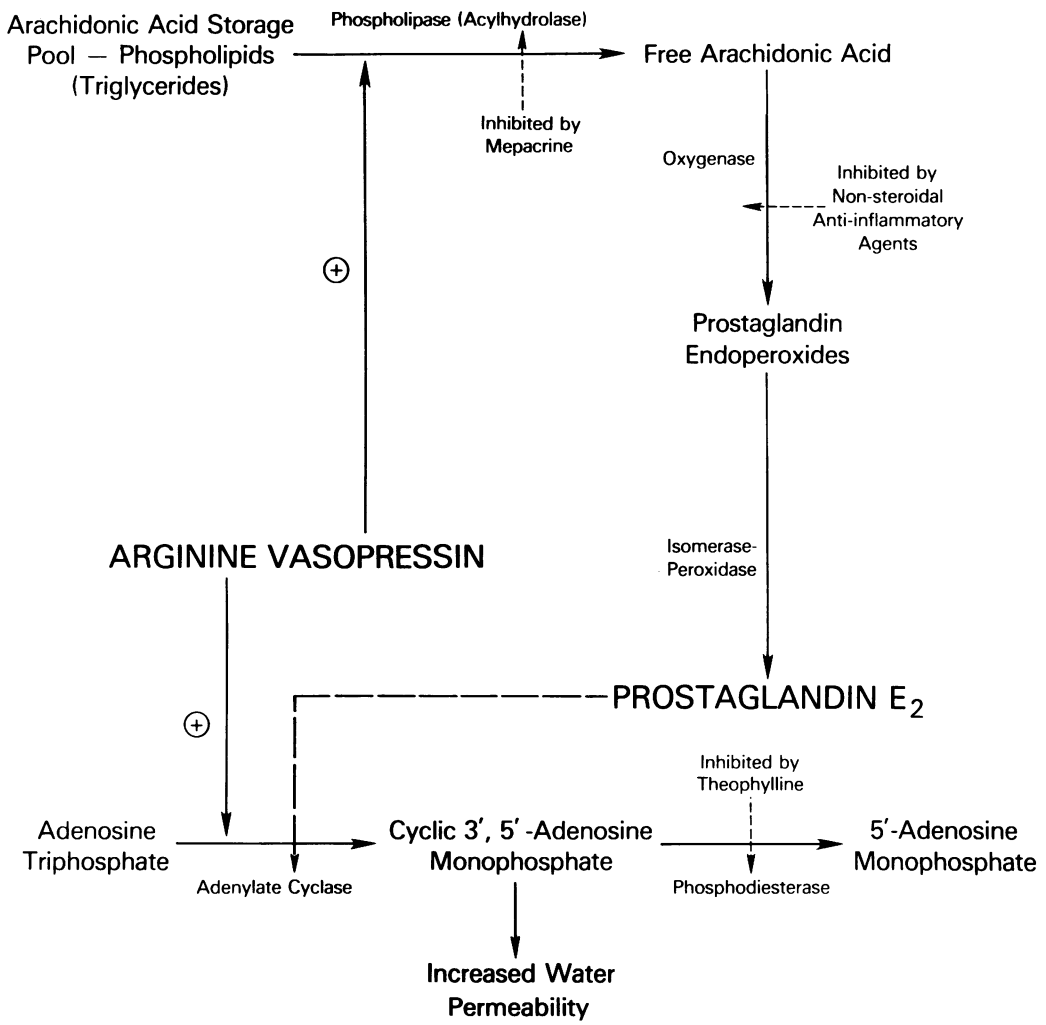

FIGURE 4 A schematized diagram of the relationship between the stimulation of PGE biosynthesis and water flow by vasopressin. In the diagram, we show arachidonic acid in the storage pool giving rise to free arachidonic acid and this going on to prostaglandin E_2, for convenience and because this is the most likely pathway. However, we have not proven unequivocally that the PGE produced by the toad urinary bladder is PGE_2.

lated PGE_2 biosynthesis in these cells in tissue culture occurs via acylhydrolase stimulation, arachidonic acid release, and subsequent PGE_2 biosynthesis (10). Endogenous prostaglandin E biosynthesis in the toad urinary bladder is similarly increased by AVP stimulation of acylhydrolase activity. Vasopressin has the dual and possibly independent effects of stimulating adenylate cyclase and stimulating PGE biosynthesis in the toad bladder.

A number of issues regarding vasopressin-stimulated PGE biosynthesis by the toad urinary bladder remain unanswered by this investigation. The mechanism of vasopressin stimulation of acylhydrolase activity is unknown. It is not known whether the same vasopressin molecule in association with its receptor stimulates both adenylate cyclase and acylhydrolase, or whether there are independent hormone-receptor complexes responsible for adenylate cyclase and acylhydrolase stimulation. In fact, the cell in which vasopressin stimulates PGE biosynthesis in the toad bladder is not known. The mammalian kidney has prostaglandin synthetase in both the renomedullary interstitial cell and the epithelium of the collecting tubule (19). It is possible that an epithelial cell in the toad urinary bladder is the site of PGE biosynthesis or that another cell type, analogous to the mammalian renomedullary interstitial cell, synthesizes PGE, which then affects the epithelial cell permeability response to vasopressin.

In Fig. 4, we have schematized the events relating PGE biosynthesis and cyclic AMP formation in the toad urinary bladder. AVP stimulates both adenylate cyclase and a phospholipase. The phospholipase releases arachidonic acid from the arachidonic acid storage pool. Inhibition of phospholipase by mepacrine diminishes arachidonic acid release and, therefore, subsequent PGE_2 biosynthesis. Free arachidonic acid is converted to the prostaglandin endoperoxides by an oxygenase, which can be inhibited by the nonsteroidal anti-inflammatory agents. After peroxidation and isomerization, prostaglandin E_2 is derived from the endoperoxides. PGE_2, in turn, inhibits the stimulation of adenylate cyclase by vasopressin, thus diminishing the formation and accumulation of cyclic AMP and thereby decreasing the water permeability response to vasopressin.

It is possible that a similar scheme (Fig. 4) may apply to the mammalian kidney. Indomethacin enhances the in vivo antidiuretic effect of vasopressin in man (20), in the dog (21), and in the rat (22). Furthermore, it has been demonstrated that urinary PGE excretion increases after vasopressin administration in rabbits (23) and rats (24). In view of the foregoing observations (10, 20–24), we suggest that vasopressin-stimulated PGE biosynthesis inhibits vasopressin-stimulated water flow in the mammalian kidney as well as the toad urinary bladder.

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