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Research Article

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Role of Prostaglandin E_2 in Mediating the Effects of pH on the Hydroosmotic Response to Vasopressin in the Toad Urinary Bladder

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ABSTRACT Acidosis inhibits the hydroosmotic response to vasopressin. Since prostaglandins are known to modulate vasopressin-stimulated water flow we investigated the role of endogenous prostaglandin E_2(PGE_2) production in the pH-dependent response of the toad urinary bladder to vasopressin. Graded acidification of the serosal media resulted in a progressive decline in vasopressin-stimulated water flow from 26.6±0.5 mg/min at pH 8.4 to 1.7±0.6 at pH 6.9. In these bladders basal PGE_2 synthesis increased from 5.09 ± 0.51 pmol/min per g hemibladder at pH 8.4 to 18.8±2.8 at pH 6.9. The addition of that concentration of PGE_2 produced by the bladder at pH 7.4 (4 nM) to bladders at pH 8.4 resulted in 62–71% of the inhibition usually seen at pH 7.4; these data suggest that basal PGE_2 production per se and not other products of prostaglandin synthesis or other pH-dependent events is responsible for the effect of acidosis. Preincubation with prostaglandin synthesis inhibitors reversed in major part the effect of serosal acidification on the response to submaximal concentrations of vasopressin and completely abolished the effect of pH on near maximal concentrations of the hormone. An increase in PGE_2 synthesis after vasopressin was not seen at any pH. These studies establish that increased basal PGE_2 synthesis plays a critical role in the pH dependence of the hydroosmotic response to vasopressin and demonstrate that factors that modulate the response to vasopressin may exert this effect by changing the basal rate of prostaglandin synthesis.

INTRODUCTION

The inhibitory effect of acidosis on the hydroosmotic response to vasopressin has been well documented by both in vitro and in vivo studies (1–10). An effect of media pH on the hydroosmotic response to vasopressin was observed in the first study of water transport in the toad urinary bladder (1). Subsequent work in this tissue established that acidification of the serosal media (pH 8.4 to 6.9) impairs the hydroosmotic response to vasopressin and theophylline but not to cyclic AMP (2–6). In the rat, acute metabolic acidosis in vivo inhibits vasopressin-stimulated free-water reabsorption, and in renal medullary slices in vitro, both vasopressin-stimulated adenylate cyclase and cyclic AMP accumulation are inhibited by acidification of the media (7). In a recent study in the toad bladder, serosal acidification (pH 6.8) led to a 60% fall in vasopressin-stimulated water flow, whereas the response to exogenous cyclic AMP was enhanced (10). Taken together these studies suggest that a major effect of acidosis is inhibition of the vasopressin-dependent cyclic AMP system.

There is also extensive evidence that endogenous prostaglandins play a major role in modulating the hydroosmotic response to vasopressin. In the toad urinary bladder and rabbit cortical collecting duct, prostaglandins E_1 and E_2 (PGE_1) (PGE_2)[1] inhibit the change in water permeability induced by vasopressin but not by cyclic AMP (11–15), whereas inhibition of

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[1] *Abbreviations used in this paper:* J_v, hydroosmotic water flow; PGE_2 prostaglandin E_2; 8-cpt-cyclic AMP, 8-(*p*-chlorophenylthio) cyclic AMP.

endogenous prostaglandin synthesis by cyclooxygenase inhibitors increases the response to vasopressin in both the toad bladder (13, 16–18) and mammalian kidney (19, 20). Additionally, PGE_2 biosynthesis has been demonstrated in the toad bladder (18, 21, 22), and basal rates of PGE_2 production have been considered to influence the subsequent response to vasopressin (22).

The purpose of the present study was to determine the possible role of PGE_2 in the pH-dependent response of the toad urinary bladder to vasopressin. Our results demonstrate that serosal acidification markedly stimulates the endogenous production of PGE_2 and contributes in major part to the inhibitory effect of acidosis on the hydroosmotic response to vasopressin.

METHODS

Paired hemibladders were removed from doubly pithed female Dominican toads (*Bufo marinus*) (National Reagents, Bridgeport, Conn.). The bladders were placed in phosphate-buffered amphibian Ringer's solution of the following composition (in mM): NaCl 103; KCl 3; $CaCl_2$ 0.5; KH_2PO_4 0.6; Na_2HPO_4 3.5; $MgSO_4$ 1.2; glucose 5, pH 8.0. In certain experiments the Ringer's was buffered further with Tris, 5mM added to the phosphate buffer. Hemibladders were mounted as sacs on glass cannulae, the mucosal surface was bathed with 4 ml of Ringer's solution diluted 1:4, and the serosal surface was bathed in 15 ml of full strength Ringer's solution bubbled with air. The pH of the serosal or mucosal solution was varied by the addition of 1 N HCl or NaOH. The pH of the media was measured before the first experimental period and at the conclusion of each experiment with a model 601A Orion Research pH meter (Orion Research Inc., Cambridge, Mass.) equiped with a 9102 glass electrode. Hydroosmotic water flow (J_v) in paired hemibladders was determined gravimetrically (1). In most experiments hemibladders were paired for differences in serosal pH. After a 30-min equilibration period the hemibladders were placed in fresh Ringer's solution and basal J_v and PGE_2 synthesis (measured as release of PGE_2 into the serosal media) were determined over a 60-min period. J_v and PGE_2 production were then measured during consecutive 45-min periods after addition of increasing concentrations of vasopressin (Pitressin) 0.5–10 mU/ml, or 8(*p*-chlorophenylthio) cyclic AMP (8-cpt-cyclic AMP) 10–50 μM, to the serosal media. Hemibladders were placed in fresh serosal media following the basal and each subsequent experimental period. In experiments using cyclooxygenase inhibitors (indomethacin, naproxen, or ibuprofen) tissues were pre-incubated (serosal media) with the inhibitor for 120 min with changes of the serosal media every 60 min. Basal (60 min), and after vasopressin measurements (45 min), of J_v and PGE_2 production were then made in the presence of the inhibitors. At the end of each period an aliquot of the serosal bath was removed and immediately frozen and stored at −20°C until the day of assay for PGE_2. No difference in the content of PGE_2 was noted when samples were assayed immediately or frozen for 1 d to 8 wk before assay.

Radioimmunoassay for PGE_2. Immunoreactive PGE_2 content was measured by the radioimmunoassay method of Dray et al. (23) and Dunn et al. (24) with minor modifications. Antiserum to PGE_2 was supplied by the Institute Pasteur, Paris, France, or Boehringer Mannheim Biochemicals, Indianapolis, Ind. Antisera from both sources had identical cross-reactivities and binding constants and yielded identical standard curves. Cross-reactivity (at $B/B_0 = 0.5$) of the PGE_2 antiserum was 3.2% with PGE_1, 0.02% with PGA_1, 0.20% with PGA_2, 0.01% with $PGF_1\ \alpha$, 0.15% with 13,14, dihydro PGE_2, 0.11% with 13,14 dihydro-15-keto PGE_2, and 0.01% with PGB_1, PGB_2, and thromboxane B_2. The assay was performed in a buffer at pH 7.4 containing 0.145 M phosphate, 0.0274 M NaCl, 0.3 mM NaN_3, and 0.1% gelatin. Standard solutions were prepared from authentic PGE_2 (gift of Dr. J. Pike, Upjohn Co., Kalamazoo, Mich.). [^{3}H]PGE_2 was obtained from New England Nuclear, Boston, Mass., (sp act 130 Ci/mmol).

All samples were assayed in duplicate using 100-μl aliquots of unextracted media. Preliminary experiments demonstrated no difference in PGE_2 content between direct immunoassay and assay following lipid extraction ($r = 0.979$, $b = 1.02$, $n = 12$, $P < 0.001$). Because of the specificity of the antiserum for PGE_2, direct assay on unextracted samples was used. Although interassay variability was <8% ($n = 18$) and intraassay variability was <4% ($n = 15$), paired experimental and control samples were always measured in the same assay. Serial dilution of random samples was performed in each assay and always showed parallelism with the standard curve ($r = 0.98$, $n = 24$). At pH 8.0, the standard addition of PGE_2 (25, 50, and 100 pg/aliquot) to samples of serosal media yielded recoveries of 98±1% ($n = 10$ for each amount). Because changes in serosal pH per se could alter the conversion of PGE_2 to PGA_2 or PGB, or conceivably could alter the metabolism of PGE_2 by bladder tissue, we compared the recovery of 100 pg PGE_2 added to the serosal media at pH 7.4 and 8.4. After 1 h of vigorous bubbling of media in the absence of toad bladders, the recovery of added PGE_2 was identical (94±1%) at pH 7.4 and 8.4 ($n = 9$). In the presence of toad bladder tissue at pH 7.4 or 8.4 the recovery of 100 pg exogenous PGE_2 (determined as total PGE_2 content minus the mean concentration produced by the bladder at each pH) was >90% ($n = 7$), indicating that serosal acidification per se did not alter either the chemical conversion or endogenous metabolism of PGE_2. In all experiments prostaglandin E_2 production was expressed as picomoles per minute per gram hemibladder with a minimal detectable rate of production of 0.04 pmol/min per g hemibladder. The agents used were: Pitressin (Parke-Davis, Morris Plains, N. J.), 8-cpt-cyclic AMP (ICN Pharmaceuticals, Inc., Covina, Calif.), indomethacin (Merck, Sharp & Dohme, West Point, Pa.), ibuprofen (Upjohn Company, Kalamazoo, Mich.), and naproxen (Syntex Laboratories, Inc., Palo Alto, Calif.).

RESULTS

Effects of serosal acidification on PGE_2 production and the hydroosmotic response to vasopressin in phosphate-buffered Ringer's. The effect of serosal pH (8.4 vs. 7.4) on J_v and PGE_2 production in the basal state and following submaximal concentrations of vasopressin are shown in Fig. 1. Basal J_v (upper panel) was not different but serosal acidification ito pH 7.4 was associated with a marked increase in the basal production of PGE_2 by the toad bladder (lower panel) (15.3±2.7 pmol/min per g hemibladder at pH 7.4 vs. 4.3±0.8 at pH 8.4, $n = 12$, $P < 0.001$). Following stim-

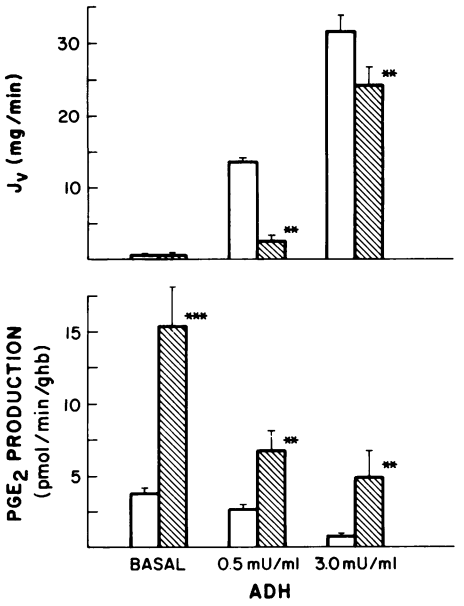

FIGURE 1 Effect of serosal media acidification (pH 7.4 vs. 8.4) on PGE_2 production and J_v in the toad urinary bladder in the basal state and after sequential addition of 0.5 and 3.0 mU/ml vasopressin. Values are mean±SEM in 12 experiments with hemibladders paired for differences in pH. Hatched bars indicate pH 7.4; open bars pH 8.4. J_v was measured gravimetrically in mg/min and PGE_2 production was expressed as picomoles per minute per gram hemibladder. Asterisks indicate P values by paired t analysis, with ** indicating $P < 0.02$ and *** indicating $P < 0.001$. ghb, gram hemibladder.

ulation with vasopressin, hemibladders at pH 7.4 (with higher basal PGE_2 production) had inhibited J_v following both 0.5 mU/ml (2.6±0.8 mg/min vs. 13.8±1.2; $P < 0.02$, 83% inhibition) and 3.0 mU/ml (24.5±2.3 vs. 31.5±1.9, $P < 0.02$, 22% inhibition). PGE_2 production following vasopressin was always higher at pH 7.4 vs. 8.4, and PGE_2 production declined with time at both pH despite the addition of vasopressin.

In separate experiments similar results were observed with higher concentrations of vasopressin at serosal pH 8.4 vs. 7.4 (Table I). Basal J_v was not different, but basal PGE_2 synthesis was again increased fourfold at pH 7.4 vs. 8.4 (17.3±1.8 pmol/min per g hemibladder vs. 4.1±1.9, $n = 9$, $P < 0.001$). Vasopressin stimulated J_v was inhibited following both 3.0 and 10 mU/ml in hemibladders at pH 7.4 with higher basal rates of PGE_2 synthesis. PGE_2 production remained higher at pH 7.4 vs. 8.4 following both concentrations of vasopressin but declined with time at both pH (17.3±1.8 pg/min per g hemibladder to 4.1±0.7 at pH 7.4, and 4.1±1.9 to 1.2±0.6 at pH 8.4).

When the serosal pH was maintained at 8.4 and comparable changes in pH (8.4 vs. 7.4) were made in the mucosal media, there was no effect on basal PGE_2 production (3.15±0.3 pmol/min per g hemibladder at mucosal pH 8.4 vs. 2.65±0.5 at mucosal pH 7.4, $n = 5$), and the J_v response to vasopressin (1.0 mU/ml) was not different (28.5±2.5 mg/min at mucosal pH 8.4 vs. 32.2±2.7 at mucosal pH 7.4). Because basal PGE_2 production was declining with time in all experiments, we assessed the effect of vasopressin per se on PGE_2 production using paired time controls that did not receive vasopressin. Serosal media was changed every 30 min for 180 min, and vasopressin (3 mU/ml) was added to the serosal media (pH 8.4) of one of each pair. PGE_2 production was not different in bladders receiving vasopressin (2.3 pmol/min per g hemibladder in vasopressin vs. 2.1 in time controls, $n = 10$, $P > 0.1$).

Effects of serosal acidification on PGE_2 production and the hydroosmotic response to 8-cpt-cyclic AMP in phosphate-buffered Ringer's. To determine if serosal acidification and the accompanying increase in basal synthesis of PGE_2 would impair the J_v response to exogenous cyclic AMP, identical experiments were performed at serosal pH 8.4 vs. 7.4 with the cyclic AMP analog 8-cpt-cyclic AMP (Fig. 2). This analog

TABLE I

Effects of Serosal Acidification on PGE_2 Production and J_v in the Basal State and after 3.0 and 10 mU/ml Vasopressin

							P values	
			pH 8.4		pH 7.4		8.4 vs. 7.4	
Conditions	Pairs	Time	J_v	PGE_2	J_v	PGE_2	J_v	PGE_2
	n	*min*	*mg/min*	*pmol/min/ghb**	*mg/min*	*pmol/min/ghb**		
Regular								
basal	9	(0–60)	0.5±0.1	4.1±1.9	0.6±0.07	17.3±1.8	NS	<0.001
3 mU/ml	9	(60–105)	31.3±2.2	3.1±0.9	21.2±2.1	8.4±2.1	<0.001	<0.02
10 mU/ml	9	(105–150)	36.5±2.1	1.2±0.6	32.5±1.4	4.1±0.7	<0.02	<0.02

* Gram hemibladder.

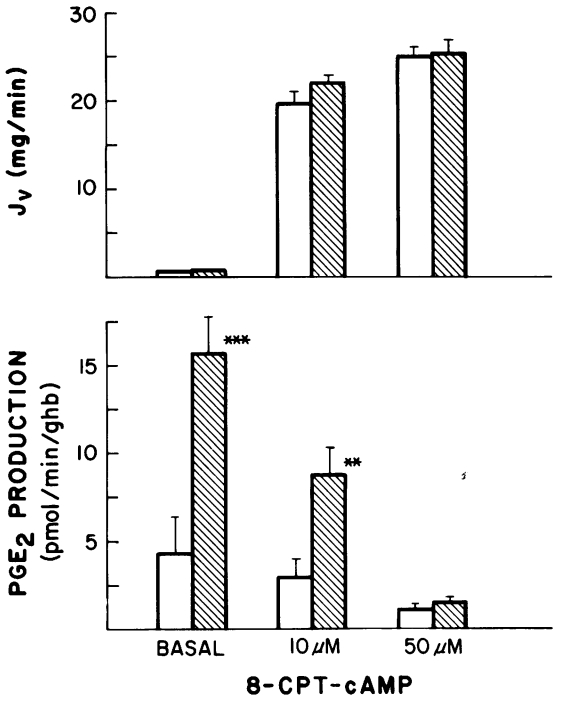

FIGURE 2 Effects of serosal media acidification (pH 7.4 vs. 8.4) on PGE_2 production and J_v in the toad urinary bladder in the basal state and after sequential addition of 10 and 50 μM 8-cpt-cyclic AMP. Values are mean±SEM in six experiments with hemibladders paired for differences in pH. Hatched bars indicate pH 7.4, open bars pH 8.4. J_v was measured gravimetrically in milligrams per minute and PGE_2 production was measured as picomoles per minute per gram hemibladder. Asterisks indicate P values by paired t analysis, with ** indicating $P < 0.02$ and *** indicating $P < 0.001$. ghb, gram hemibladder.

of cyclic AMP was used because low concentrations of 8-cpt-cyclic AMP are capable of fully mimicking the effect of vasopressin in the toad urinary bladder (25) and cortical collecting tubule (26). Although basal PGE_2 synthesis was again elevated in hemibladders at serosal pH 7.4 (15.6±2.3 pmol/min per g hemibladder vs. 4.4±1.9, $n = 6$, $P < 0.001$), in contrast to the response to vasopressin, the subsequent J_v response to submaximal concentrations of 8-cpt-cyclic AMP was not impaired (Fig. 2). As in the previous experiments PGE_2 production declined with time in bladders incubated at both pH 8.4 and 7.4. In these and previous experiments using a phosphate buffer Ringer's solution, serosal pH was observed to change 0.07–0.2 pH units during the 150-min experiment.

Effects of sequential decreases in serosal pH (8.4 to 6.9) on basal PGE_2 production and subsequent hydroosmotic response to vasopressin (0.5 mU/ml) in Tris-buffered Ringer's. The previous experiments suggested a direct relationship between serosal pH, basal PGE_2 synthesis, and the J_v response to vasopressin in the toad bladder. To further establish these relationships, separate sets of experiments were performed at serosal pH 8.4, 7.9, 7.65, 7.4, and 6.9. For these experiments an identical amphibian Ringer's was used except that 5 mM Tris was added; this buffer was found to hold the chosen pH essentially free of drift throughout the experiment (<0.03 pH unit change over 150 min). Bladders were paired for the minimal interval of pH over the pH range studied. Fig. 3 illustrates the effect of decreasing serosal pH on J_v after 0.5 mU/ml vasopressin. Serosal acidification resulted in a progressive decline in vasopressin-stimulated J_v from 26.6±0.5 mg/min at pH 8.4, to 13.6±1.4 at pH 7.65, and to 1.7±0.6 at pH 6.9. Basal PGE_2 synthesis was not different between pH 8.4 and 7.9, but PGE_2 synthesis increased from 5.1±0.9 pmol/min per g hemibladder to 18.8±2.8 over the pH range 7.9 to 6.9.

Effects of addition of exogenous PGE_2 on the subsequent response to vasopressin at pH 8.4. In the previous experiments basal PGE_2 synthesis at pH 7.4 averaged 16.0±0.2 pmol/min per g hemibladder in phosphate-buffered Ringer's ($n = 27$) and 16.1±1.7 in Tris-buffered Ringer's ($n = 28$). These values correspond to a final serosal PGE_2 concentration of ~1.5 ng PGE_2/ml serosal media (4 nM). To determine the specific effect of this concentration of PGE_2 on vasopressin-stimulated water flow in the absence of other possible pH-dependent factors, J_v was measured in

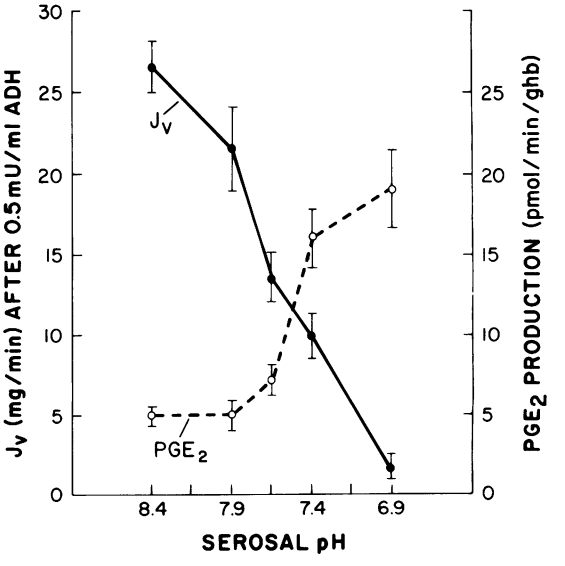

FIGURE 3 Effects of graded decreases in serosal media pH (8.4 to 6.9) on basal PGE_2 production and subsequent hydroosmotic response to vasopressin (0.5 mU/ml). Values are mean±SEM. Hemibladders were paired for the minimal interval of pH with 12–28 hemibladders at each pH. ADH, antidiuretic hormone; ghb, gram hemibladder.

paired hemibladders at serosal pH 8.4 and at serosal pH 8.4 to which 1.5 ng PGE_2/ml had been added. Thus, these hemibladders were exposed to identical serosal pH and any difference observed in J_v could be attributed solely to serosal PGE_2 content and not to other factors considered to be pH dependent. The addition of 1.5 ng PGE_2/ml serosal media to bladders at pH 8.4 resulted in marked inhibition of J_v (11.2±1.5 vs. 19.6±5.2, n = 8, $P < 0.01$); this lower J_v is ~65% of the inhibition usually observed at pH 7.4 (Fig. 4). Similar results were observed in three additional experiments; the addition of 1.5–3.0 ng PGE_2/ml serosal media at pH 8.4 inhibited J_v in the range of 62–71% of the inhibition usually seen at pH 7.4 alone. Thus, in experiments at pH 8.4 the addition of concentrations of exogenous PGE_2 usually produced at pH 7.4 inhibited J_v substantially, but not completely to the levels observed at pH 7.4 alone.

Effects of cyclooxygenase inhibitors on PGE_2 production and the subsequent response to vasopressin at serosal pH 8.4 vs. 7.4. Hemibladders were paired for pH (7.4 vs. 8.4) and the cyclooxygenase inhibitor was added for 120 min before the usual basal (60-min) and after vasopressin (45-min) periods. In separate control experiments, bladders were paired for pH (7.4 vs. 8.4) without inhibitors. During the first 60 min of preincubation with inhibitors, PGE_2 was detected in low concentration (0.7–3.5 pmol/min per g hemibladder, n = 10), but was not detected in the serosal media after the second 60 min of preincubation. Fig. 5 demonstrates that a 2-h preincubation with 1 μM indomethacin or naproxen at pH 7.4 reverses the inhibition

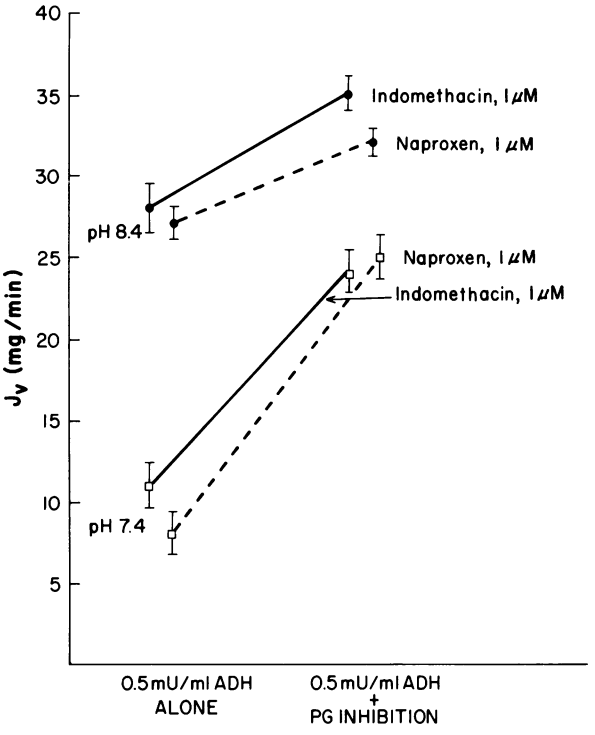

FIGURE 5 Effects of cyclooxygenase inhibitors on the subsequent J_v response to vasopressin (0.5 mU/ml) at serosal pH 7.4 vs. 8.4. Hemibladders (n = 6–10/group) were paired for differences in pH and the cyclooxygenase inhibitor was added for 120 min before measurement of J_v. In separate experiments hemibladders (n = 8) were paired for differences in serosal pH without inhibitors. ADH, antidiuretic hormone.

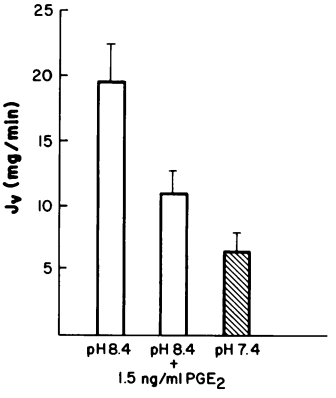

FIGURE 4 Effects of addition of exogenous PGE_2 on the subsequent response to vasopressin (0.5 mU/ml) at serosal pH 8.4. The concentration of PGE_2 added (1.5 ng/ml; 4 nM) was identical to the concentration of PGE_2 released into the serosal media at pH 7.4 in previous studies. Hemibladders (n = 8) were paired for pH 8.4 alone vs. pH 8.4 with 1.5 ng/ml exogenous PGE_2. J_v after 0.5 mU/ml at pH 7.4 was determined in eight separate hemibladders. Open bars indicate pH 8.4; hatched bars pH 7.4.

of vasopressin-stimulated J_v usually seen at this pH. (J_v of 7.8±1.2 and 10.9±1.3 mg/min at pH 7.4 alone, increasing to 24±1.5 and 25±1.4 at pH 7.4 with indomethacin and naproxen, respectively). The latter values are not statistically different from vasopressin-stimulated J_v usually seen at pH 8.4, in the absence of prostaglandin inhibitors. However, following pretreatment with indomethacin and naproxen (1 μM), water flow at pH 8.4 increased above that observed during prostaglandin inhibition at pH 7.4 ($P < 0.01$). While the increase in J_v after 0.5 mU/ml vasopressin during inhibition of prostaglandin synthesis was much greater at pH 7.4 vs. 8.4 (~150 vs. 28% increase) a small prostaglandin-independent pH effect persisted at this submaximal concentration of vasopressin. Similar results were observed with ibuprofen. After incubation with ibuprofen (1 μM) J_v following 0.5 mU/ml vasopressin was 26.7±3.8 mg/min at pH 7.4 and 37.2±2.3 at pH 8.4 (n = 5/group; $P < 0.02$). However, when bladders were exposed to a concentration of vasopressin of 3 mU/ml, 120-min incubation with indomethacin (10 μM) abolished the effect of pH on J_v (35.2±2.3 at pH

8.4 vs. 34.9±1.9 at pH 7.4, $n = 10$ each group, $P > 0.1$). These findings demonstrate that inhibition of prostaglandin synthesis reverses in major part the effect of serosal pH on the response to low concentrations of vasopressin, but that a small prostaglandin-independent pH effect remains. Under the conditions of the present study this prostaglandin-independent effect of pH was reversed by near maximal concentrations of vasopressin.

DISCUSSION

The present experiments demonstrate that basal prostaglandin synthesis, specifically the synthesis of PGE_2, plays a critical role in the well-established pH dependence of the hydroosmotic response to vasopressin in the toad urinary bladder. This conclusion is supported by the following: (*a*) acidification of the serosal media from pH 8.4 to 6.9 results in a progressive increase in basal PGE_2 synthesis accompanied by a progressive and marked decline in the J_v response to vasopressin; (*b*) preincubation with inhibitors of prostaglandin synthesis reverses in major part the effects of serosal pH on the subsequent response to submaximal concentrations of vasopressin; (*c*) the addition of that concentration of PGE_2 found in the serosal media at pH 7.4 to serosal media at pH 8.4 results in 62–71% of the inhibition of J_v usually observed at pH 7.4.

The inhibitory effect of serosal acidification on vasopressin-stimulated water flow has been investigated extensively in previous studies. In the toad bladder this inhibition has been observed in multiple buffer systems, i.e., when serosal pH is reduced by bubbling with CO_2, by addition of strong acid (HCl), or by removal of bicarbonate (1–8). In addition, acute metabolic acidosis in vivo has been shown to inhibit the response to vasopressin in both the amphibian bladder (9) and mammalian kidney (7). In a study describing cyclic AMP as the intracellular messenger of vasopressin, Orloff and Handler (3) observed that while the hydroosmotic response in the toad bladder to vasopressin and theophylline was impaired, the response to cyclic AMP was not inhibited by serosal acidification (pH 7.6 to 6.5). Gulyassy and Edelman (6) demonstrated that decreasing serosal pH (from 8.4 to 6.9) was associated with enhancement of cyclic AMP-stimulated J_v but that at lower pH (to 6.3) the response to cyclic AMP was also inhibited. Recent studies by Carvounis et al. (10) have extended these results by demonstrating that serosal acidification to pH 6.8 inhibited the response to vasopressin and enhanced the response to cyclic AMP without changing the permeability to urea. The results of our experiments are therefore in agreement with most previous studies in demonstrating that serosal acidification (pH 8.4 to 6.9) inhibits the response to vasopressin but not to cyclic AMP and its analogs. Taken together, these studies emphasize that a major effect of acidification on the action of vasopressin occurs at a step proximal to the formation of cyclic AMP.

Previous workers have considered several sites at which pH might alter the hydroosmotic response to vasopressin. The pH optimum both for binding of vasopressin to plasma membranes (pH 8.0–8.5) and for vasopressin-sensitive adenylate cyclase (pH 7.5) favor inhibition of the action of the hormone at lower pH (27, 28). Studies by Beck and Kim (7) were consistent with these predictions since acid media inhibited both vasopressin-stimulated adenylate cyclase and cyclic AMP accumulation in the rat renal medulla. The present study demonstrates that a specific mechanism by which reduction in serosal pH impairs the response to vasopressin is a pH-dependent increase in the basal production of PGE_2. This conclusion is supported by our demonstration that inhibition of prostaglandin synthesis reversed in major part the effect of serosal acidification on submaximal concentrations of vasopressin. Furthermore, in experiments in which pH was kept constant at 8.4, thereby excluding direct pH effects on hormone binding and adenylate cyclase, the addition of that concentration of PGE_2 produced at pH 7.4 (4 nM) resulted in 62–71% of the inhibition usually seen at pH 7.4; these experiments further demonstrate that PGE_2 per se and not other products of prostaglandin synthesis, including arachidonic acid, the intermediate endoperoxide PGH_2, or thromboxane A_2 is responsible for this effect. However, both the experiments with inhibitors of prostaglandin synthesis and with direct addition of PGE_2 are consistent with the notion that other prostaglandin-independent, pH-dependent factors may contribute to inhibition of submaximal concentrations of vasopressin since neither maneuver (inhibition of synthesis or direct addition of PGE_2) completely reversed the effects of pH at low concentrations of the hormone.

In this study an increase in the synthesis of PGE_2 by the toad bladder following the addition of vasopressin was not observed. Zusman et al. (18) previously reported that comparable concentrations of vasopressin increased production of PGE in the toad urinary bladder by 6- to 10-fold. An increase in PGE synthesis subsequent to the administration of vasopressin was also reported by Burch et al. (21); these workers found lower basal rates of PGE production and also used simultaneous time controls not receiving vasopressin (whereas Zusman et al. compared PGE production before and after vasopressin). Both laboratories measured PGE synthesis after alkaline conversion of PGE to PGB using a radioimmunoassay for PGB. In our study measurements of prostaglandin production in the basal state and following antidiuretic hormone were made sequentially, as in the study by Zusman et al. In contrast to their findings, the addition of vaso-

pressin in concentrations up to 10 mU/ml did not increase the production of PGE_2 (Fig. 1 and Table I). Rather, the production of PGE_2 was highest in the basal state and fell with time despite the administration of vasopressin. We also observed no effect of vasopressin (3 mU/ml) on PGE_2 production when simultaneous time controls were used. Extensive studies on this question were recently reported by Bisordi et al. (22) using an identical specific antibody for PGE_2 as in our study. These workers failed to detect stimulation of PGE_2 by vasopressin in the toad urinary bladder using a variety of techniques including simultaneous time controls. Our results are in agreement with Bisordi et al. (22) indicating that under the usual study conditions in the toad bladder, PGE_2 production is not stimulated by low concentrations of vasopressin. However, our results provide the first direct evidence that factors that modulate the response to vasopressin may exert this effect by changing the basal rate of prostaglandin synthesis.

Several lines of evidence suggest that the mechanism by which the basal production of PGE_2 inhibits the response to vasopressin in the toad bladder is by inhibition of vasopressin-sensitive adenylate cyclase. First, PGE_1 and PGE_2 inhibit the water flow response to vasopressin and theophylline, but not to cyclic AMP (11–14). Second, in the intact bladder both with and without theophylline in the media, concentrations of PGE_1 that inhibit vasopressin-stimulated J_v result in a $> 50\%$ inhibition of vasopressin-stimulated accumulation of cyclic AMP suggesting an effect on adenylate cyclase (29). Lipson and Sharp (12) earlier proposed that PGE_1 either competitively inhibits the action of vasopressin on adenylate cyclase or binds to a different receptor and decreases the affinity of the vasopressin receptor. It should be emphasized that in contrast to the amphibian urinary bladder the relationships between prostaglandin precursors, primary prostaglandins, other metabolites of arachidonic acid, and the action of vasopressin may be more complex in the mammalian kidney. In sections of rat renal medulla high concentrations of PGE_2 (0.8 mM) increase adenylate cyclase and are additive with vasopressin (30) whereas arachadonic acid and the endoperoxide PGH_2 inhibit both basal and vasopressin-stimulated adenylate cyclase (31). In studies of isolated medullary collecting tubules from the rat kidney Jackson et al. (32) found no effect of PGE_2 on basal or vasopressin-stimulated adenylate cyclase suggesting that other products of prostaglandin synthesis may regulate vasopressin-sensitive adenylate cyclase in the mammalian collecting duct.

It is likely that the mechanism by which serosal acidification increases the production of PGE_2 is related to a change in intracellular pH. Intracellular pH in the toad bladder, as determined by the distribution of [^{14}C]5,5-dimethyl-2,4-oxalidinedione (DMO), has been shown to parallel changes in serosal media pH (33, 34), whereas mucosal acidification, which had no effect on PGE_2 production or the response to vasopressin in the present study, is without effect on intracellular pH (33). Two possible sites for an effect of intracellular pH on prostaglandin synthesis are the activity of phospholipase A_2 and the cyclooxygenase enzymes (prostaglandin synthetase activity). In whole rabbit kidney microsomes, a narow pH optimum at 7.5 has been reported for prostaglandin synthetase whereas microsomes from fresh rabbit renal medulla gave two pH optima at 7.0 and 9.0 (35). A third possibility is that serosal acidification may increase PGE_2 synthesis subsequent to a change in the intracellular concentration of calcium. Recent studies suggest that calcium may play a central role in controlling prostaglandin and thromboxane biosynthesis (36). The calcium ionophore A23187 has been shown to stimulate prostaglandin synthesis in several tissues, including the rat renal medulla and cultured renal epithelial cells (36, 37), and we have observed that A23187 markedly increases the production of PGE_2 in the toad urinary bladder.[2] Since extracellular acidosis has been shown to increase intracellular calcium activity in other tissues (38, 39), it is possible that changes in cytosolic calcium concentration mediate the effect of pH on prostaglandin production in the toad bladder.

The present studies provide the first evidence that factors that modulate the hydroosmotic response to vasopressin may exert this effect by changing the basal rate of prostaglandin synthesis. Studies in the toad urinary bladder are usually performed at pH 7.4 to 8.0. Our work demonstrates that this is a critically sensitive range for an effect of media pH on the basal production of PGE_2.

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